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Antibacterial Activity of Sophoraflavanone G Isolated from the Roots of *Sophora flavescens*

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Abstract This study investigated the antibacterial activities of sophoraflavanone G from *Sophora flavescens* in combination with two antimicrobial agents against oral bacteria. The combined effect of sophoraflavanone G and the antimicrobial agents was evaluated using the checkerboard method to obtain a fractional inhibitory concentration (FIC) index. The sophoraflavanone G+ampicillin (AM) combination was found to have a synergistic effect against *S. mutans*, *S. sanguinis*, *S. sobrinus*, *S. gordonii*, *A. actinomycetemcomitans*, *F. nucleatum*, *P. intermedia*, and *P. gingivalis*, whereas the sophoraflavanone G+gentamicin (GM) combination had a synergistic effect against *S. sanguinis*, *S. criceti*, *S. anginosus*, *A. actinomycetemcomitans*, *F. nucleatum*, *P. intermedia*, and *P. gingivalis*. Neither combination exhibited any antagonistic interactions (FIC index>4). In particular, the MICs/MBCs for all the bacteria were reduced to one-half ~ one-sixteenth as a result of the drug combinations. A synergistic interaction was also confirmed by time-kill studies for nine bacteria where the checkerboard suggested synergy. Thus, a strong bactericidal effect was exerted through the drug combinations, plus *in vitro* data suggested that sophoraflavanone G combined with other antibiotics may be microbiologically beneficial rather than antagonistic.

Keywords: *Sophora flavescens*, sophoraflavanone G, antibacterial activity, checkerboard method, time-kill method, synergic effect

Dental plaque is a film of microorganisms on the tooth surface that plays an important part in the development of caries and periodontal diseases [9, 26, 31]. The further accumulation of plaque around the gingival margin and subgingival region can lead to a shift in its microbial composition from streptococcus-dominated to a larger number of *Actinomyces* spp. and increased number of capnophilic and obligatory anaerobic bacteria, such as *Porphyromonas gingivalis* [23, 24, 31, 37]. Several studies have already reported on the antibacterial effects of teeth-cleaning chewing sticks on cariogenic bacteria and periodontal pathogens, particularly bacteroides species, and their inhibitory action on dental plaque formation [1, 32, 38]. Frequently used antibacterial chemicals include povidone iodine products, chlorhexidine, and cetylpyridinium chloride, plus natural antibacterial substances have also attracted attention [2, 3, 13, 21, 25, 28, 29].

Sophorae Radix, the dried roots of *Sophora flavescens* AITON (Leguminosae), is an Oriental traditional medicine that has well-known antibacterial, antiviral, antiprotozoal, antiinflammatory, and antipyretic effects and is used as an insecticide and for the treatment of skin and mucosal ulcers, sores, diarrhea, gastrointestinal hemorrhages, arrhythmia, and eczema [6, 11, 16, 17, 19, 35, 39–41]. In addition to flavonoids with the regular prenyl side chains, *S. flavescens* also produces diverse flavanones with lavandulyl chains, irregular monoterpenoid groups, such as kurarinone and sophoraflavanone G [10, 12, 27, 30]. Recent pharmaceutical studies have shown that the lavandulyl side chain is essential for the antitumor activity and phospholipase-Cγ1-inhibition activity of the flavonoids isolated from this plant [7, 22]. Sophoraflavanone G, one of the main lavandulylated flavanones isolated from the dried roots of *S. flavescens*, is known to possess antimalarial, antimicrobial, antiviral, and antioxidant activities, and to inhibit the production of nitric oxide and prostaglandin E_2 in lipopolysaccharide-treated RAW cells [4, 8, 15, 17, 34].

The roots of *S. flavescens* used in the present study were originally collected in October 2001 from Jinan, Jeonbuk Province, Korea. The authenticity of the plant was confirmed by Y. S. Ju, College of Oriental Medicine, Woosuk University, and

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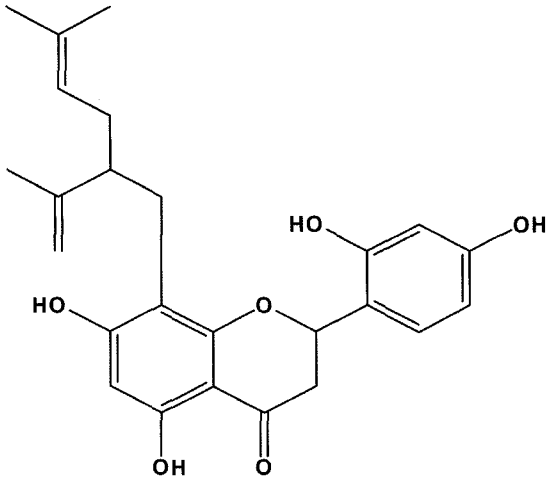

Fig. 1. Structure of 5,7,2',4'-tetrahydroxy-8-lavandulylflavanone (sophoraflavanone G) isolated from *Sophora flavescens*.

a voucher specimen (JS01-3) was deposited in the Herbarium of the Department of Bio and Medicinal Chemistry, College of Natural Science, Mokwon University. Sophoraflavanone G was isolated from the roots of *S. flavescens* based on the method described previously [27]. The sophoraflavanone G isolated from the dried roots of *S. flavescens* was identified based on a comparison with the spectral data in reported literature [12, 27] (Fig. 1). Sophoraflavanone G: $C_{25}H_{28}O_6$ (M_R: 424); pale yellow needle; $[\alpha]_D^{25}$ -49° (*c* 1.0 in MeOH); UV (MeOH) λ_{max}=340, 293 nm.

The oral bacterial strains used in this study were *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC 10556), *Streptococcus sobrinus* (ATCC 27607), *Streptococcus ratti* (KCTC: Korean collection for type cultures 3294), *Streptococcus criceti* (KCTC 3292), *Streptococcus anginosus* (ATCC 31412), *Streptococcus gordonii* (ATCC 10558), *Actinobacillus actinomycetemcomitans* (ATCC 43717), *Fusobacterium nucleatum* (ATCC 10953), *Prevotella intermedia* (ATCC 25611), and *Porphylomonas gingivalis* (ATCC 33277). A Brain-Heart Infusion broth supplemented with 1% yeast extract (Difco Laboratories, Detroit, MI, U.S.A.) was used for all the bacterial strains, except *P. intermedia and P. gingivalis.* The minimum inhibitory concentrations (MICs) were determined as the lowest test sample concentration that resulted in a complete inhibition of visible growth in the broth [14, 20, 33], whereas the minimum bactericidal concentrations (MBCs) were determined as the lowest concentration of sophoraflavanone G that killed 99.9% of the test bacteria when plating on the

Table 1. Checkerboard assay of sophoraflavanone G and ampicillin against oral bacteria.

Strains	Agent	MIC/MBC (μg/ml)		FIC[b] (μg/ml)	FICI[2]	Outcome
		Alone	Combination[a]			
S. mutans	Sophoraflavanone G	3.2/3.2	0.4 /0.4	0.125	0.375	Synergistic
ATCC 25175	Ampicillin	0.25/0.5	0.0625/0.125	0.25		
S. sanguinis	Sophoraflavanone G	3.2/3.2	0.8/1.6	0.25	0.5	Synergistic
ATCC 10556	Ampicillin	1/2	0.25/0.5	0.25		
S. sobrinus	Sophoraflavanone G	3.2/3.2	0.8/1.6	0.25	0.31	Synergistic
ATCC 27607	Ampicillin	0.25/0.5	0.0156/0.0312	0.063		
S. ratti	Sophoraflavanone G	1.6/3.2	0.8/0.8	0.5	1	Additive
KCTC 3294	Ampicillin	1/2	0.5/0.5	0.5		
S. criceti	Sophoraflavanone G	1.6/3.2	0.4/0.8	0.25	0.75	Additive
KCTC 3292	Ampicillin	0.5/1	0.25/0.25	0.5		
S. anginosus	Sophoraflavanone G	3.2/6.4	0.8/0.8	0.25	0.75	Additive
ATCC 31412	Ampicillin	1/1	0.5/0.5	0.5		
S. gordonii	Sophoraflavanone G	0.8/0.8	0.1/0.2	0.125	0.375	Synergistic
ATCC 10558	Ampicillin	1/2	0.025/0.5	0.25		
A. actinomycetemcomitans	Sophoraflavanone G	3.2/3.2	0.2/0.4	0.063	0.31	Synergistic
ATCC 43717	Ampicillin	32/64	8/16	0.25		
F. nucleatum	Sophoraflavanone G	6.4/12.8	0.4/0.8	0.063	0.5	Synergistic
ATCC 51190	Ampicillin	4/4	1/1	0.25		
P. intermedia	Sophoraflavanone G	3.2/6.4	1.6/1.6	0.25	0.5	Synergistic
ATCC 49049	Ampicillin	4/8	1/2	0.25		
P. gingivalis	Sophoraflavanone G	0.2 /0.8	0.1/0.1	0.25	0.31	Synergistic
ATCC 33277	Ampicillin	0.25/0.5	0.0156/0.0312	0.063		

[a]The checkerboard test was performed as previously described [5]. The MICs and MBCs of sophoraflavanone G with ampicillin against oral bacteria are indicated.

[b]The interaction was defined as synergistic if the FIC index was less than or equal to 0.5, additive if the FIC index was greater than 0.5 and less than or equal to 1.0, indifferent if the FIC index was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FIC index was greater than 2.0 [5, 18].

Table 2. Checkerboard assay of sophoraflavanone G and gentamicin against oral bacteria.

Strains	Agent	MIC/MBC (μg/ml)		FIC[b] (μg/ml)	FICI[2]	Outcome
		Alone	Combination[a]			
S. mutans	Sophoraflavanone G	3.2/3.2	0.8/0.8	0.25	0.75	Additive
ATCC 25175	Gentamicin	8/16	4/4	0.5		
S. sanguinis	Sophoraflavanone G	3.2/6.4	0.4/0.4	0.125	0.375	Synergistic
ATCC 10556	Gentamicin	64/64	16/16	0.25		
S. sobrinus	Sophoraflavanone G	3.2/3.2	1.6/3.2	0.5	0.75	Additive
ATCC 27607	Gentamicin	4/8	1/2	0.25		
S. ratti	Sophoraflavanone G	1.6/3.2	0.8/0.8	0.5	1	Additive
KCTC 3294	Gentamicin	16/32	8/8	0.5		
S. criceti	Sophoraflavanone G	1.6/3.2	0.4/0.4	0.25	0.281	Synergistic
KCTC 3292	Gentamicin	8/16	0.25/0.25	0.031		
S. anginosus	Sophoraflavanone G	3.2/6.4	0.8/0.8	0.25	0.281	Synergistic
ATCC 31412	Gentamicin	32/32	1/2	0.031		
S. gordonii	Sophoraflavanone G	0.8/0.8	0.1/0.1	0.125	0.63	Additive
ATCC 10558	Gentamicin	32/32	16/16	0.5		
A. actinomycetemcomitans	Sophoraflavanone G	3.2/3.2	0.8 /0.8	0.25	0.5	Synergistic
ATCC 43717	Gentamicin	4/8	1/1	0.25		
F. nucleatum	Sophoraflavanone G	6.4/12.8	1.6/1.6	0.25	0.5	Synergistic
ATCC 51190	Gentamicin	2/4	0.5/0.5	0.25		
P. intermedia	Sophoraflavanone G	3.2/6.4	0.4/0.8	0.125	0.375	Synergistic
ATCC 49049	Gentamicin	16/32	4/4	0.25		
P. gingivalis	Sophoraflavanone G	0.2 /0.8	0.05/0.1	0.25	0.31	Synergistic
ATCC 33277	Gentamicin	256/512	16/32	0.063		

[a]The checkerboard test was performed as previously described [5]. The MICs and MBCs of sophoraflavanone G with gentamicin against oral bacteria are indicated.

[b]The interaction was defined as synergistic if the FIC index was less than or equal to 0.5, additive if the FIC index was greater than 0.5 and less than or equal to 1.0, indifferent if the FIC index was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FIC index was greater than 2.0 [5, 18].

appropriate agar plate. In addition, the antibacterial effects of combining sophoraflavanone G with certain antibiotics were assessed using a checkerboard test, as previously described [5, 14]. The antimicrobial combinations assayed included sophoraflavanone G from *S. flavescens* plus ampicillin or gentamicin. As such, the fractional inhibitory concentration index (FICI) was determined as the sum of the FICs of each drug, which in turn was defined as the MIC of each drug when used in combination, divided by the MIC of the drug when used alone. The interaction was then defined as synergistic if the FIC index was less than or equal to 0.5, additive if the FIC index was greater than 0.5 and less than or equal to 1.0, indifferent if the FIC index was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FIC index was greater than 2.0 [5, 18]. The MIC/MBC of sophoraflavanone G was found to be either 0.4/0.8 or 1.6/3.2 μg/ml, the MIC/MBC of ampicillin either 0.25/0.5 or 32/64 μg/ml, and the MIC/MBC of gentamicin either 0.5/1 or 256/512 μg/ml (Tables 1 and 2). When combined with sophoraflavanone G, the MIC/MBC of ampicillin was reduced ≥8-fold for *S. mutans*, *S. sanguinis*, *S. sobrinus*, *S. gordonii*, *A. actinomycetemcomitans*, *F. nucleatum*, *P. intermedia*, and *P. gingivalis*, reflecting a synergistic effect, as defined by the FICI≤0.5. The addition of sophoraflavanone G led to a reduced single dilution for *S. ratti*, *S. criceti*, and *S. anginosus*, as defined by the FICI≤0.5-1 (Table 1). The combination of gentamicin and sophoraflavanone G resulted in a decrease in the MIC/MBC for all the bacteria, where the MIC/MBC of 2-256/4-512 μg/ml for gentamicin became 0.25-32/0.25-32 μg/ml. The FICI classified the combination of sophoraflavanone G and gentamicin as additive for *S. mutans*, *S. sobrinus*, *S. ratti*, and *S. gordonii* and synergistic for *S. sanguinis*, *S. criceti*, *S. anginosus*, *A. actinomycetemcomitans*, *F. nucleatum*, *P. intermedia*, and *P. gingivalis* (Table 2).

Many attempts have already been made to eliminate *S. mutans* from the oral flora [9, 23]. For example, antibiotics, such as ampicillin, chlorhexidine, erythromycin, penicillin, tetracycline, and vancomycin, have been shown to be very effective in preventing dental caries [1, 2, 25, 29, 38], whereas various flavonoid derivatives from *S. flavescens*, such as quercetin, sophoraflavanone G, and kaempferol, have been found to exhibit antimicrobial and antimalaria activity [4, 7, 17]. In addition, phytoalexins, defensive compounds produced by plants against microbial infections, have been purified from *Sophora exigua* (Leguminosae) and their growth inhibitory effects on oral cariogenic bacteria determined *in vitro* [36].

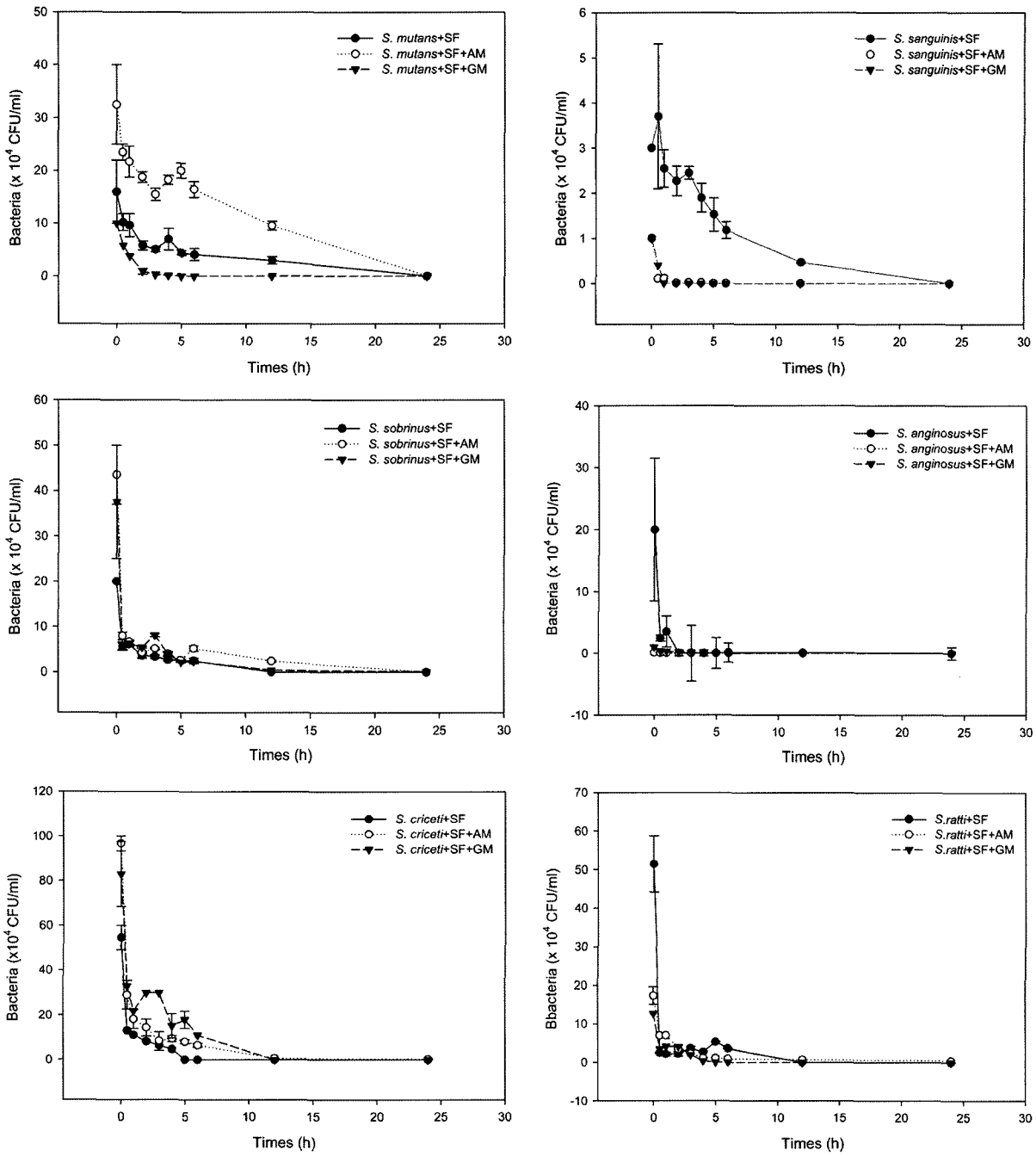

Fig. 2. Time-kill curves for MIC of sophoraflavanone G alone and when combined with MIC of ampicillin and gentamicin against *S. mutans*, *S. sanguinis*, *S. sobrinus*, *S. anginosus*, *S. criceti*, and *S. ratti*.
Bacteria were incubated with sophoraflavanone G (●), sophoraflavanone G+ampicillin (○), and sophoraflavanone G+gentamicin (▼) over time. Data points are the mean values±SEM of six experiments. CFU, colony-forming units.

The bactericidal activities of the drugs investigated in this study as regards oral bacteria were also evaluated using time-kill curves. As such, tubes containing sophoraflavanone G and oral bacteria were incubated at 37°C in an anaerobic chamber and viable counts performed 0, 0.5, 1, 2, 3, 4, 5, 6, 12, and 24 h after the addition of the antimicrobial agents. Agar plates were incubated for up to 48 h in an anaerobic chamber at 37°C. The colony counts were performed in duplicate, and the means taken. Cultures of all the bacteria, with a cell density of 10^5–10^6 CFU/ml, were exposed to the MIC of sophoraflavanone G alone and with ampicillin or gentamicin several times. When exposed to sophoraflavanone G alone, the rate of killing CFU/ml increased after 1 h, whereas the combination of

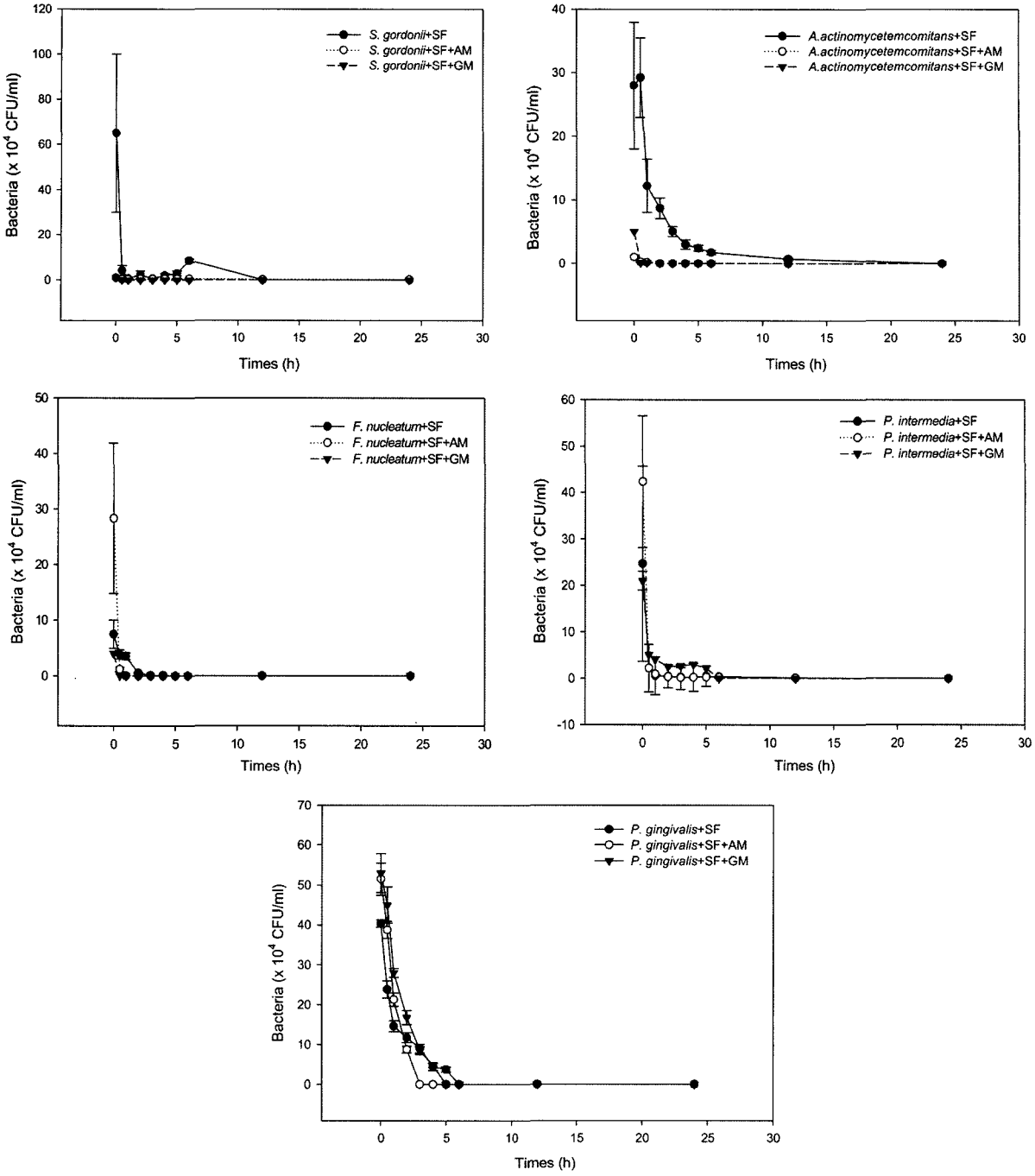

Fig. 3. Time-kill curves for MIC of sophoraflavanone G alone and when combined with MIC of ampicillin and gentamicin against *S. gordonii*, *A. actinomycetemcomitans*, *F. nucleatum*, *P. intermedia*, and *P. gingivalis*.
Bacteria were incubated with sophoraflavanone G (●), sophoraflavanone G+ampicillin (○), and sophoraflavanone G+gentamicin (▼) over time. Data points are the mean values±SEM of six experiments. CFU, colony-forming units.

sophoraflavanone G and ampicillin or gentamicin produced a more rapid rate of killing after 30 min (Figs. 2 and 3). Furthermore, the combination of sophoraflavanone G and ampicillin or gentamicin killed all the bacteria within 5 h, except for *S. mutans*. Thus, the drug combinations exhibited a strong bactericidal effect. The antibacterial agents currently used to prevent dental caries include xylitol, tea extracts, essential oils, and antibiotics. Xylitol, a natural sweetener derived from xylose, is presently widely used in chewing gum, toothpaste, and mouthwash [1, 2, 25, 28, 29].

Accordingly, the present findings suggest that sophoraflavanone G fulfills the conditions required for

novel cariogenic bacteria and periodontal pathogens as a bacteroide species drug, and may be useful for the treatment of oral bacteria infections. However, for medicinal purposes, the safety and toxicity of this compound still need to be addressed. The difference in susceptibility may also allow the formulation of products that will selectively kill or inhibit certain organisms, while having a minimal effect on the commensal microorganisms.

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