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# Screening and Isolation of Antibiotic Resistance Inhibitors from Herb Materials-Resistance Inhibition of Volatile Components of Korean Aromatic Herbs#

**Chung Kyu Lee, Hyekyung Kim, Kyung Ho Moon and Kuk Hyun Shin***

*College of Pharmacy, Kyungsung University, Pusan 608-736 and *Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea*

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The resistance inhibitory activities of 54 odorant mixtures(essential oil) from 41 Korean aromatic herbs were tested against multi-drug resistant *Staphylococcus aureus* SA2, which has resistances to 10 usual antibiotics including chloramphenicol. As results, combinations of 28 kinds of samples from 21 herbs and chloramphenicol have resistance inhibitory activities in dose dependent manner.

**Key words** : Volatile components, Resistance inhibition, Antibiotics resistance, Combination with antibiotics

## INTRODUCTION

Since the introduction of penicillin, many compounds have been developed as antimicrobial agents or antibiotics. But newly investigated potent antimicrobial agent soon loose its activity. One of the main reasons disappearing the effectiveness is due to the development of resistance to antibiotics by microorganism. In order to maintain the effectiveness of antibiotic, the reduction of resistance is thought to be more valuable than to select newer and stronger antibiotics. As well as the numerous synthetic compounds, many natural products were found to possess antibiotic activities (Yashphe, *et al.*, 1979; Sharma, *et al.*, 1980; Janssen, *et al.*, 1984; Onawunmi, *et al.*, 1984; Paster, *et al.*, 1990; Garg, *et al.*, 1992; Soliman, *et al.*, 1994; Marotti, *et al.*, 1994; Gundidza, *et al.*, 1994; Shin, 1996).

But their activities proved ineffective in resistance acquired microorganism. So some portion of efforts to develop new antibiotics should be oriented to find resistant inhibitors or reducers. As the interaction of β-lactam antibiotic with β-lactamase was reported by Bush and Sykes (1984), many attempts were made to elucidate the nature of resistance. But few efforts were exerted to develop the resistance inhibitors. Augmentine, a combined preparation of amoxicilline and clavulanic acid, is a good example of resistance inhibitory preparation. To investigate such preparations, we attempted to search the resistance inhibitors from herbal materials (Kim, *et al.*, 1995; Park, *et al.*, 1997). According to the results of previous studies, some odorant or nonpolar herbal components, when combined with antibiotics, showed potent inhibitory activities against multi-drug resistant microorganisms such as *Staphylococcus aureus* SA2, which has resistance to 10 usual antibiotics including chloramphenicol (Cm). In present study we examine 54 odorant samples (essential oil mixtures from 41 species) for the resistance inhibitory activity against the strain. As results, we found out that 28 samples, when combined with Cm, have significant resistance inhibitory activities in dose-dependant manner.

## MATERIALS AND METHODS

### Plant material and sample preparation

The collection of Korean aromatic herbs, botanical identifications and extraction of volatile components using Karlsruker apparatus from corresponding plant parts were carried out by Shin (1996). Each prepared volatile sample (mixture) was dissolved in 0.5 ml of absolute ethanol, and was added to liquid medium. The multi-drug resistant strain *S. aureus* SA2 was isolated from hospitalized patients and cultivated in our laboratory. The liquid medium, consisted of tryptic soy broth (TSB, containing 1.7% Bactotrypton, 0.3% Bactosoyton, 0.25% Bactodextrose, 0.5% sodium chloride

*Correspondence to*: Chung Kyu Lee, College of Pharmacy, Kyungsung University, Pusan 608-736, Korea

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and 0.25% dipotassium phosphate, Difco), was placed in 15 ml capped tube and pasteurized (Kim, 1995).

### *In vitro* evaluation of antimicrobial activity and resistance inhibition

The bacterial strain was cultivated in TSB with 50 μg/ml of Cm for sustaining the resistance and suppression of other bacterial strain at 37°C for 12 hrs. Each sample of various dose and 50 μg/ml of Cm were added to 5 ml of TSB medium with $10^5$ cells of *S. aureus* SA2. The mixture was vortexed thoroughly and then incubated at 37°C for 24 hrs. After incubation, turbidity of incubate dued to the growth of microorganism was measured spectrophotometrically. The resistance inhibitory effect was expressed by comparing the growth in sample treated group with control group.

## RESULTS AND DISCUSSION

In the view point of resistance inhibition, Jedličková *et al.* (1992) attempted to potentiate the activities of

**Table I.** Antimicrobial (AM) and resistance inhibitory (RI) activities of volatile components from Korean herbs against multidrug-resistant *S. aureus* SA2.

| Species (Family) treated[a] | Part | AM[b] (Growth % of Control) | RI[c] (Growth % of Control) | Antimicrobial activities reported[d] |
|---|---|---|---|---|
| *Acanthopanax koreanum* (Araliaceae) | stem | 94.39 | 101.92 | Sa+++ |
| *Ainsliama acerifolia* (Compositae) | root | 28.89 | 1.50 | - |
| *Akebia quinata* (Lardizabalaceae) | flower | 103.75 | 100.53 | - |
| *Angelica dahurica* (Umbelliferae) | root | 92.81 | 1.19 | - |
| *Angelica gigas* (Umbelliferae) | leaf | 98.45 | 91.48 | - |
| | root | 103.13 | 93.88 | Sa+++; Ps+ |
| *Angelica koreana* (Umbelliferae) | root | 96.55 | 94.27 | - |
| *Angelica tenuissima* (Umbelliferae) | root | 13.85 | 0.63 | - |
| *Artemisia iwayomogi* (Compositae) | leaf | 1.83 | 0.11 | Sa+++; Ps+++ |
| *Asiasarum sieboldii* (Aristolochiaceae) | stem | 50.97 | 0.27 | - |
| | root | 1.10 | 3.53 | - |
| *Aster scaber* (Compositae) | leaf | 98.96 | 4.22 | - |
| *Cinnamomum japonicum* (Lauraceae) | leaf | 51.43 | 0.78 | - |
| *Clerodendron trichotomum* (Verbenaceae) | leaf | 97.95 | 0.14 | Sa+++; Ps++ |
| *Codonopsis lanceolata* (Campanulaceae) | leaf | 99.74 | 89.97 | - |
| *Dendropanax morbifera* (Araliaceae) | leaf | 103.44 | 37.84 | Sa+++; Ps+++ |
| *Dictamnus dasycarpus* (Rutaceae) | stem | 101.38 | 94.37 | - |
| | leaf | 99.70 | 18.35 | Ps+ |
| | root | 97.02 | 31.45 | - |
| *Gardenia jasminoides* (Rubiaceae) | flower | 98.21 | 0.91 | - |
| *Houttuynia cordata* (Saururaceae) | whole | 103.44 | 98.48 | Ps+ |
| *Juniperus rigida* (Cupressaceae) | leaf | 96.33 | 105.59 | Sa+++; Ps+++; Ef++ |
| *Ligusticum wallichii* (Umbelliferae) | rhyzome | 101.95 | 94.66 | Ps+; Ef+ |
| *Ligustrum japonicum* (Oleaceae) | flower | 98.21 | 4.01 | - |
| | stem | 5.72 | 2.00 | - |
| | leaf | 97.02 | 1.96 | - |
| *Lindera obtusiloba* (Lauraceae) | flower | 105.03 | 1.54 | - |
| | stem | 100.29 | 0.80 | Sa+++; Ps+++; Ef++ |
| | leaf | 37.17 | 88.25 | Sa++ |
| *Magnolia sieboldii* (Magnoliaceae) | flower | 105.36 | 99.67 | Sa+++; Ps+++ |
| *Paulownia coreana* (Scrophulariaceae) | flower | 97.73 | 100.66 | - |
| *Peucedanum japonicum* (Umbelliferae) | root | 94.18 | 96.72 | Sa+++; Ps++ |
| *Philadelphus schrenkii* (Saxifragaceae) | stem | 96.33 | 95.59 | - |
| | leaf | 104.48 | 3.67 | Ps+ |
| | flower | 103.75 | 8.34 | - |

**Table I.** Continued

| Species (Family) treated[a] | Part | AM[b] (Growth % of Control) | RI[c] (Growth % of Control) | Antimicrobial activities reported[d] |
|---|---|---|---|---|
| *Pinus densiflora* (Pinaceae) | leaf | 100.82 | 98.88 | Sa+++; Ps+++ |
| *Pinus koraiensis* (Pinaceae) | leaf | 104.07 | 93.80 | Sa+++; Ps+++ |
| *Pittosporum tobira* (Pittosporaceae) | leaf | 66.18 | 0.91 | Sa+++; Ps++ |
| *Poncirus trifoliata* (Rutaceae) | fruit | 96.10 | 0.33 | Ps+; Ef+ |
| *Prunus padus* (Rosaceae) | stem | 95.01 | 85.77 | Sa+++; Ps++ |
| | leaf | 98.70 | 35.05 | - |
| *Ptestyrax corymbasa* (Styracaceae) | flower | 57.80 | 1.95 | - |
| *Rosa multiflora* (Rosaceae) | stem | 3.49 | 0.67 | Ps+; Ef+ |
| | leaf | 5.64 | 64.51 | - |
| | flower | 102.83 | 0.69 | - |
| *Rosa davurica* (Rosaceae) | flower | 100.00 | 96.68 | Ef+ |
| *Sorbus alnifolia* (Rosaceae) | flower | 89.33 | 96.96 | - |
| *Staphylea bumalda* (Staphyleaceae) | flower | 100.54 | 56.98 | - |
| *Styrax japonicus* (Styracaceae) | flower | 96.79 | 101.35 | - |
| *Syringa dilatata* (Oleaceae) | flower | 100.26 | 59.73 | - |
| *Valeriana fauriei* (Valerianaceae) | root | 25.44 | 12.26 | Sa+++; Ps+++ |
| *Vitex negundo* var. *incisa (Verbenaceae)* | flower | 96.52 | 1.50 | Sa+ |
| *Zanthoxylum ailanthoides (Rutaceae)* | leaf | 102.53 | 79.91 | - |
| *Zanthoxylum piperitum (Rutaceae)* | leaf | 99.74 | 2.31 | Sa+++; Ps+++ |

[a]Each volatile component obtained from the plant part was treated as 50 μg/ml of liquid medium.
[b]Antimicrobial activity is that of sample itself without addition of antibiotics.
[c]Resistance inhibitory activity is that of sample in combination with 50 μg/ml of chloramphenicol
[d]For details confer Shin(1996). Inhibition: +++, more than 95%; ++, more than 80%; +, more than 60% and -, less than 60% of control at 100 μg/ml of extract, respectively. Microorganism: Sa, *S. aureus* ATCC25923; Ps, *Pseudomonas aeruginosa* ATCC 27853 and Ef, *Enterococcus faracali* ATCC29212.

antibiotics using natural products, such as 1,8-cineol, linalool and terpinen-4-ol. They found that the combinations of these essential oil components with antibiotics, such as amikacin, gentamicin or tobramycin, strongly inhibited the growth of microorganisms, such as *E. coli, Staphylococcus* spp. and *Pseudomonas* spp. But they didn't explain the mechanism of such effects. According to Shin (1996), 24 samples (volatile mixtures from 23 herbs) among the tested 54 samples showed antimicrobial effects against the *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC 27853 and/or *Enterococcus faracali* ATCC29212. For example, volatile components from the stem of *Acanthopanax koreanum* (Araliaceae) potently inhibited the growth of *S. aureus* ATCC25923 *in vitro.* But in the present study using antibioitics resistant *S. aureus* SA2, samples didn't inhibited the growth of microorganism. Among the tested samples, some of them (eight samples) showed inhibitory activities in the growth of *S. aureus* SA2 (less than 30% of control in growth, Table I). Such results imply that many of antimicrobial samples can't affect the resistance acquired microorganism. So we tried to verify the activity of samples combined with antibiotics for resistance inhibition. As also shown in Table I, 28 samples among the tested 54

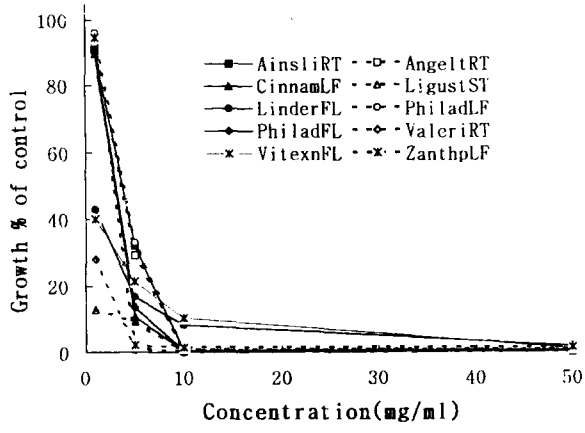


**Fig. 1.** Dose-dependent inhibition in the growth of antibiotics resistant *S. aureus* SA2 by samples of group A in combination of 50 μg/ml of Cm. Legends: AinsliRT, root of *Ainsliama acerifolia;* AngeltRT, root of *Angelica tenuissima;* CinnamLF, leaf of *Cinnamomum japonicum;* LigustST, stem of *Ligustrum japonicum;* LinderFL, flower of *Lindera obtusiloba;* PhiladLF, leaf of *Philadelphus schrenkii;* PhiladFL, flower of *Philadelphus schrenkii;* ValeriRT, root of *Valeriana fauriei;* VitexnFL, flower of *Vitex negundo* var. *incisa* and ZanthpLF, leaf of *Zanthoxylum piperita.*

samples showed resistance inhibition less than 30% of control in growth when combined with 50 μg/ml of Cm,

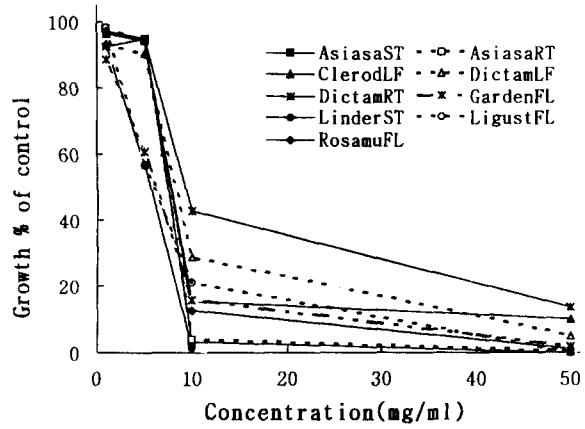


**Fig. 2.** Dose-dependent inhibition in the growth of antibiotics resistant *S. aureus* SA2 by samples of group B in combination of 50 μg/ml of Cm. Legends: AsiasaST, stem of *Asiasarum sieboldii*; AsiasaRT, root of *Asiasarum sieboldii*; ClerodLF, leaf of *Clerodendron trichotomum*; DictamLF, leaf of *Dictamnus dasycarpus*; DictamRT, root of *Dictamnus dasycarpus*; GardenFL, flower of *Gardenia jasminoides*; LigustFL, flower of *Ligustrum japonicum*; LinderST, stem of *Lindera obtusiloba* and RosamuFL, flower of *Rosa multiflora*.

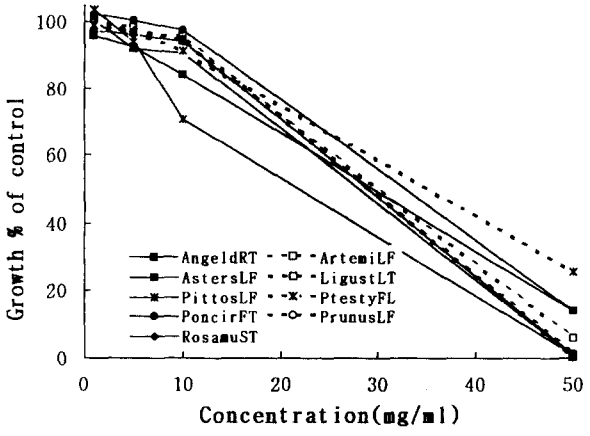


**Fig. 3.** Dose-dependent inhibition in the growth of antibiotics resistant *S. aureus* SA2 by samples of group C in combination of 50 μg/ml of Cm. Legends: AngeldRT, root of *Angelica dahurica*; ArtemiLF, leaf of *Artemisia iwayomogi*; AstersLF, leaf of *Aster scaber*; LigustLT, leaf and twig of *Ligustrum japonicum*; PittosLF, leaf of *Pittosporum tobira*; PoncirFT, fruit of *Poncirus trifoliata*; PrunusLF, leaf of *Prunus padus*; PtestyFL, flower of *Ptestyrax corymbasa* and RosamuST, stem of *Rosa multiflora*.

which mean the reduction of resistance.

To confirm the resistance inhibitory activities by the samples in combination with antibiotics, repeated tests with selected samples were carried out in various dose. As results, their activities were ascertained dose-dependently and could be divided into three types in the inhibitory doses. Ten samples of Group A including components from the root of *Ainsliama acerifolia* have 50% inhibition concentration ($IC_{50}$) below than 5 μg/ml approximately (Fig. 1). Group B of nine samples including the root of *Asiasarum sieboldii* have $IC_{50}$ values below 10 μg/ml(Fig. 2) and group C of nines samples including the root of *Angelica dahurica* have $IC_{50}$ values more than or near 30 μg/ml (Fig. 3).

The chemical features of active samples are, as screened by Shin (1996) using GC-Mass system (Hitachi D-2500 and Hewlett-Packard 5890 II) and Wiley/NBS library (McLafferty and Stauffer, 1988), found to be essential oil constituents. They are some terpenoids (such as β-cubebene from the root of Ainsliama acerifolia, 1,8-cineol and linalool from the leaf of *Cinnamomum japonicum* and β-elemene from the leaf of *Zanthoxylum piperitum*) and/or other compounds (benzaldehyde from the leaf and twig of *Prunus padus* and geyerine from the root of *Ainsliama acerifolia*). The mode or mechanism of resistance inhibitory activities and chemical characteristics will be elucidated later by continuing works in our labaratory.

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