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Antibacterial Activity of Water Soluble Components of *Elfvingia applanata* Alone and in Combinations with Quinolones

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A preparation of water soluble components (EA) was made from carpophores of *Elfvingia applanata* (Pers.) Karst and its *in vitro* antibacterial activity on a number of bacterial species was examined by macrobroth dilution assay. Among 16 species of bacteria tested, the most potent antibacterial activity was observed against *Staphylococcus epidermidis* and *Proteus vulgaris*, of which MICs were 1.25 mg/ml. To investigate the antibacterial effects in combinations of EA with quinolone antibiotics, such as ciprofloxacin, enoxacin, lomefloxacin, norfloxacin, and ofloxacin, the fractional inhibitory concentrations (FICs) and the fractional inhibitory concentration indices (FICIs) for four bacterial strains were determined by macrobroth dilution checkerboard assay. Combinations of EA and quinolones exhibited either additive or indifferent effects of antibacterial activity in most instances. However, both synergistic and antagonistic effects were not observed in any cases.

KEYWORDS: ***Elfvingia applanata*, Macrobroth dilution checkerboard assay, Minimal inhibitory concentration (MIC), Fractional inhibitory concentration (FIC), Fractional inhibitory concentration index (FICI)**

Basidiomycetes are known as a treasure-trove of numbers of antimicrobial substances such as cinnabarin (Chung *et al.*, 1978), scorodonin (Anke *et al.*, 1980), phlebiakauranol aldehyde (Anke *et al.*, 1987), 4-O-methylmelleolide, and judeol (Donnelly *et al.*, 1985). Since the chemical structures of these components appeared to be quite different from the antimicrobial drugs currently used in the clinical field, they impose some possibility of candidate for developing a new class of antibiotics which might be valuable in tackling the problems of antibiotic resistant bacterial strains.

As of oriental folk medicine, the carpophores of *Elfvingia applanata* (Pers.) Karst (Polyporaceae) of Basidiomycetes have been used in treatment of various ailments including cancers as like the carpophores of *Ganoderma lucidum* (Kim and Kim, 1990) were widely supplemented to cancer patients. *E. applanata* seems to be very valuable biomaterial it contains biologically active components such as bitter triterpenoids (Nishitoba *et al.*, 1988), alusenone and friedelin (Protiva *et al.*, 1980), α-D-glucan and β-D-glucan (Mizuno *et al.*, 1981; Usui *et al.*, 1983). Recently, components which modulate humoral immune response were detected in a fraction (FDP) passed by DEAE cellulose ion exchange column obtained from aqueous extract of *E. applanata* (Kim *et al.*, 1994a). Not only this, reports on antibacterial and antiviral activities of the aqueous extract of *E. applanata* were also made (Kim *et al.*, 1994b; Rym *et al.*, 1999). And it is worthwhile to mention that a significant toxicity of these components was not detected in the acute toxicity test (Kim *et al.*, 1994c).

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To characterize the antimicrobial components of *E. applanata* in more detail, the antibacterial activity of the water soluble components (EA) prepared from the carpophores of *E. applanata*, was examined against sixteen species of bacteria. Then, the antibacterial effects of EA in combinations with quinolon antibiotics such as ciprofloxacin, enoxacin, lomefloxacin, norfloxacin, and ofloxacin were investigated in this study.

Materials and Methods

Materials. The carpophores of *E. applanata* (Pers.) Karst. (Polyporaceae) were purchased from a commercial supplier of Cheongju and authenticated by Dr. W. H. Park, Seoul National Industrial University. A voucher specimen (No. CPM 319) has been deposited at the Medicinal Plants Herbarium at Chungbuk Nat'l University.

Extraction of water soluble components. The dried carpophores of *E. applanata* (dry weight; 500 g) were extracted with hot water for 8 h. Steps of concentration of the extract followed by freeze lyophilization yielded ca. 20 grams of dark brownish powder, EA.

Bacterial strains and antibiotics. As gram positive bacteria, *Bacillus anthracis* ATCC 11966, *B. cereus* ATCC 11778, *B. subtilis* ATCC 6051, *Micrococcus luteus* ATCC 4698, *Staphylococcus aureus* ATCC 25923 and *S. epidermidis* ATCC 14990 were used. As gram negative bacteria, *Escherichia coli* ATCC 10586, *Enterobacter aerogenes* ATCC 13048, *Klebsiella oxytoca* ATCC 8724, *K. pneumonia* ATCC 13882, *Proteus vulgaris* ATCC 6509, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella thompson*

ATCC 8391, *S. typhimurium* ATCC 23564, *Serratia marcescens* ATCC 29633 and *Shigella sonnei* ATCC 11060 were used. Quinolone antibiotics, ciprofloxacin (CIP), enoxacin (ENO), lomefloxacin (LOM), norfloxacin (NOR) and ofloxacin (OFL) were supplied from Cheil Food and Chemical Inc. (Seoul, Korea).

The overnight (18 hr) bacterial cultures, *S. marcescens* grown at 26°C, *B. cereus* and *M. luteus* at 30°C and others at 37°C were prepared. Each culture was adjusted to 30% transmission at 540 nm (UV Spectronic 21) with Mueller-Hinton broth (Difco Co., Detriot, MI. USA) to give approximately about 10^8 CFU/m*l* and then further diluted 1 to 10 for culture seeding. EA was prepared in Mueller-Hinton broth at a concentration of 6 mg/m*l*. Quinolone antibiotics were dissolved in either DMSO (dimethyl sulfoxide) or Tween-80^R at lower than 2%, giving no apparent effect on bacterial growth. The concentrations of the stock solutions of quinolones were as follows; ciprofloxacin (860 μg/m*l*), enoxacin (1,000 μg/m*l*), lomefloxacin (586 μg/m*l*), norfloxacin (1,000 μg/m*l*) and ofloxacin (1,000 μg/m*l*).

Determination of the minimal inhibitory concentrations. The minimal inhibitory concentrations (MICs) of EA and quinolones were determined by macrobroth dilution method as suggested by National Committee for Clinical Laboratories Standard (NCCLS, 1993). One m*l* of sterile Mueller-Hinton broth was placed in a row of 10 sterile tubes (13 × 100 mm). One of either EA (6 mg/m*l*) or quinolone solutions was added to the first tube and then two-fold serial dilutions were made upto ninth tubes. The tenth tube was not included in two-fold serial dilution and used as a blank. Then, 50 μ*l* of bacterial inoculum (5×10^5 bacterial cells) was added to each tubes and incubated for 18 hours at the appropriate temperatures. The MICs were defined as the lowest concentration of either EA or antibiotics exhibiting no visual turbidity due to bacterial growth. To obtain more precise MICs, the same procedure was repeated within a vicinity of MICs of each EA and quinolone solutions.

Antibacterial effects of EA in combinations with quinolones. The antibacterial effects of EA in combinations with quinolones were measured by macrobroth dilution checkerboard assay (Eliopoulos and Moellering, 1991). *B. subtilis* ATCC 6051, *M. luteus* ATCC 4698, *P. aeruginosa* ATCC 10145 and *S. sonnei* ATCC 11060 were chosen for the assay of antimicrobial combinations. The concentration of each materials in combination regimen was ranged from 5-7 dilution below the MIC to twice as much as the MIC. Antibiotic activity of combined regimen was evaluated on the basis of fractional inhibitory concentration (FIC), fractional inhibitory concentration indices (FICI) and isobologram. FICs were defined as "MIC of drug A (or B) in combination/MIC of A (or B) alone". FICI was the sum of FICs of A and B (Norden, 1982). The values of FICI ≤ 0.5 were considered as synergistic, FICI values between 0.5 and 1.0 as additive, FICI values above 1.0 upto 2.0 were considered as indifferent and FICI greater than 2.0 as antagonistic each other (Otsuki and Nishino, 1996).

Results and Discussion

The MICs of EA against a numbers of bacteria are shown in Table 1 and those of quinolone antibiotics against four

Table 1. Minimal inibitory concentrations (MICs) of EA[a]

Strain	MIC (mg/m*l*)
Bacillus anthracis ATCC 11966	5.00
Bacillus cereus ATCC 11778	2.50
Bacillus subtilis ATCC 6051	4.00
Micrococcus luteus ATCC 4698	2.00
Staphylococcus aureus ATCC 25923	1.75
Staphylococcus epidermidis ATCC 14990	1.25
Escherichia coli ATCC 10586	5.00
Enterobacter aerogenes ATCC 13048	3.25
Klebsiella pneumonia ATCC 13882	5.00
Klebsiella oxytoca ATCC 8724	4.50
Proteus vulgaris ATCC 6509	1.25
Pseudomonas aeruginosa ATCC 10145	2.00
Salmonella thompson ATCC 8391	4.50
Salmonella typhimurium ATCC 23564	5.00
Serratia marcescens ATCC 29633	5.00
Shigella sonnei ATCC 11060	3.25

[a]EA : water soluble components of *Elfvingia applanata* carpophores. The data are expressed as mean values of four separate experiments.

Table 2. Minimal inhibitory concentrations (MICs) of quinolones used for antimicrobial combinations

Strain	MIC (μg/m*l*)				
	CIP[a]	ENO	LOM	NOR	OFL
Bacillus subtilis ATCC 6051	0.25[b]	1.00	0.50	0.50	0.25
Micrococcus luteus ATCC 4698	2.00	32.00	8.00	16.00	2.00
Pseudomonas aeruginosa ATCC 10145	0.03	0.13	0.06	0.25	0.03
Shigella sonnei ATCC 11060	0.25	2.00	1.00	4.00	0.50

[a]NOR : norfloxacin, OFL : ofloxacin, CIP : ciprofloxacin, ENO : enoxacin, LOM : lomefloxacin.
[b]The data are expressed as mean values of four separate experiments.

bacterial species are in Table 2. EA exhibited the most potent antibacterial activity against gram positive *S. epidermidis* as well as against gram negative *Proteus vulgaris*, of which MICs were 1.25 mg/ml. Potent antibacterial activity against *P. vulgaris* would be worthy to mention since a report was made that antibacterial components obtained from *Poria cocos* were effective against only gram positive bacteria (Lee *et al.*, 1982). However, the EA seemed to be more active against gram positive cocci than gram positive rods, such as *Bacillus* spp. Potent antibacterial activities of EA on *P. vulgaris* as well as *P. aeruginosa*, the opportunistic pathogens, which are very notorious for multiple drug resistance, would suggest one to pursue further studies on the antibacterial components of *E. applanata*, *Basiodiomyces* fungus.

Due to the potent antibacterial activity of quinolone antibiotics (Neu, 1990), clinical use of quinolones has tremendously expanded over a few decades. However, their clinical application has been imposing the problems of eliciting bacterial drug-resistance and cross-resistance with other antibiotics in addition to some side effects (Goto *et al.*, 1990). In order to lessen such trouble imposed by quinolones, combinations of quinolones and other synthetic and/or semi-synthetic antibiotics have been practiced in clinical use (Lewin and Smith, 1989). However, emergance of the drug resistant bacterial strains and drug side effects remain unsolved yet. Because of lesser chance of eliciting bacterial cross-resistance by combined use of the antimicrobial components of natural origin with synthetic antibiotics, it would be worthy to pursue the combined effects of antibacterial components of *Basidiomyces* with quinolones. As only few reports were available for antimicrobial combinations with components from *Basidiomycetes* (Yoon *et al.*, 1994; Kim *et al.*, 1994b), we investigated antibacterial effect of EA with combination of quinolones by macrobroth dilution checkerboard assay and compiled data in Table 3. As seen in Table 3 combinations of EA with quinolone antibiotics, neither synergistic ($0.5 \geq$ FICI) nor antagonistic effect (FICI > 2.0) was observed at any case. The combination of EA and ciprofloxacin (CIP) showed lower FICI than other combinations. The combination of EA and enoxacin (ENO) showed FICI of 2.0, an indifferent effect, against all strains tested.

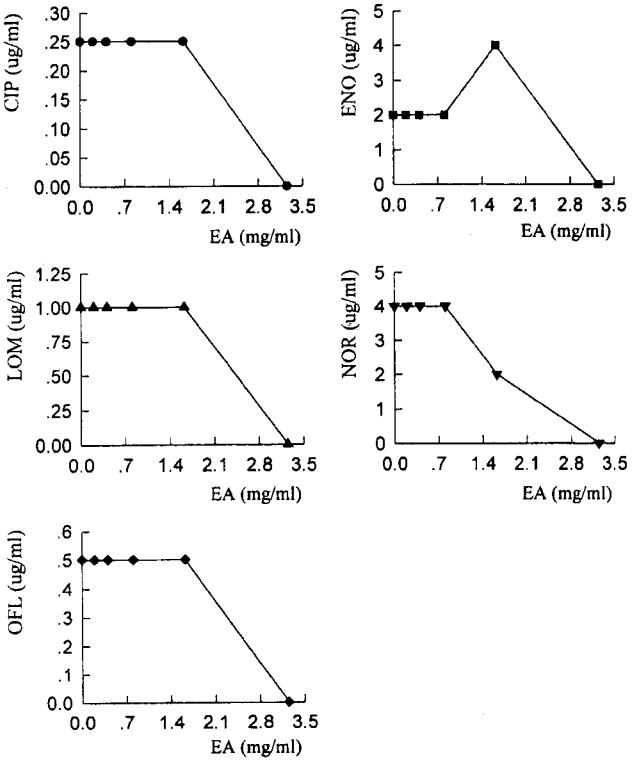

Fig. 1. Isobolograms of the FICs obtained with checkboard combinations of EA and quinolones against *Shigella sonnei*. FIC was defined as additive MIC of drug A and B/MIC of either A or B alone. EA : water soluble components of *Elfvingia applanata* carpophores, CIP : ciprofloxacin, ENO : enoxacin, LOM : lomefloxacin, Nor: norfloxacin, OFL : ofloxacin.

It was rather interesting that FICs of EA on *S. sonnei* were comparably lower than on other bacterial strains. All combinations seemed to show the indifferent effects, FICIs, 1.063 and 2.000.

Since combination of EA with either ciprofloxacin (CIP), lomefloxacin (LOM), norfloxacin (NOR) or ofloxacin (OFL) showed the lowest FICI (1.063) against *S. sonnei*, we choose the FIC values obtained by checkerboard combination of EA and five of quinolone antibiotics against *S. sonnei*, one of well known enteropathogens, to construct iso- bolograms (Fig. 1). Except a combination of EA with ENO, other combinations depicted a similar

Table 3. FICs and FICIs of EA with quinolones in antimicrobial combinations

Strain	FIC[a]		FICI[b]	FIC		FICI	FIC		FICI	FIC		FICI	FIC		FICI
	EA[c]	CIP[d]		EA	ENO[e]		EA	LOM[f]		EA	NOR[g]		EA	OFL[h]	
Bacillus subtilis ATCC 6051	0.063	1.000	1.063	1.000	1.000	2.000	1.000	1.000	2.000	1.000	1.000	2.000	1.000	1.000	2.000
Micrococcus luteus ATCC 4698	0.063	1.000	1.063	1.000	1.000	2.000	1.000	1.000	2.000	0.063	1.000	1.063	1.000	1.000	2.000
Pseudomonas aeruginosa ATCC 10145	1.000	1.000	2.000	1.000	1.000	2.000	0.063	1.000	1.063	1.000	1.000	2.000	1.000	1.000	2.000
Shigella sonnei ATCC 11060	0.063	1.000	1.063	1.000	1.000	2.000	0.063	1.000	1.063	0.063	1.000	1.063	0.063	1.000	1.063

[a]FIC : fractional inhibitory concentration, [b]FICI : fractional inhibitory concentration index, [c]EA : water soluble components *Elfvingia applanata* carpophores, [d]CIP : ciprofloxacin, [e]ENO : enoxacin, [f]LOM : lomefloxacin, [g]NOR : norfloxacin, [h]OFL : ofloxacin.
The data are expressed as mean values of four separate experiments.

hyperbolic curve against *S. sonnei* as one might expect.

In conclusion, the antimicrobial combinations of EA and five quinolone antibiotics exhibited either additive or indifferent effects, but neither synergism nor antagonism. There was a report on *in vivo* safety of EA (Kim *et al.*, 1994c), but further studies are warranted for EA to be used in combinational therapy with quinolones for aiming dosage reduction of the toxic antibiotics.

References

Anke, T., Kupka, J., Schamm, G. and Steglich, W. 1980. Antibiotics from Basidiomycetes X. Scorodonin, a new antibacterial and antifungal metabolite from *Marasmius scorodonius* (F_R)F_R. *J. Antibiotics* **33**: 463-467.

Anke, T., Casser, I. and Steglich, W. 1987. Antibiotics from Basidiomycetes. 26. Phlebiakauranol aldehyde, an antifungal and cytotoxic metabolite from *Punctularia atropurpurascens. J. Antibiotics* **40**: 443-449.

Chung, K. S., Shim, M. J. and Kim, B. K. 1978. Studies on the constituents of the higher fungi of Korea (XI). an antibiotic component and a sterol of *Coriolus sanguineus* Fr. *Arch. Pharm. Res.* **1**: 33-40.

Demain, A. L. and Solomon, N. A. 1983. *Antibiotics Containing the Beta-Lactam Structure* Part II. Spring-Verlag Berlin Heidelberg New York Tokyo. pp. 438.

Donelly, D., Abe, F., Conveney, D., Fukuda, N. and O'Reilly, J. 1985. Antibacterial sesquiterpene aryl esters from *Armillaria mellea. J. Natural Products* **48**: 10-16.

Eliopoulos, G. M. and Moellering, R. C. Jr. 1991. *Antimicrobial combinations.* In *Lorian V.* (ed.), *Antibiotics in Labolatory Medicine.* The Williams & Wilkins Co., Baltimore, pp. 432-492.

John, C. C. 1994. Treament failure with use of a third generation cephalosporin for penicillin-resistanct pneunococcal meningitis : case report and review. *Clin. Infect. Diseases* **18**: 188-193.

Kim, S. S. and Kim, Y. S. 1990. *Korean Mushrooms*, Yupoong Publishing Co, Seoul, pp. 300-301.

Kim, Y. S., Ryu, K. H., Mo, Y. K., Lee, C. K. and Han, S. S. 1994a. Effects of the antitumor component, F-D-P, isolated from *Elfvingia applanata* on the immune response. *Kor. J. Pharmacogn.* **25**: 348-355.

Kim, Y. S., Rym, K. H., Lee, C. K. and Han, S. S. 1994b. Antimicrobial activity of *Elfvingia applanata* extract alone and in combination with some antibiotics. *Yakhak Hoeji* **38**: 742-748.

Kim, Y. S., Kang, J. K., Lee, C. K. and Han, S. S. 1994c. Effect of *Elfvingia applanata* exract on the acute toxicity in mice. *Yakhak Hoeji.* **38**: 756-762.

Koehler, M., Bubala, H., Lacheta, L., Daton-Cholewa, J. and Janik-Moszant, A. 1989. Side effects of cefoperazone (3rd generation cephalosporin) in a child sith acute lymophoblastic leukemia. *Pediatria Polska.* **64**: 497-499.

Lee, K. S., Lee, M. W. and Lee, J. Y. 1982. Sutdies on the antibacterial activity of *Poria cocos. Korean J. Mycol.* **10**: 27-31.

Mizuno, T., Hayashi, K., Arakawa, M., Shinkai, K., Shimizu, M. and Tanaka, M. 1981. Host-mediated antitumor polysaccharides. III. Studies on fractionation, chemical structure, and antitumor activity of water-soluble homoglucans isolated from kofukisaruno koshikake, the fruit body of *Ganoderma applanatum. Shizuoka Daigaku Nogakubu Kenkyu Hokoku* 49-64.

National Committee for Clinical Laboratory Standards, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard-3rd edn M7-A3.* NCCLS, Villanova, PA., 1993.

Neu, H. C. 1990. Third generation cephalosporins. Safety profiles after 10 years of clinical use. *J. Clin. Phamrmacol.* **30**: 396.

Nishitoba, T., Goto, S., Sato, H. and Sakamura, S. 1989. Bitter triterpenoids from the fungus *Ganoderma applanatum. Phytochemistry* **28**: 193-197.

Norden, C. W. 1982. Problems in determination of antimicrobial synergism *in vitro. Review of Infectious Disease* **4**: 276-281.

Protiva, J., Skorkovska, H., Urban, J. and Vystrcil, A. 1980. Triterpenens and steroids from *Ganoderma applanatum. Coll. Czech. Chem. Commun.* **45**: 2710-2713.

Rym, K. H., Eo, S. K., Kim, Y. S., Lee, C. K. and Han, S. S. 1999. Antiviral activity of water soluble substance from *Elfvingia applanata. Kor. J. Pharmacogn.* **30**: 25-33.

Usui, T., Iwasaki, Y., Mizuno, T., Tanaka, M., Shinkai, K. and Arakawa, M. 1983. Isolation and characterization of antitumor active β-D-glucans from the fruit bodies of *Ganoderma applanatum. Carbohydr. Res.* **115**: 273-280.

Yoon, S. Y., Eo, S. K., Kim, Y. S., Lee, C. K. and Han, S. S. 1994. Antimicrobial activity of *Ganoderma lucidum* extract alone and in combination with some antibioitcs. *Arch. Pharm. Res.*, **17**: 438-442.