

## Screening of Oriental Herbal Medicines for Antibacterial Activities

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**Abstract** – The water extracts of oriental herbal medicines which have been clinically used to treat bacterial infections in Korea were screened for *in vitro* antibacterial activity by the paper disc assay method. Two Gram positive bacteria, *Staphylococcus aureus* SG511, *Bacillus subtilis* ATCC 6633 and two Gram negative bacteria, *Escherichia coli* 055, *Pseudomonas aeruginosa* 9027 were used as test organisms. Among 83 of the extracts tested, 25 were active against *Staphylococcus aureus* SG 511, 9 were active against *Bacillus subtilis* ATCC 6633, while none showed inhibitory activity against *Escherichia coli* 055 and *Pseudomonas aeruginosa* 9027. Among them, Hwangyonhaedoktang plus hwangyon, Chongwisan, and Ssangbaksan showed remarkably potent antibacterial activity.

**Key words** – Oriental herbal medicine (Hanbang), antibacterial activity, *Staphylococcus aureus* SG511, *Bacillus subtilis* ATCC 6633, *Escherichia coli* 055, *Pseudomonas aeruginosa* 9027, paper disc assay method.

### Introduction

The Oriental countries including Korea, China and Japan have been using traditional medicine to cure many diseases since ancient times. Because of long history and experience in the usage of herbal medicines, development of new drugs derived from herbal medicines may avoid the side effects or toxicities that synthetic drugs might have. Therefore, traditional medicines can be considered as a gold mine for new drug development. Although, many studies on antibacterial activities of extracts or the components of plants growing or cultivated in

Korea have been reported (Cha,1977, Woo *et al.*, 1979, Bae *et al.*, 1987), no report has been made so far on the oriental herbal medicines which have been used clinically to treat bacterial infections in Korea. In order to develop new antibacterial agents from oriental herbal medicines, water extracts of 83 of oriental herbal medicines were screened for *in vitro* antibacterial activity by the paper disc assay method (Kahn *et al.*, 1980, Acar and Goldstein 1991). Among them, water extracts of Hwangyonhaedoktang plus hwangyon, Chengwisan, and Ssangbaksan showed remarkably potent antibacterial activity. Fractionations and activity-guided purifications of

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**Table 1.** Antibacterial activity of extracts of various herbal medicines

Species	Antibacterial activity (mg/mL) <sup>a</sup>											
	<i>S. aureus</i> SG511			<i>B. subtilis</i> ATCC6633			<i>E. coli</i> 055			<i>P. aeruginosa</i> 9027		
	200	100	50	200	100	50	200	100	50	200	100	50
Soungtang	-	-	-	-	-	-	-	-	-	-	-	-
Ssangbaeksan	+++	++	-	++	++	-	-	-	-	-	-	-
Taebangpungtang	-	-	-	-	-	-	-	-	-	-	-	-
Taechongnyongtang	-	-	-	-	-	-	-	-	-	-	-	-
Taeganghwaltang	-	-	-	-	-	-	-	-	-	-	-	-
Taehwangmokdanpitang	++	-	-	++	-	-	-	-	-	-	-	-
Taesihotang	++	-	-	+	-	-	-	-	-	+	-	-
Tanggwijomtongtang	-	-	-	-	-	-	-	-	-	-	-	-
Tanglisodogum	-	-	-	-	-	-	-	-	-	-	-	-
Tansanbohyoltang	-	-	-	-	-	-	-	-	-	-	-	-
Toinsunggitang	+	-	-	+	+	-	-	-	-	-	-	-
Tojoksan	-	-	-	-	-	-	-	-	-	-	-	-
Tungyongtang	-	-	-	-	-	-	-	-	-	-	-	-
Uiiintang	-	-	-	-	-	-	-	-	-	-	-	-
Uiinbujapaejangtang	-	-	-	-	-	-	-	-	-	-	-	-
Yongdamsagantang	++	-	-	-	-	-	-	-	-	-	-	-
Yotaektonggitang	-	-	-	-	-	-	-	-	-	-	-	-
Youngsonjetongum	-	-	-	-	-	-	-	-	-	-	-	-

<sup>a</sup>The antibacterial activity is represented as follows: - no inhibitory zone was formed; +, inhibitory zone was formed with 8 mm; ++, 9~12 mm; +++, 13~16 mm; +++++, more than 17 mm in diameter. <sup>b</sup>ten times dosed Hwangbaek was added to the Hwangyonhaedoktang. <sup>c</sup>ten times dosed Hwangyon was added to the Hwangyonhaedoktang.

active crude extracts are under way.

## Materials and Methods

**Preparation of Extracts** – Since oriental herbal medicines have been clinically taken as water extracts, each sample was extracted with 300 ml of boiling water for 2 hours, in an open vessel. The hot extract was filtered and lyophilized and the resulting extract was tested. Samples of 200 mg each were dissolved in water, then diluted to appropriate concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml) and filtered with a microfilter (Stervix 0.22  $\mu\text{m}$ ) prior to testing.

**Test Organisms** – Two Gram positive bacteria, *Staphylococcus aureus* SG511, *Bacillus subtilis* ATCC 6633 and two Gram negative bacteria, *Escherichia coli* 055, *Pseudomonas aeruginosa* 9027 were used as test organisms.

**Antibacterial Activity** – Each strain was cultured in 10 ml of Fleish Extract Broth (Beef Extract 10 g, Bacto Peptone 10 g, NaCl 3 g,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  2 g/l distilled water) at 37°C for 18 hours. After 18 hours cultivation, the turbidity of bacterial suspensions was adjusted with the same sterile broth as Fleish Extract Broth from 0.02 to 0.03 optical density (OD) at 540 nm and then used for the tests. For the paper disc assay method, 2 ml of the bacterial suspensions was poured uniformly into the plate (size 245 mm  $\times$  245 mm  $\times$  20 mm, Nunc) made of 88 ml of Müeller Hinton agar as media. The paper discs (6 mm in diameter, Difco) containing 2 mg, 1 mg and 0.5 mg of samples were carefully placed on the plate and the samples were allowed to diffuse for 1 hour at 4°C in a refrigerator. Further cultivation was followed at 37°C for 18 hours and the clear inhibition zone was observed.

## Results and Discussion

The antibacterial activity of 83 of oriental herbal medicines, most of which are comm-

only used to treat bacterial infections in Korea were screened by the paper disc assay method. The antibacterial activity was evaluated on the basis of the following criterion: an inhibitory zone below 8.0 mm in diameter is negative in antibacterial action and that over 8.0 mm is positive (Bauer *et al.*, 1966). The latter is further classified by three grades based on their activity. Table 1 summarizes the results of antibacterial activity of 83 of oriental herbal medicines. About 25 extracts showed antibacterial activity against *S. aureus* SG 511, 9 were active against *B. subtilis* ATCC 6633, but none showed antibacterial activity against Gram negative bacteria, *E. coli* 055, and *P. aeruginosa* 9027. Among these extracts, Hwangyonhaedoktang plus hwangyon, Chongwisan, and Ssangbaksan showed potent antibacterial activity. It is of interest to compare the antibacterial activity of Hwangyonhaedoktang plus hwangyon with that of Hwangyonhaedoktang. Both of them consist of Coptidis Rhizoma, Gardeniae Fructus, Phellodendri Cortex, and Scutellariae Radix (Ge Hong, 1978). But the amount of Coptidis Rhizoma of Hwangyonhaedoktang plus hwangyon is ten times as much dosed as that of Hwangyonhaedoktang. Therefore, we assumed that the ingredients of Coptidis Rhizoma of Hwangyonhaedoktang play a major role in the improvement of antibacterial activity. The results of this *in vitro* study are the first trial to elucidate the effectiveness of herbal medicines which are clinically used to treat bacterial infections. Fractionations and activity-guided purifications of active extracts are under way.

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## References

Acar, J. F., Goldstein, F. W., *Antibiotics in Labora-*

- tory Medicine* (Lorian, V.ed.), Williams & Wilkins, Baltimore, 1991, pp. 17-52.
- Bae, K., Byun, J., Screening of Leaves of Higher Plants for Antibacterial Action. *Kor. J. Pharmacogn.*, **18**, 1-4 (1987).
- Bauer, A. W., Kirby, W. M. M., Sherris, J. C., Turck, M., Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Amer. J. Clin. Pathol.*, **45**, 493-496 (1966).
- Cha, S. M., Potential Anticancer and Antibacterial Medicinal Plants, A Statistical Evaluation of Their Frequencies of Appearance in Oriental Medicine Formularies. *Kor. J. Pharmacogn.*, **8**, 1-15 (1977).
- Ge Hong, Shi Hou Fang, Ji Wen Shu Ju, Taipei, 1978, pp. 38-39.
- Khan, M. R., Ndaalio, G., Nkunya, M. H. H., Wevers, H. and Sawhney, A. N., Studies on African Medicinal Plants Part I. Preliminary Screening of Medicinal Plants for Antibacterial Activity. *Planta Med.*, **40**(Supplement), 91-97 (1980).
- Woo, W. S., Lee, E. B., Han, B. H., Biological Evaluation of Korean Medicinal Plants(III). *Arch. Pharm. Res.*, **2**, 127-188 (1979).

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