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Screening and Isolation of Antibiotics Resistance Inhibitors from Herb Materials. II - Inhibitory Effects of 'Chwinamool' (*Aster scaber*)

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Abstract – Repeated column chromatographic works of hexane fraction of *Aster scaber* afforded some volatile mixtures of two or three components, which possess potent inhibitory activity (more than 90% of control at 50 μg/ml) to the resistance of multi-drug resistant *Staphylococcus aureus* SA2 when combined with chloramphenicol (50 μg/ml).

Key words – *Aster scaber*; Compositae; antibiotics resistance inhibition; combined preparation; *Staphylococcus aureus* SA2

In spite of continuing development of new antibiotics, man suffers from resistance of microorganism as ever. On the other hand, some researchers endeavor to use combination of natural products and antibiotics to reduce the resistance.[1,2] Previously, we reported the inhibitory actions of 21 herb materials and especially of their hexane fractions on the resistance of *Staphylococcus aureus* SA2,[2] which has multi-drug resistance to 10 usual antibiotics,[3-5] such as ampicillin, chloramphenicol (Cm), clindamycin, erythromycin, gentamicin, kanamycin, methicillin, streptomycin, tetracycline and tobramycin. It was found that the combination of hexane fraction of edible plant *Aster scaber* (Compositae, 'Chwinamool' as commonly called in Korea) and Cm has the most potent activity especially in reduction of resistance.[2] As a continuous study on it, we tried to elucidate the active principle of the inhibitory effect of the components of the plant on the resistance of the strain to Cm.

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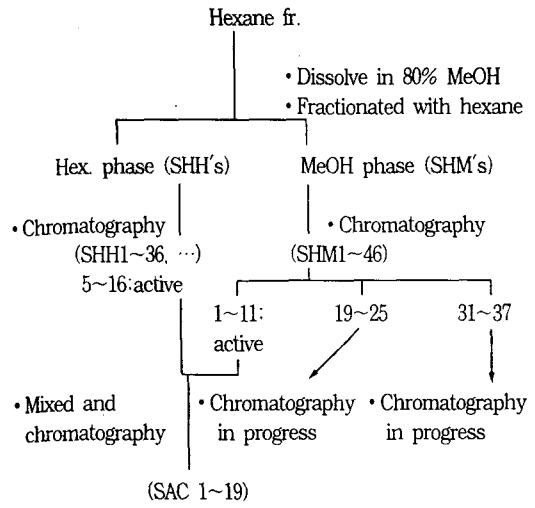
MATERIALS AND METHODS

Herb materials, extraction and fractionation – In the previous report,[2] we identified the edible plant 'Chwinamool' as *Synurus deltoides* (Compositae), but thereafter we confirmed the plant as *Aster scaber* Thunb. pharmacognostically. The voucher specimen is deposited in the Laboratory of Pharmacognosy, Kyungsung University (No. 943 A). From the aerial part of the plant (dry wt. 1.33 kg), methanolic extract (540 g) was obtained through routine work. By partitioning with *n*-hexane, chloroform, ethyl acetate and butanol in sequence, corresponding fractions (75 g, 12 g, 38 g and 57 g, respectively) and finally water raction (358 g) were obtained from methanolic extract. The active hexane fraction was dissolved in 80% MeOH and partitioned again with hexane to give upper hexane phase (SHH's, 22 g) and MeOH phase (SHM's, 40 g). The SHH's and SHM's were separately applied to sil-



Scheme I. Futher fractionation and column chromatographic procedure of hexane fraction of *A. scaber* to afford active eluates monitered by resistance inhibition activities against *S. aureus* SA2.

ica gel column chromatography (solvent system: petroleum benzene-chloroform=10-1 → 1-1) through monitoring with resistance inhibition activity, as shown in Scheme I.

Sample preparation - Each sample was dissolved in absolute ethanol, and were added to liquid medium.

Bacterial strain - *Staphylococcus aureus* SA2, which was isolated from hospitalized patient in the Pusan area, was cultivated in our laboratory.[2)]

Liquid Medium - Tryptic soy broth (TSB, containing 1.7% Bactotrypton, 0.3% Bacto-soyton, 0.25% Bacto-dextrose, 0.5% Sodium chloride and 0.25% Dipotassium phosphate, Difco) was placed in 15 ml capped tube and pasteurized.

***In vitro* resistance inhibitory effect** - Resistance inhibitory effect was determined according to methods of Japanese Chemotherapy Committee with slight modification.[6)] The bacterial strain was cultivated in TSB with 50 μg/ml of antibiotics for sustaining the resistance and suppression of other bacterial strain at 37℃ for 12 hrs. Each sample and antibiotics (50 μg/ml of Cm) were added to 5 ml of TSB medium with an inoculum size of 10^5 cells of *S. aureus* SA2. The medium was vortexed thoroughly and then incubated at 37℃ 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

Resistance inhibitory activities of column chromatographic eluates - The hexane fraction of *A. scaber*, oily liquid, consisted of more than 10 components on TLC (solvent: petroleum benzene-ethyl acetate=10-1). Among over 36 eluates of hexane phase, SHH's 5~16 were likely to be active but the amount of the eluates were not enough for further chemical works, they were mixed together with SHM's 1~11, which showed similar TLC pattern. The

Table I. Inhibitory effects of column chromatographic eluates of *Aster scaber* combinated with Cm[a] indicated by the growth of *S. aureus* SA2[b]

Eluates[c]	Growth % of Control	Eluates[c]	Growth % of Control
SHH 1	103.73	SHH 25	100.85
SHH 2	102.96	SHH 26	100.85
SHH 3	92.32	SHH 27	104.95
SHH 4	100.85	SHH 28	105.31
SHH 17	2.38	SHH 29	99.44
SHH 18	3.02	SHH 30	94.15
SHH 19	97.01	SHH 31	95.44
SHH 20	103.61	SHH 32	95.12
SHH 21	105.68	SHH 33	95.35
SHH 22	102.65	SHH 34	95.24
SHH 23	107.95	SHH 35	101.44
SHH 24	107.56	SHH 36	93.93

[a]Chloramphenicol conc., 50 μg/ml. [b]10^5 cells/5 ml medium. [c]Sample conc., 50 μg/ml.

Table II. Inhibitory effects of column chromatographic eluates of *Aster scaber* combined with Cm[a] indicated by the growth of *S. aureus* SA2[b]

Eluates[c]	Growth % of Control	Eluates[c]	Growth % of Control
SHM 12	100.10	SHM 30	95.50
SHM 13	94.53	SHM 31	2.11
SHM 14	101.49	SHM 32	33.56
SHM 15	100.50	SHM 33	16.50
SHM 16	99.50	SHM 34	6.91
SHM 17	103.98	SHM 35	2.45
SHM 18	100.00	SHM 36	0.94
SHM 19	22.43	SHM 37	0.74
SHM 20	8.94	SHM 38	0.78
SHM 21	5.26	SHM 39	0.57
SHM 22	35.14	SHM 40	89.50
SHM 23	2.99	SHM 41	98.80
SHM 24	0.62	SHM 42	92.20
SHM 25	7.17	SHM 43	92.20
SHM 26	92.53	SHM 44	1.31
SHM 27	92.04	SHM 45	1.15
SHM 28	107.96	SHM 46	0.93
SHM 29	104.98		

[a]Chloramphenicol conc., 50 μg/ml. [b]10^5 cells/5 ml medium. [c]Sample conc., 50 μg/ml.

mixed eluates were applied to column chromatography again to afford SAC's 1~19. SHH's 17 and 18, having different TLC pattern from preceding eluates, also showed potent inhibition.

On the other hand, MeOH phase gave 46 sub-eluates of different TLC patterns. They could be subdivided into three group of effectiveness in resistance inhibition (Table II) and difference in TLC pattern as SHM's 1~11, 19~25 and 31~39. As the amount of SHM's 1~11 and SHH's 5~16 were not enough to be applied to further tests and showed similar TLC pattern, they were mixed together and pplied to column chromatography (solvent system: petroleum benzene-chloroform=10-1 → 1-1) as mentioned above to give 19 sub-eluates (SAC 1~19). SAC's 2, 4~8, 11 and 13 revealed resistance inhibitory activities at level of 50 μg/ml (Table III).

Up to above results, we had felt a necessity to confirm and compare the activities of SAC's and SHM's directly. These eluates, with two or three spots on TLC (solvent system: petroleum benzene-ethyl acetate=10-1) showed few differences in pattern. For example, activities of SHM's 21~23 and 31~37 in Table II and SAC's 11~13 in Table III are significantly different each other, so they seemed to have different constituents.

Table III. Inhibitory effects of column chromatographic eluates of Aster scaber combined with Cm[a] indicated by the growth of *S. aureus* SA2[b]

Eluates[c]	Growth % of Control	Eluates[c]	Growth % of Control
SAC 1	77.74	SAC 11	10.33
SAC 2	14.02	SAC 12	104.35
SAC 3	98.49	SAC 13	7.39
SAC 4	8.73	SAC 14	111.63
SAC 5	3.86	SAC 15	118.27
SAC 6	17.63	SAC 16	118.58
SAC 7	3.86	SAC 17	105.84
SAC 8	2.61	SAC 18	114.47
SAC 9	108.88	SAC 19	113.39
SAC 10	113.39		113.39

[a]Chloramphenicol conc., 50 μg/ml. [b]10^5 cells/5 ml medium. [c]Sample conc., 50 μg/ml.

Table IV. Reconfirmative results in inhibitory effects of column chromatographic eluates of *Aster scaber* combined with Cm[a] indicated by the growth of *S. aureus* SA2[b]

Eluates[c]	Growth % of Control	Eluates[c]	Growth % of Control
SAC 4	32.88	SHM 19	48.98
SAC 5	95.39	SHM 20	5.93
SAC 6	83.96	SHM 21	0.86
SAC 7	3.35	SHM 22	1.08
SAC 8	8.57	SHM 23	0.94
SAC 9	97.25	SHM 24	1.73
SAC 10	100.00	SHM 25	1.98
SAC 11	6.22	SHM 31	0.75
SAC 12	9.90	SHM 32	0.63
SAC 13	4.38	SHM 33	0.42
		SHM 34	0.88
		SHM 35	1.42
		SHM 36	1.06
		SHM 37	0.74

[a]Chloramphenicol conc., 50 μg/ml. [b]10^5 cells/5 ml medium. [c]Sample conc., 50 μg/ml.

Results of reconfirmative test were agreed well to their TLC pattern (Table IV).

Chemical characteristics of effective subeluates-The TLC (developer: petroleum benzene-EtOAc=20-1 or 10-1) patterns of effective eluates SAC's and SHM's show two or three spots under UV (365 nm) or vaniline-sulfuric acid. According to our unpublished results, essential components of odorant or edible plants revealed the resistance inhibitory activities. To elucidate the mode of resistance inhibition and active principles, further works are in progress.

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