Arch. Pharm. Res. Vol. 17, No. 6, pp. 438-442, 1994

Antimicrobial Activity of *Ganoderma lucidum* Extract Alone and in Combination with Some Antibiotics

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(Received September 9, 1994)

Antimicrobial activity of GL (the aqueous extract from the carpophores of *Ganoderma lucidum* $(F_R)K_{ARST}$) was tested *in vitro* against Gram positive and Gram negative bacteria by serial broth dilution method, and the antimicrobial activity was expressed by minimal inhibitory concentration (MIC). Among fifteen species of bacteria tested, the antimicrobial activity of GL was the most potent against *Micrococcus luteus* (MIC, 0.75 mg/ml). To investigate the effects of antimicrobial combinations of GL with four kinds of antibiotics (ampicillin, cefazolin, oxytetracycline and chloramphenicol), the fractional inhibitory concentration index (FICI) was determined by checkerboard assay for each strain. The antimicrobial combinations of GL with four antibiotics resulted in additive effect in most instances, synergism in two instances, and antagonism in two instances. Synergism was obvserved when GL was combined with cefazolin against *Bacillus subtilis* and *Klebsiella oxytoca*.

Key words: *Ganoderma lucidum*, Carpophores, Antimicrobial activity, Minimal inhibitory concentration (MIC), Checkerboard assay, Fractional inhibitory concentration index (FICI), Antimicrobial combinations

INTRODUCTION

Ganoderma lucidum$(F_R)K_{ARST.}$, which belongs to Polyporaceae of a basidiomycetous fungus, has been used as one of traditional folk remedies in Korea as well as in other Asian countries (Kim *et al.*, 1986; Kim *et al.*, 1990). *G. lucidum* has been reported to have a variety of biologically active components such as nucleoside class inhibiting aggregation of platelets (Kubo *et al.*, 1986; Shimizu *et al.*, 1985), ganoderic acid A, B, C and D inhibiting histamine-release (Kohda *et al.*, 1985), ganoderal A, ganoderol A and B, ganoderic acid K and S inhibiting angiotensin converting enzyme and stabilizing blood pressure (Kanmatsuse *et al.*, 1985; Morigawa *et al.*, 1986), ganoderan A, B and C decreasing blood sugar (Hikino *et al.*, 1985; Hikino *et al.*, 1989), polysaccharides having antitumor activity (Miyazaki *et al.*, 1981; Sone *et al.*, 1985; Hyun *et al.*, 1990) and immunomodulatory activity (Maeda *et al.*, 1971; Shin *et al.*, 1985; Lee *et al.*, 1990).

Several investigators identified antimicrobial components from basidiomycetous fungi. Chung *et al.* (Chung *et al.*, 1978) isolated and identified cinnabarin from *Coriolus sanguineus* grown in Korea. Anke *et al.* (Anke *et al.*, 1980; Anke *et al.*, 1986) isolated phlebiakauranol aldehyde and scorodonin from *Punctularia atropurpurascens* and *Marasmius scorodonius*, respectively, and their antimicrobial activities were investigated against bacteria, yeast and fungi. 4-O-methylmelleolide and judeol, which showed strong antimicrobial activity against Gram positive bacteria, were also isolated from *Armillaria mellea* (Donnelly *et al.*, 1985). The antimicrobial components isolated from basidiomycetous fungi appeared to be different from the existing antibiotics in structure, showing a possibility of development to a new class of antibiotics that may be used in the treatment of antibiotic-resistant strains.

The antimicrobial activity of GL, the aqueous extract from the carpophores of *G. lucidum*, was first noted by Yoon (Yoon D. S., 1959) who reported on the antimicrobial activities of the saline-extracted components of eighty-one selected mushrooms. Chung *et al.* (Chung *et al.*, 1986) also reported on the antimicrobial activity of ten selected basidiomycetous fungi including *G. lucidum*. Recently, Kim *et al.* (1993) reported that GL had anti-HIV activity. To characterize the antimicrobial activity of *G. lucidum* further, the antimicrobial activity of GL was examined against fifteen species of bacteria, and then the effects of antimicrobial com-

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binations with four kinds of antibiotics such as ampicillin, cefazolin, oxytetracycline and chloramphenicol were investigated in the present study.

MATERIALS AND METHODS

Materials

The carpophores of *Ganoderma lucidum*(F_R)$K_{ARST.}$ was purchased from Cheongju-city market. Nutrient broth and agar were purchased from Difco Co. Test antibiotics, which are ampicillin sodium, cefazolin sodium, oxytetracycline HCl and chloramphenicol, were purchased from Chong Kun Dang Co.

Test Strains

Test strains subcultured in our laboratory were used. As Gram positive bacteria, *Bacillus anthracis* ATCC 6603, *Bacillus cereus* ATCC 27348, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341 and *Staphylococcus aureus* ATCC 25923 were used. As Gram negative bacterial species, *Escherichia coli* ATCC 259 22, *Klebsiella oxytoca* ATCC 8724, *Klebsiella pneumoniae* ATCC 10031, *Proteus vulgaris* ATCC 27853, *Salmonella tompson* ATCC 10256, *Salmonella typhi* ATCC 6229, *Salmonella typhimurium* ATCC 14028, and *Serratia marcescens* ATCC 27117 were used.

Preparation of GL

To prepare GL (the aqueous extract from the carpophores of *G. lucidum*), the carpophores were dried, disrupted, and then extracted with distilled water in a water bath at 90-100℃ for 8 hours. After filtration, extraction was twice repeated in the same condition, The filtrates were combined, and concentrated by a vacuum evaporator. The resulting semifluid material was lyophilized by a freeze dryer to yield a dark brownish powder.

Preparation of Stock Solution of GL and Antibiotics

GL was dissolved in nutrient broth in a concentration of 5 mg/ml. Ampicillin, cefazolin and oxytetracycline were dissolved in nutrient broth in a concentration of 1,000 μg/ml. Chloramphenicol was dissolved in DMSO and diluted with nutrient broth to a concentration of 1,000 ug/ml where DMSO did not inhibit the growth of bacteria.

Antimicrobial Activity

To prepare bacterial inocula, *Serratia marcescens* was incubated at 26℃, *Bacillus cereus* and *Micrococcus luteus* were incubated at 30℃, and other strains were incubated at 37℃ for 18 hours. The cultured bacteria were adjusted with nutrient broth until transmission was about 30% at 540 nm by using UV-spectronic 21, and diluted 1 to 100 again (Gary *et al.*, 1979).

The antimicrobial activity was determined by serial broth dilution method (Ericsson *et al.*, 1971). Initially, test materials were subjected to twofold step dilution with nutrient broth, and dispensed to nine test tubes, respectively. 50 μl of bacterial inoculum was added to each test tube, and incubated for 18 hours. The MICs of test materials were defined as the lowest concentration exhibiting no visual turbidity.

Antimicrobial Combinations

The antimicrobial combinations of GL with four kinds of antibiotics were tested by checkerboard assay (Victor Lorian, 1985). The species of selected bacteria were *B. anthracis, B. subtilis, S. aureus, E. coli, K. oxytoca, P. vulgaris, S. typhi*. The concentration tested for each antimicrobial agent typically ranged from 5 to 7 dilutions below the MIC to twice the MIC (or higher if antagonism was suspected), using 2-fold dilutions of each antimicrobial agent. 50 ul of bacterial inoculum was added to each test tube, and incubated for 18 hours. The effects of antimicrobial combinations were evaluated by isobologram, fractional inhibitory concentration (FIC) and fractional inhibitory concentration index (FICI) (Elion *et al.*, 1954; Hallander *et al.*, 1982; Jadavji *et al.*, 1984). Synergism was assumed when the MIC of each component in one or more combinations was one-fourth or less of its original MIC ($FICI \leq 0.5$). Antagonism was assumed when the MIC of either component was increased twofold or more over its original MIC or when the MICs of both components were increased one or more compared to their respective MICs ($FICI \geq 2.0$). All other results were considered additive or indifferent.

RESULTS AND DISCUSSION

Antimicrobial Activity

The antimicrobial activity of GL against Gram positive and negative bacteria were shown in Fig. 1 and Fig. 2, respectively. Among five species of Gram positive bacteria tested, the most prominent antimicrobial activity of GL was shown in *Micrococcus luteus* and its MIC was 0.75 mg/ml. The antimicrobial activity of GL was tested against ten species of Gram negative bacteria. GL showed relatively strong antimicrobial activity against *Proteus vulgaris* (MIC=1.25 mg/ml) and *Escherichia coli* (MIC=1.75 mg/ml), and six species of bacteria were shown to have MIC values larger than 5 mg/ml. As indicated in Fig. 1 and Fig. 2, the antimicrobial activity of GL was more potent against Gram positive bacteria than against Gram negative bacteria.

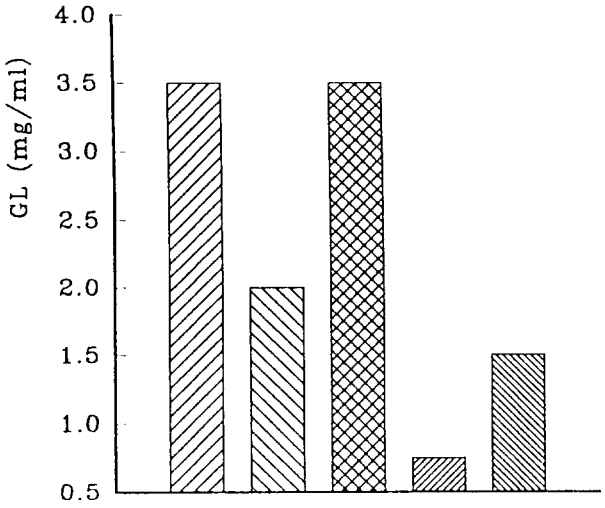

Fig. 1. MICs of GL against gram positive bacteria.
GL: extract of Ganoderma lucidum, ▨: *Bacillus anthracis* ATCC 6603, ▧: *Bacillus cereus* ATCC 27348, ▩: *Bacillus subtilis* ATCC 6633, ▨: *Micrococcus luteus* ATCC 9341, ▧: *Staphylococcus aureus* ATCC 25923.

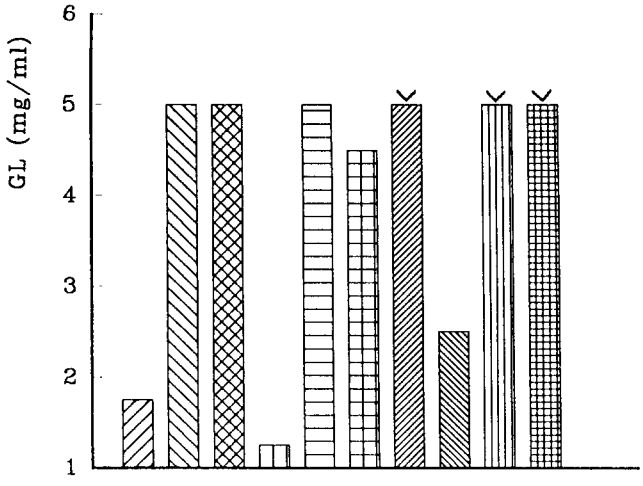

Fig. 2. MICs of GL against gram negative bacteria.
GL: extract of *Ganoderma lucidum*, ▨: *Escherichia coli* ATCC 25922, ▧: *Klebsiella oxytoca* ATCC 8724, ▩: *Klebsiella pneumoniae* ATCC 10031, ▯: *Proteus vulgaris* ATCC 6509, ▭: *Providencia rettgeri* ATCC 936, ⊞: *Pseudomonas aeruginosa* ATCC 27853, ▨: *Salmonella tompson* ATCC 10256, ▧: *Salmonella typhi* ATCC 6229, ▥: *Salmonella typhimurium* ATCC 14028, ▩: *Serratia marcescens* ATCC 27117.

The MICs of four kinds of antibiotics were shown in Table I. Four kinds of antibiotics generally inhibited the bacterial growth at lower concentrations against *Bacillus subtilis*. Robbins (Robbins *et al.*, 1947) and his coworkers, and Lee (Lee *et al.*, 1982) and his coworkers proposed that most antimicrobial components of a basidiomycetous fungus were potent against Gram positive bacteria only. However, our experiment showed that GL had good antimicrobial action on *Proteus vulgaris* and *Escherichia coli* belonging to Gram negative bacteria.

Table I. MICs of test antibiotics used for antimicrobial combinations

Strains	MIC (μg/ml)			
	ABPC	CEZ	OTC	CM
Bacillus anthracis ATCC 6603	1.00	0.25	0.06	4.00
Bacillus subtilis ATCC 6633	0.13	0.50	0.06	4.00
Staphylococcus aureus ATCC 25923	1.00	0.25	0.13	8.00
Escherichia coli ATCC 25922	256.00	32.00	0.50	4.00
Klebsiella oxytoca ATCC 8724	8.00	2.00	0.50	1.00
Proteus vulgaris ATCC 6509	0.50	0.25	2.00	4.00
Salmonella typhi ATCC 6229	4.00	1.00	0.50	4.00

ABPC: ampicillin, CEZ: cefazolin, OTC: oxytetracycline, CM: chloramphenicol

Table II. FICs and FICIs of GL with ampicillin

Strains	FIC		FICI
	GL	ABPC	
Bacillus anthracis ATCC 6603	0.50	1.00	1.50
Bacillus subtilis ATCC 6633	0.50	1.00	1.50
Staphylococcus aureus ATCC 25923	0.50	1.00	1.50
Escherichia coli ATCC 25922	0.50	1.00	1.50
Klebsiella oxytoca ATCC 8724	0.10	0.50	0.60
Proteus vulgaris ATCC 6509	1.00	1.00	2.00
Salmonella typhi ATCC 6229	0.50	1.00	1.50

GL: extract of *Ganoderma lucidum*, ABP: ampicillin

Table III. FICs and FICIs of GL with cefazolin

Strains	FIC		FICI
	GL	CEZ	
Bacillus anthracis ATCC 6603	0.25	0.48	0.73
Bacillus subtilis ATCC 6633	0.25	0.24	0.49
Staphylococcus aureus ATCC 25923	0.07	0.48	0.55
Escherichia coli ATCC 25922	0.50	0.50	1.00
Klebsiella oxytoca ATCC 8724	0.25	0.25	0.50
Proteus vulgaris ATCC 6509	1.00	1.00	2.00
Salmonella typhi ATCC 6229	0.50	0.50	1.00

GL: extract of *Ganoderma lucidum*, CEZ: cefazolin

Antimicrobial Combinations

The antimicrobial combinations of GL with four kinds of antibiotics were performed by checkerboard assay, and the results were summerized in Table II, III, IV and V. In combination with ampicillin, additive effect was shown in *Klebsiella oxytoca*, and indifferent effects were shown in five species of bacteria including *Escherichia coli*, but antagonism was shown in *Proteus vulgaris* only. The antimicrobial combinations of GL with cefazolin showed synergism in *Bacillus subtilis* and *Klebsiella oxytoca*, and additive effects in four

Table IV. FICs and FICIs of GL with oxytetracycline

Strains	FIC		FICI
	GL	OTC	
Bacillus anthracis ATCC 6603	0.06	0.50	0.56
Bacillus subtilis ATCC 6633	0.50	1.00	1.50
Staphylococcus aureus ATCC 25923	0.25	0.50	0.75
Escherichia coli ATCC 25922	0.50	0.50	1.00
Klebsiella oxytoca ATCC 8724	0.10	0.50	0.60
Proteus vulgaris ATCC 6509	0.50	0.06	0.56
Salmonella typhi ATCC 6229	0.50	1.00	1.50

GL: extract of *Ganoderma lucidum*, OTC: oxytetracycline

Table V. FICs and FICIs of GL with chloramphenicol

Strains	FIC		FICI
	GL	CM	
Bacillus anthracis ATCC 6603	0.50	0.50	1.00
Bacillus subtilis ATCC 6633	0.50	1.00	1.50
Staphylococcus aureus ATCC 25923	0.50	1.00	1.50
Escherichia coli ATCC 25922	0.50	0.50	1.00
Klebsiella oxytoca ATCC 8724	0.50	1.00	1.50
Proteus vulgaris ATCC 6509	0.50	0.50	1.00
Salmonella typhi ATCC 6229	0.50	1.00	1.50

GL: extract of *Ganoderma lucidum*, CM: chloramphenicol

species of bacteria including *Staphylococcus aureus*. Indifferent effects were not found, and antagonism was found in *Proteus vulgaris* only. Fig. 3 is the isobolograms of GL in combination with cefazolin against *Bacillus subtilis* and *Klebsiella oxytoca*. The antimicrobial combinations with oxytetracycline showed additive effects in five species of bacteria and indifferent effects in two species of bacteria. Chloramphenicol showed additive effects in three species of bacteria and indifferent effects in four species of bacteria including *Staphylococcus aureus*. As indicated in the results of checkerboard assay, the antimicrobial combinations of GL with cefazolin showed increased antimicrobial activity except against *Proteus vulgaris*. The antimicrobial combinations of GL with ampicillin showed less effect than with other antibiotics. On the other hand, it is interesting that the antimicrobial combinations of GL with ampicillin and cefazolin, which belongs to β-lactam antibiotics, equally showed antagonism in *Proteus vulgaris*.

The antimicrobial combinations of GL with four kinds of antibiotics showed synergism or additive effects in several pathogenic bacteria as described in the results of checkerboard assay. Moreover, GL appeared to have very low toxicity when administrated *in vivo* (Kim *et al.*, 1986). Therefore, GL may be able to be used as a restorative drug that can permit a significant reduction in the dosage of the toxic antimicrobial agents without compromising antimicrobial activity.

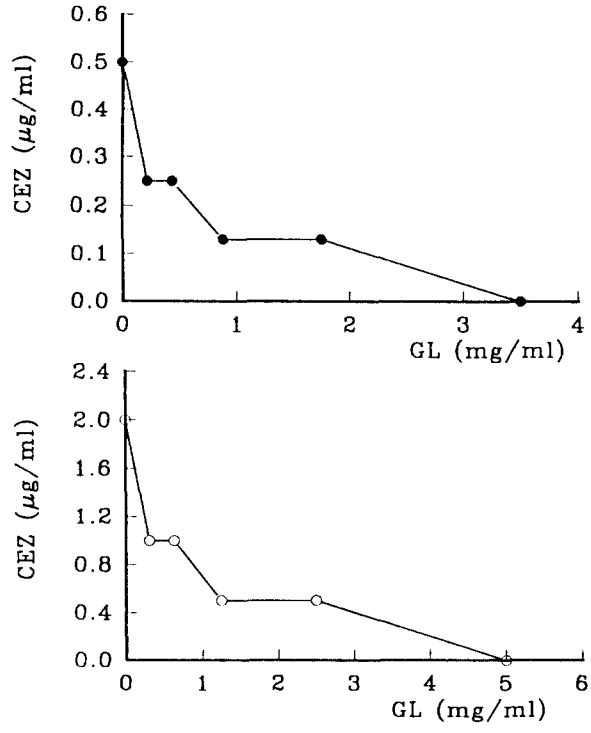

Fig. 3. Isobolograms of antimicrobial combinations of GL with cefazolin against *Bacillus subtilis* ATCC 6633 (upper) and *Klebsiella oxytoca* ATCC 8724 (lower).
GL: extract of *Ganoderma lucidum*, CEZ: cefazolin.

ACKNOWLEDGEMENT

This paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1993.

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