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# Antimicrobial Activity of Extract and Fractions from *Drynaria fortunei* Against Oral Bacteria

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One of the traditional Korean medicine, *Drynaria fortunei* (*D. fortunei*) is one of candidates known to be effective for the treatment of inflammation, hyperlipemia, arteriosclerosis, rheumatism, and gynecological diseases such as osteoporosis and bone resorption. The present study investigated the antimicrobial activity of methanol (MeOH) extract and *n*-butanol (*n*-BuOH), chloroform ($CHCl_3$), and ethyl acetate (EtOAc) fractions of *D. fortunei* against oral bacteria. The *n*-BuOH and $CHCl_3$ fractions (MICs, 0.0078 to 0.3125 mg/ml; MBCs, 0.019 to 0.625 mg/ml) were demonstrated as strong antibacterial activity than the MeOH extract and EtOAc fraction. The combination effects of *n*-BuOH fraction with ampicillin or gentamicin were synergistic against some oral bacteria. We suggest that *D. fortunei* could be employed as a natural antibacterial agent in oral care products.

**Key Words:** *Drynaria fortunei*, Antibacterial activity, Minimum inhibitory concentrations/minimum bactericidal concentrations

## INTRODUCTION

One of the traditional Korean medicine, *Drynaria fortunei* (*D. fortunei*; Gol-Se-Bo in Korean and Gu-Sui-Bu in Chinese) is one of candidates known to be effective for the treatment of inflammation, hyperlipemia, arteriosclerosis, rheumatism, and gynecological diseases such as osteoporosis and bone resorption in oriental medicine (5,17,20). *D. fortunei* is also commonly used to manage disorders of orthopedics and has been claimed to have therapeutic effects on bone healing (5,17). Liu et al. has shown that *D. fortunei* has an antioxidant effect on rat osteoblasts from hydrogen peroxide-induced death and may promote bone recovery under similar pathologic conditions (8). Liu reported that *D. fortunei* increases the attachment and growth of human gingival fibroblasts on *in vitro* (7). The water extract of *D. fortunei* has been reported to significantly protect against ototoxicity caused by treptomycin, streptomycin and kanamycin in human and the progression of bone loss induced by ovariectomy in rats (9,18). Moreover, it was also shown that *D. fortunei* extracts are shown to be potent inhibitors of the degradation of denaturated collagen by cathepsin K and of bone resorption in an *in vitro* model (6).

In the present study, the antimicrobial activities of *D. fortunei* extract and fractions against oral bacteria through several analyses were investigated.

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## MATERIAL AND METHODS

### 1. Plant material and preparation of crude plant extracts

The *D. fortunei* was purchased from the herbal medicine cooperative association of Jeonbuk Province, Korea, in November 2004. The identifier was confirmed by Dr. Young-Sung Ju at the College of Oriental Medicine, Woosuk University. A voucher specimen (no. JS12A) has been

deposited at the Division of Medicinal Chemistry and Cosmetics, Mokwon University. The dried and powered roots (1.2 kg) of *D. fortunei* were extracted by repeated refluxing with methanol (MeOH) (2×6 L) for 4 h at 80℃. The combined MeOH extract (12 L) was clarified by filtration and evaporated to obtain dark brown syrup (210 g). The MeOH extract was suspended in water ($H_2O$) and partitioned with chloroform ($CHCl_3$), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH) successively. The organic solvent extracts were dried *in vacuum* at 45℃ to yield the $CHCl_3$ soluble fraction (0.23 g), EtOAc soluble fraction (8.94 g) and *n*-BuOH soluble fraction (22.47 g).

**2. Microbial strains**

The antimicrobial activity of the MeOH extract and $CHCl_3$, EtOAc, and BuOH fractions of *D. fortunei* against oral bacteria: *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC 10556), *Streptococcus sobrinus* (ATCC 27607), *Streptococcus ratti* (KCTC 3294), *Streptococcus criceti* (KCTC 3292), *Streptococcus anginosus* (ATCC 31412) and *Streptococcus gordonii* (ATCC 10558), *Actinobacillus actinomycetemcomitans* (ATCC 43717), *Fusobacterium nucleatum* (ATCC 51190), *Prevotella intermedia* (ATCC 49046), and *Porphyromonas gingivalis* (ATCC 33277) was determined by the broth dilution method. Brain heart infusion (BHI; Difco Laboratories, Detroit, MI, USA) was used for all bacteria. For *A. actinomycetemcomitans*, *F. nucleatum*, and *P. intermedia*, BHI broth supplemented with 1% yeast extract (Difco) was used. For *P. gingivalis*, BHI broth containing 1 μg/ml hemin (Sigma, St. Louis, MO, USA) and 1 μg/ml menadione (Sigma) was used.

**3. Minimum inhibitory concentrations/minimum bactericidal concentrations assay**

The minimum inhibitory concentrations (MICs) were determined for the MeOH extract and $CHCl_3$, EtOAc, and BuOH fractions of *D. fortunei* by the broth dilution method, and were carried out in triplicate. The antibacterial activities were examined after incubation at 37℃ for 18~24 h and *P. gingivalis* for 2~3 days under anaerobic conditions. MICs were determined as the lowest concentration of test samples that resulted in a complete inhibition of visible growth in the broth. Following anaerobic incubation of MICs plates, the minimum bactericidal concentrations (MBCs) were determined on the basis of the lowest concentration of the extract and several fractions of *D. fortunei* that kills 99.9% of the test bacteria by plating out onto each appropriate agar plate. Ampicillin and gentamicin were used as standard antibiotics in order to compare the sensitivity with

**Table 1.** Minimum inhibitory concentrations and minimum bactericidal concentrations (mg/ml) of the extract and several fractions of *Drynaria fortunei* for some oral bacteria

| Strains | *Drynaria fortunei* extract and fractions MICs/MBCs (mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | MeOH | *n*-BuOH | $CHCl_3$ | EtOAc | Ampicillin | Gentamicin |
| *S. mutans* ATCC 25175 | 5/5 | 0.3125/0.625 | 0.078/0.156 | 5/10 | $4/4\times10^{-3}$ | $8/8\times10^{-3}$ |
| *S. sanguinis* ATCC 10556 | 2.5/5 | 0.3125/0.625 | 0.078/0.156 | 5/10 | $32/32\times10^{-3}$ | $8/16\times10^{-3}$ |
| *S. sobrinus* ATCC 27607 | 2.5/2.5 | 0.019/0.039 | 0.078/0.156 | 2.5/2.5 | $2/2\times10^{-3}$ | $4/8\times10^{-3}$ |
| *S. ratti* KCTC 3294 | 5/5 | 0.0078/0.019 | 0.156/0.3125 | 5/10 | $4/4\times10^{-3}$ | $4/8\times10^{-3}$ |
| *S. criceti* [1]KCTC 3292 | 2.5/2.5 | 5/10 | 0.156/0.3125 | 2.5/2.5 | $4/4\times10^{-3}$ | $8/8\times10^{-3}$ |
| *S .anginosus* ATCC 31412 | 2.5/2.5 | 0.0078/0.019 | 0.019/0.039 | 2.5/5 | $4/4\times10^{-3}$ | $16/16\times10^{-3}$ |
| *S. gordonii* ATCC 10558 | 1.25/2.5 | 0.156/0.3125 | 0.039/0.156 | 0.3125/0.3125 | $1/2\times10^{-3}$ | $2/4\times10^{-3}$ |
| *A. actinomycetemcomitans* ATCC 43717 | 5/5 | 1.25/2.5 | 2.5/5 | 5/10 | $64/64\times10^{-3}$ | $2/2\times10^{-3}$ |
| *F. nucleatum* ATCC 51190 | 2.5/5 | 1.25/2.5 | 1.25/1.25 | 2.5/2.5 | $0.25/0.25\times10^{-3}$ | $16/32\times10^{-3}$ |
| *P. intermedia* ATCC 49046 | 0.019/0.039 | 0.019/0.019 | 0.019/0.039 | 0.039/0.039 | $32/32\times10^{-3}$ | $0.5/1\times10^{-3}$ |
| *P. gingivalis* ATCC 33277 | 0.625/0.625 | 0.019/0.039 | 0.019/0.019 | 1.25/1.25 | $0.5/1\times10^{-3}$ | $256/512\times10^{-3}$ |

[1] Korean Collection for Type Cultures

the extract and several fractions of *D. fortunei* against test bacteria.

### 4. Checker board dilution test

The antibacterial effects of a combination of the MeOH extract or *n*-BuOH fraction, which exhibited the highest antimicrobial activity, and antibiotics were assessed by the checkerboard test as previously described (2,4). The antimicrobial combinations assayed included the MeOH extract or *n*-BuOH fraction plus ampicillin or gentamicin.

Fractional inhibitroy concnetration (FIC) was calculated by MICs of combination X + Y and FIC index was calculated by (MIC of combination X + Y) / (MIC of drug X alone) + (MIC of combination X + Y) / (MIC of drug Y alone). The interaction was defined as synergistic if the fractional inhibitory concentration index (FICI) was less than or equal to 0.5, additive if the FICI was greater than 0.5 and less than or equal 1.0, indifferent if the FICI was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FICI was greater than 2.0 (2).

### 5. Time-kill curves

Bactericidal activities of the drugs under study were also evaluated using time-kill curves on oral bacteria. Tubes

**Table 2.** The MeOH extract and *n*-BuOH fraction of *Drynaria fortunei* with or without ampicillin against some oral bacteria

| Strains | MIC[1] | MIC (mg/ml) | | FIC[2] (mg/ml) | | FICI[3] | | Outcome | |
|---|---|---|---|---|---|---|---|---|---|
| | | MeOH | *n*-BuOH | MeOH | *n*-BuOH | MeOH | *n*-BuOH | MeOH | *n*-BuOH |
| *S. mutans* ATCC 25175 | Alone | 1.25 | 0.039 | 0.25 | 0.125 | 0.5 | 0.375 | Synergistic | Synergistic |
| | Ampicillin | $1\times10^{-3}$ | $0.5\times10^{-3}$ | 0.25 | 0.25 | | | | |
| *S. sanguinis* ATCC 10556 | Alone | 0.625 | 0.039 | 0.25 | 0.25 | 0.375 | 0.5 | Synergistic | Synergistic |
| | Ampicillin | $4\times10^{-3}$ | $2\times10^{-3}$ | 0.125 | 0.25 | | | | |
| *S. sobrinus* ATCC 27607 | Alone | 1.25 | 0.0045 | 0.5 | 0.25 | 0.623 | 0.31 | Additive | Synergistic |
| | Ampicillin | $0.25\times10^{-3}$ | $0.125\times10^{-3}$ | 0.123 | 0.063 | | | | |
| *S. ratti* KCTC 3294 | Alone | 1.25 | 0.019 | 0.25 | 0.5 | 0.75 | 1 | Additive | Additive |
| | Ampicillin | $2\times10^{-3}$ | $1\times10^{-3}$ | 0.5 | 0.5 | | | | |
| *S. criceti* KCTC 3292 | Alone | 0.625 | 1.25 | 0.25 | 0.25 | 0.75 | 0.75 | Additive | Additive |
| | Ampicillin | $2\times10^{-3}$ | $1\times10^{-3}$ | 0.5 | 0.5 | | | | |
| *S. anginosus* ATCC 31412 | Alone | 0.3125 | 0.019 | 0.25 | 0.25 | 0.75 | 0.75 | Additive | Additive |
| | Ampicillin | $2\times10^{-3}$ | $2\times10^{-3}$ | 0.5 | 0.5 | | | | |
| *S. gordonii* ATCC 10558 | Alone | 0.3125 | 0.019 | 0.25 | 0.125 | 0.5 | 0.375 | Synergistic | Synergistic |
| | Ampicillin | $0.25\times10^{-3}$ | $0.125\times10^{-3}$ | 0.25 | 0.25 | | | | |
| *A. actinomycetemcomitans* ATCC 43717 | Alone | 1.25 | 0.156 | 0.25 | 0.063 | 0.375 | 0.31 | Synergistic | Synergistic |
| | Ampicillin | $8\times10^{-3}$ | $4\times10^{-3}$ | 0.125 | 0.25 | | | | |
| *F. nucleatum* ATCC 51190 | Alone | 0.3125 | 0.078 | 0.125 | 0.063 | 0.625 | 0.5 | Additive | Synergistic |
| | Ampicillin | $0.125\times10^{-3}$ | $0.063\times10^{-3}$ | 0.5 | 0.25 | | | | |
| *P. intermedia* ATCC 49049 | Alone | 0.0045 | 0.0045 | 0.25 | 0.25 | 0.5 | 0.5 | Synergistic | Synergistic |
| | Ampicillin | $8\times10^{-3}$ | $4\times10^{-3}$ | 0.25 | 0.25 | | | | |
| *P. gingivalis* ATCC 33277 | Alone | 0.039 | 0.0025 | 0.063 | 0.25 | 0.313 | 0.31 | Synergistic | Synergistic |
| | Ampicillin | $0.125\times10^{-3}$ | $0.063\times10^{-3}$ | 0.25 | 0.063 | | | | |

[1] The MICs of MeOH extract and *n*-BuOH fraction of *Drynaria fortunei* with or without ampicillin against oral bacteria are indicated.
[2] Fractional inhibitory concentration (FIC) was calculated by the combined MICs of the MeOH extract and *n*-BuOH fraction with or without ampicillin.
[3] Fractional inhibitory concentration index (FICI) = (MIC of combination X + Y) / (MIC of drug X alone) + (MIC of combination X + Y) / (MIC of drug Y alone).

containing Mueller-Hinton (Difco) supplemented to which antibiotics had been added at concentrations of the MICs were inoculated with a suspension of the test strain, giving a final bacterial count between $0.5\sim1\times10^6$ CFU/ml. Thereafter the tubes were incubated at 37℃ in anaerobic chamber and viable counts were performed at 0, 0.5, 1, 2, 3, 4, 5, 6, 12 and 24 h after addition of antimicrobial agents, on agar plates incubated for up to 48 h in anaerobic chamber at 37℃. Antibiotic carryover was minimized by washings by centrifugation and serial 10-fold dilution in sterile phosphate-buffered saline, pH 7.3. Colony counts were performed in duplicate, and means were taken. The solid media used for colony counts were BHI agar for streptococci, *A. actinomycetemcomitans*, and *F. nucleatum* and BHI agar containing hemin and menadione for *P. intermedia* and *P. gingivalis*.

## RESULTS

### 1. Antimicrobial activity of *D. fortunei* extract and fractions

The results of the antimicrobial activity (Table 1) showed that the MeOH extract and $CHCl_3$, EtOAc, and *n*-BuOH fractions exhibited antimicrobial activities against all oral bacteria tested (MICs, 0.0078 to 5 mg/ml; MBCs, 0.019 to

**Table 3.** The MeOH extract and *n*-BuOH fraction of *Drynaria fortunei* with or without gentamicin against some oral bacteria

| Strains | MIC[1] | MIC (mg/ml) | | FIC[2] (mg/ml) | | FICI[3] | | Outcome | |
|---|---|---|---|---|---|---|---|---|---|
| | | MeOH | *n*-BuOH | MeOH | *n*-BuOH | MeOH | *n*-BuOH | MeOH | *n*-BuOH |
| *S. mutans* ATCC 25175 | Alone | 1.25 | 0.019 | 0.25 | 0.125 | 0.75 | 0.5 | Additive | Synergistic |
| | Ampicillin | $4\times10^{-3}$ | $2\times10^{-3}$ | 0.5 | 0.25 | | | | |
| *S. sanguinis* ATCC 10556 | Alone | 0.625 | 0.039 | 0.25 | 0.25 | 0.31 | 0.31 | Synergistic | Synergistic |
| | Ampicillin | $4\times10^{-3}$ | $2\times10^{-3}$ | 0.5 | 0.25 | | | | |
| *S. sobrinus* ATCC 27607 | Alone | 0.3125 | 0.0025 | 0.5 | 0.25 | 1 | 1 | Additive | Additive |
| | Ampicillin | $0.5\times10^{-3}$ | $0.5\times10^{-3}$ | 0.123 | 0.063 | | | | |
| *S. ratti* KCTC 3294 | Alone | 0.625 | 0.009 | 0.25 | 0.5 | 0.75 | 0.75 | Additive | Additive |
| | Ampicillin | $1\times10^{-3}$ | $1\times10^{-3}$ | 0.5 | 0.5 | | | | |
| *S. criceti* KCTC 3292 | Alone | 0.625 | 0.625 | 0.25 | 0.25 | 0.75 | 0.75 | Additive | Additive |
| | Ampicillin | $0.5\times10^{-3}$ | $0.25\times10^{-3}$ | 0.5 | 0.5 | | | | |
| *S. anginosus* ATCC 31412 | Alone | 0.3125 | 0.019 | 0.25 | 0.25 | 0.375 | 0.375 | Synergistic | Synergistic |
| | Ampicillin | $1\times10^{-3}$ | $0.5\times10^{-3}$ | 0.5 | 0.5 | | | | |
| *S. gordonii* ATCC 10558 | Alone | 0.156 | 0.019 | 0.25 | 0.125 | 0.31 | 0.31 | Synergistic | Synergistic |
| | Ampicillin | $1\times10^{-3}$ | $1\times10^{-3}$ | 0.25 | 0.25 | | | | |
| *A. actinomycetemcomitans* ATCC 43717 | Alone | 0.625 | 0.078 | 0.25 | 0.063 | 0.5 | 0.5 | Synergistic | Synergistic |
| | Ampicillin | $0.5\times10^{-3}$ | $0.5\times10^{-3}$ | 0.125 | 0.25 | | | | |
| *F. nucleatum* ATCC 51190 | Alone | 0.625 | 0.039 | 0.125 | 0.063 | 0.5 | 0.5 | Synergistic | Synergistic |
| | Ampicillin | $4\times10^{-3}$ | $2\times10^{-3}$ | 0.5 | 0.25 | | | | |
| *P. intermedia* ATCC 49049 | Alone | 0.009 | 0.0025 | 0.25 | 0.25 | 0.31 | 0.31 | Synergistic | Synergistic |
| | Ampicillin | $0.25\times10^{-3}$ | $0.125\times10^{-3}$ | 0.25 | 0.25 | | | | |
| *P. gingivalis* ATCC 33277 | Alone | 0.0045 | 0.0025 | 0.063 | 0.25 | 0.75 | 0.5 | Additive | Synergistic |
| | Ampicillin | $32\times10^{-3}$ | $8\times10^{-3}$ | 0.25 | 0.063 | | | | |

[1] The MICs of MeOH extract and *n*-BuOH fraction of *Drynaria fortunei* with or without gentamicin against oral bacteria are indicated.
[2] Fractional inhibitory concentration (FIC) was calculated by the combined MICs of the MeOH extract and *n*-BuOH fraction with or without gentamicin.
[3] Fractional inhibitory concentration index (FICI) = (MIC of combination X + Y) / (MIC of drug X alone) + (MIC of combination X + Y) / (MIC of drug Y alone).

20 mg/ml). The $CHCl_3$ and *n*-BuOH fractions showed the strongest antimicrobial activity against all the oral bacteria (MICs, 0.0078 to 0.3125 mg/ml; MBCs, 0.019 to 0.625 mg/ml), except *S. criceti*, *A. actinomycetemcomitans*, and *F. nucleatum* (MICs/MBCs values; 1.25 to 5/1.25 to 10 mg/ml). The MICs/MBCs for ampicillin were found to be either 0.25 to 64/0.25 to 64 μg/ml; for gentamicin, either 0.5 to 256/1 to 512 μg/ml.

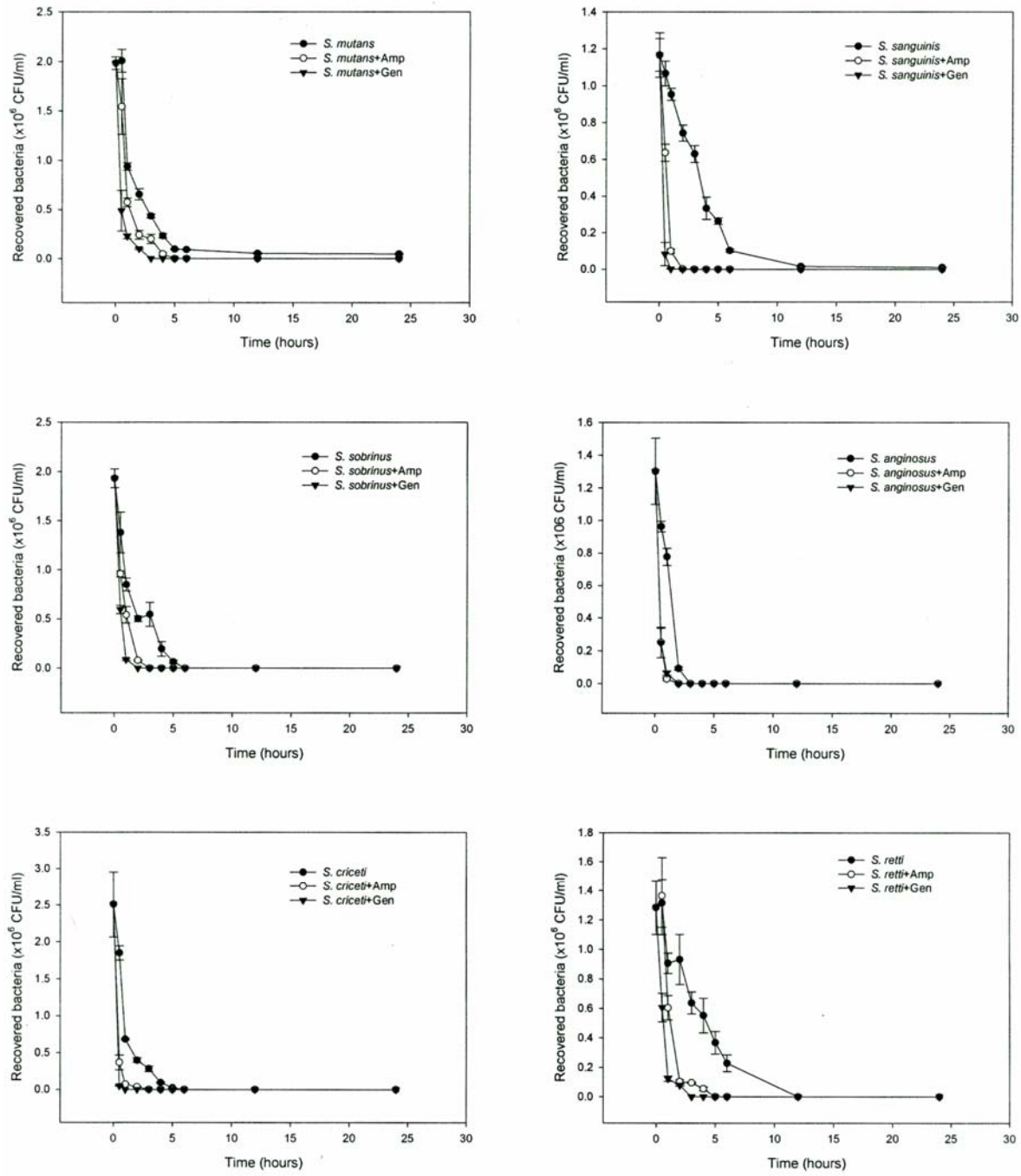


**Figure 1.** Time-kill curves of MICs of *n*-BuOH fraction alone and its combination with MICs of ampicillin or gentamicin against. *S. mutans*, *S. sanguinis*, *S. sobrinus*, *S. anginosus*, *S. criceti*, and *S. ratti*. Bacteria were incubated with *n*-BuOH fraction along (●), n-BuOH fraction with ampicillin (○), and n-BuOH fraction with gentamicin (▼) over time. Data points are the mean values ± S.E.M. of four experiments. CFU, colony-forming units.

### 2. Synergistic effects of *D. fortunei* with antibiotics

The antibacterial activities of MeOH extract or *n*-BuOH fraction combination with ampicillin or gentamicin against oral bacteria were presented in Tables 2 and 3, respectively. In combination with MeOH extract, the MICs for ampicillin were reduced ≥ 4-8-fold in *S. mutans*, *S. sanguinis*, *S. gordonii*, *A. actinomycetemcomitans*, *P. intermedia*, and *P.*

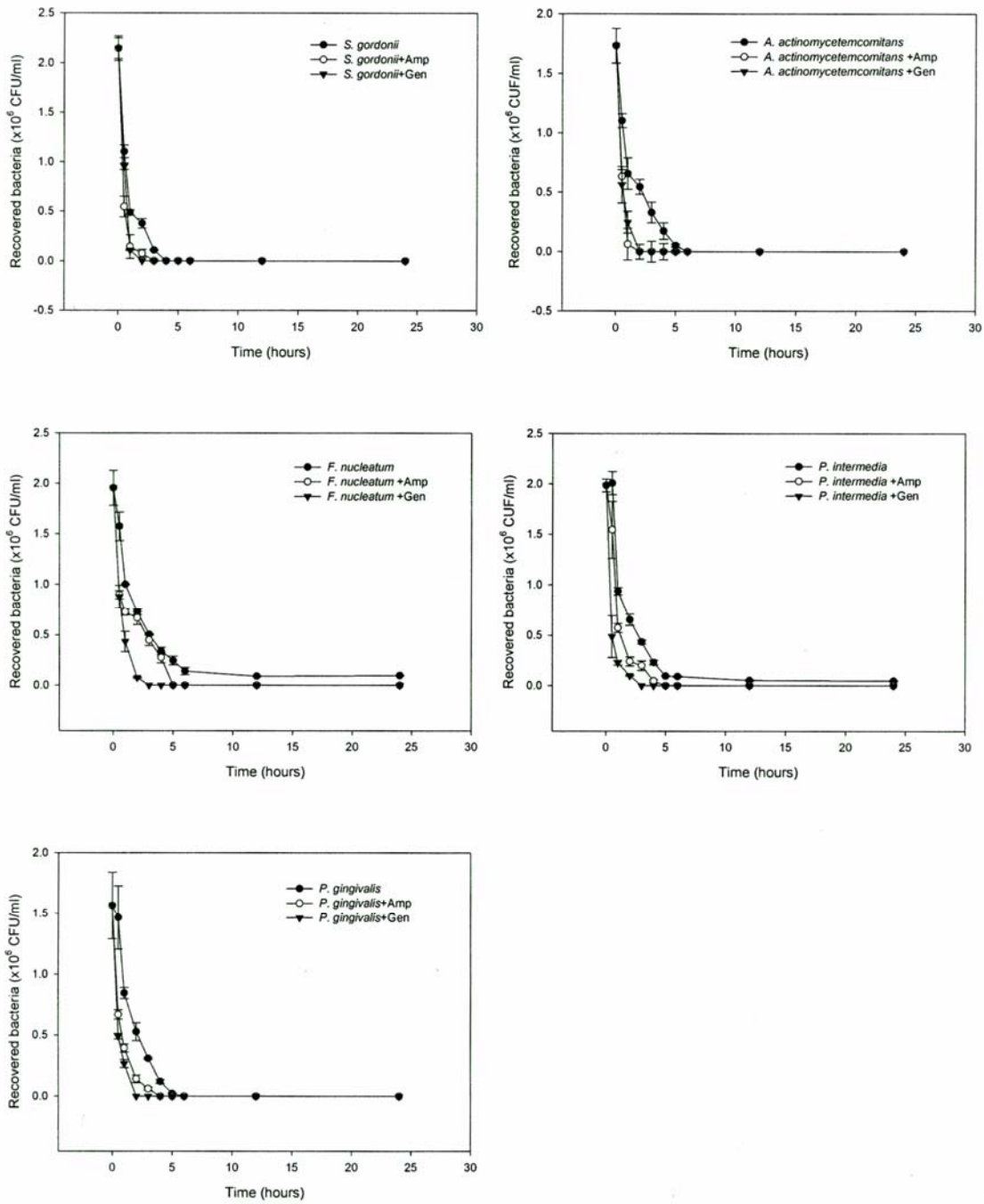


**Figure 2.** Time-kill curves of MICs of *n*-BuOH fraction alone and its combination with MICs of ampicillin or gentamicin against *S. gordonii*, *A. actinomycetemcomitans*, *F. nucleatum*, *P. intermedia*, and *P. gingivalis*. Bacteria were incubated with *n*-BuOH fraction along (●), *n*-BuOH fraction with ampicillin (○), and *n*-BuOH fraction with gentamicin (▼) over time. Data points are the mean values ± S.E.M. of four experiments. CFU, colony-forming units.

*gingivalis*, producing a synergistic effect as defined by FICI ≤ 0.375~0.5. The additive effect of MeOH extract led to a reduction of a single or double dilution in *S. sobrinus*, *S. ratti*, *S. criceti*, *S. anginosus*, and *F. nucleatum*, and as defined by FICI ≤ 0.625~0.75. In combination with *n*-BuOH fraction, the MICs for ampicillin were reduced ≥ 4-16-fold in all bacteria, except *S. ratti*, *S. criceti*, and *S. anginosus*, producing a synergistic effect as defined by FICI ≤ 0.31~0.5. The combination of gentamicin and MeOH extract resulted in the decrease in MICs for all bacteria, with the MICs of 0.0045~1.25 μg/ml for gentamicin becoming 0.25~32 μg/ml. The FICI classified the combination of MeOH extract or *n*-BuOH franction and gentamicin as synergistic for all bacteria, except additive in *S. ratti*, *S. criceti*, and *S. anginosus* (Table 3).

**3. Time-kill effects of oral bacteria**

The bactericidal effects of *n*-BuOH fraction with ampicillin or gentamicin against oral bacteria were confirmed by time-kill curve experiments (Fig. 1, 2). The cultures of all bacteria, with a cell density of $10^6$ CFU/ml, were exposed to MICs of *n*-BuOH fraction alone and with ampicillin or gentamicin. We observed that *n*-BuOH fraction alone resulted in increased rate of killing by time dependent manner, with a more rapid rate of killing by *n*-BuOH fraction with ampicillin or gentamicin more than *n*-BuOH fraction alone. A strong bactericidal effect was exerted in drug combinations.

## DISCUSSION

The results were observed that the $CHCl_3$ and *n*-BuOH fractions of *D. fortunei* were showed the strongest antimicrobial activity against all the oral bacteria (MICs, 0.0078 to 0.3125 mg/ml; MBCs, 0.019 to 0.625 mg/ml), except *S. criceti*, *A. actinomycetemcomitans*, and *F. nucleatum* (MICs/MBCs values; 1.25 to 5/1.25 to 10 mg/ml). In preliminary investigation, the $CHCl_3$ fraction was shown the strong antimicrobial activity more than *n*-BuOH fraction, but indicated the high cytotoxicity effect in human cells. Moreover, in combination with *n*-BuOH fraction, the MICs for ampicillin or gentamicin were reduced ≥ 4-16-fold in all bacteria, except *S. ratti*, *S. criceti*, and *S. anginosus*, producing a synergistic effect as defined by FICI ≤ 0.31~0.5.

The use of antimicrobial agents such as ampicillin, chlorhexidine, erythromycin, penicillin, tetracycline, and vancomycin to suppress cariogenic bacteria, and thereby to inhibit the development of caries, seems to be a rational approach and the World Health Organization (WHO) has recommended and encouraged the use of chewing sticks as an effective tool for oral hygiene (15,19). Recently, many researchers reported that many plant extracts or derivatives have been incorporated into commercial toothpastes and mouthwashes to treat oral diseases related to caries or periodontal diseases (11,16). Some of the polyphenols isolated from plants exhibit anticaries activity either due to growth inhibition against mutans streptococci or due to the inhibition of glucosyltransferases (3,13). *D. fortunei* contains several flavonoids of which biological activities are reported as antinephrotoxicity, kidney primary epithelial tubular cell regeneration, and inhibition of gentamicin ototoxicity and bone resorption (6,9,10).

Previously, researchers reported that several essential oils from plants had antibacterial activity against oral bacteria (1). The oolong tea polyphenols possess a strong antiglucosyltransferase activity and inhibit experimental dental caries in species pathogen-free rats infected with mutans streptococci (12,13). Cacao beans, which form the main constituent of chocolate, contain some polyphenols which exhibit antiglucosyl transferase activity (14). In this study, we observed that *n*-BuOH fraction with ampicillin or gentamicin resulted a more rapid rate of killing than *n*-BuOH fraction alone. The *D. fortunei* is composed of many flavonoids then, thus it may perhaps indicate strong antibacterial activity against oral bacteria.

These findings suggest that the *D. fortunei* fulfills the conditions required for a novel agent against cariogenic bacteria and periodontal pathogens and could be employed as a natural antibacterial agent in oral care products.

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