Synergistic antimicrobial activity of apigenin against oral Pathogens

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Abstract**—** *Although a broad range of biological and pharmacological activities of apigenin have been reported, the mechanism(s) behind its antibacterial effects are not fully understood. In this study, we investigated the synergistic antibacterial activity of apigenin in combination with existing antimicrobial agents against oral bacteria. The combination effect of apigenin was evaluated against oral bacteria, either alone or with antibiotics, via broth dilution method and checkerboard and time kill assay. MIC/MBC values for apigenin, ampicillin, gentamicin, erythromycin, and vancomycin against all the tested bacteria ranged between 50-200/100-800 microg/mL, 0.0313-16/0.125-32 microg/mL, 2-256/4-512 microg/mL, 0.008-32/0.016-64 microg/mL, and 0.25-64/1-128 microg/mL, respectively. Checkerboard assay revealed synergistic activity in the combination of apigenin with antibiotics at fractional inhibitory concentration index (FICI) <0.5. 1-6 hours of treatment with 1/2 MIC of apigenin with 1/2 MIC of antibiotics resulted from an increase of the rate of killing in units of CFU/mL to a greater degree than was observed with alone. These results suggest that the apigenin is important in the antibacterial actions of oral pathogen agents.*

Keywords**— Apigenin, antibacterial activity, oral pathogen bacteria, Synergistic effect, Minimum inhibitory concentrations (MICs), Minimum bactericidal concentrations (MBCs)**

I. INTRODUCTION

Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases [1, 2]. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases [3, 4]. The association between oral diseases and the oral microbiota is well established [5]. Of the more than 750 species of bacteria that inhabit the oral cavity, a number are implicated in oral diseases [6-8]. The development of dental caries involves acidogenic and aciduric gram-positive bacteria, primarily the mutans streptococci (*Streptococcus mutans* and *S. sobrinus*), lactobacilli, and actinomycetes, which metabolize sucrose to organic acids (mainly lactic acid) that dissolve the calcium phosphate in teeth, causing decalcification and eventual decay [7, 9]. In contrast, periodontal diseases are subgingival conditions that have been linked to anaerobic gram-negative bacteria such as *Porphyromonas gingivalis*, Actinobacillus sp., Prevotella sp., and Fusobacterium sp. [10, 11]. In periodontal diseases, the areas at or below the gingival crevice become infected causing a cellular inflammatory response of the gingiva and surrounding connective tissue [12, 13]. These inflammatory responses can manifest as gingivitis (extremely common and seen as bleeding of the gingival or gum tissues) or periodontitis (the inflammatory response results in loss of collagen attachment of the tooth to the bone and in loss of bone) [13, 14].

Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopeias as agents used to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens [15-19]. Flavonoids have also been shown to exhibit broader bioactivities such as protection of vascular integrity, antihepatotoxicity, anti-inflammatory activity, antitumor effect, antiallergic properties, and antimicrobial effects [20-22]. Apigenin (4,5,7-trihydroxyflavone), a flavone subclass of flavonoid widely distributed in many herbs, fruits, and vegetables, is a substantial component of the human diet and has been shown to possess a variety of biological characteristics, including anticancer, antibacterial, antioxidant, anti-apoptosis, and anti-inflammatory [23-26]. The low antibacterial activity of apigenin alone in Gram-negative bacteria may be due to the presence of lipopolysaccharide and protein-rich outer membrane which covers and protects the internal peptidoglycan wall [27]. The in vitro anti-inflammatory effect of apigenin was studied in many cases. The apigenin decreased cytokines, TNF-α, IL-1β, and IL-6 in LPS-stimulated human peripheral blood mononuclear cells [28]. Apigenin has been reported to suppress cell proliferation in various cell types, and such an anti-proliferative effect has been shown to be associated with PI3K/Akt pathway [29, 30]. Although a broad range of biological and pharmacological activities of apigenin have been reported, the mechanism(s) behind its antibacterial effects are not fully

understood. In this study, we investigated the synergistic antibacterial activity of apigenin in combination with existing antimicrobial agents against oral bacteria.

II. MATERIALS AND METHODS

2.1 Bacterial strains

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. Brain-Heart Infusion (Difco Laboratories, Detroit, MI) broth supplemented with 1% yeast extract (Difco) was used for all bacterial strains except *P. intermedia* and *P. gingivalis.* For *P. intermedia* and *P. gingivalis*, BHI broth containing hemin 1 μg/mL (Sigma, St. Louis, MO, USA) and menadione 1 μg/mL (Sigma) was used.

2.2 Minimum inhibitory concentrations/minimum bactericidal concentrations assay

The minimum inhibitory concentrations (MICs) were determined for apigenin by the broth dilution method [18], and were carried out in triplicate. The antibacterial activities were examined after incubation at 37℃ for 18 h (facultative anaerobic bacteria), for 24 h (microaerophilic bacteria), and for 1-2 days (obligate anaerobic bacteria) 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. MIC_{50}s and MIC_{90}s, defined as MICs at which, 50 and 90%, respectively of oral bacteria were inhibited, were determined. Following anaerobic incubation of **MICs** plates, the minimum bactericidal concentrations (MBCs) were determined on the basis of the lowest concentration of apigenin that kills 99.9% of the test bacteria by plating out onto each appropriate agar plate. Ampicillin, gentamicin, erythromycin, and vancomycin (Sigma) were used as standard antibiotics in order to compare the sensitivity of apigenin against oral bacteria.

2.3 Checker-board dilution test

The antibacterial effects of a combination of apigenin, which exhibited the highest antimicrobial activity, and antibiotics were assessed by the checkerboard test as previously described [18]. The antimicrobial combinations assayed included apigenin with antibiotics, ampicillin, gentamicin, erythromycin, and vancomycin. Serial dilutions of two different antimicrobial agents were mixed in cation-supplemented Mueller-Hinton broth. After 24-48 h of incubation at 37°C, the MICs were determined to be the minimal concentration at which there was no visible growth and MBCs were determined on the basis of the lowest concentration of apigenin that kills 99.9% of the test bacteria by plating out onto each appropriate agar plate. The fractional inhibitory concentration (FIC)/ fractional bactericidal concentration (FBC) index was calculated according to the equation: FIC/FBC index=FIC/FBC_A+FIC/FBC_B=(MIC/MBC of drug A in combination/MIC/MBC of drug A alone)+(MIC/MBC of drug B in combination/MIC/MBC of drug B alone). The FIC and FBC index are the sum of the FICs and FBCs of each of the drugs, which in turn is defined as the MIC and MBC of each drug when it is used in combination divided by the MIC and MBC of the drug when it is used alone. The interaction was defined as synergistic if the FIC and FBC index was less than or equal to 0.5, additive if the FIC and FBC index was greater than 0.5 and less than or equal 1.0, indifferent if the FIC and FBC index was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FIC and FBC index was greater than 2.0.

2.4 Time-kill curves

Bactericidal activities of the drugs under study were also evaluated using time-kill curves on oral bacteria. Tubes containing Mueller-Hinton supplemented to which antibiotics had been added at concentrations of the MIC_{50} were inoculated with a suspension of the test strain, giving a final bacterial count between $5\sim6.6\times10^6$ CFU/ml. The tubes were thereafter incubated at 37°C in an 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°C. 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 and BHI agar containing hemin and menadione for *P. intermedia* and *P. gingivalis*.

III. RESULTS AND DISCUSSION

The main etiological factor of dental caries and periodontal disease is dental plaque [31-33]. Therefore, it is reasonable to search for natural products that have antiplaque properties and antimicrobial activity against oral pathogens [16, 19, 34-35]. Apigenin was evaluated for their antimicrobial activities against eleven common bacterial species present in the oral cavity. The results of the antimicrobial activity showed that apigenin exhibited antimicrobial activities against cariogenic bacteria (MICs, 25 to 200 μg/mL; MBCs, 100 to 800 μg/mL), against periodontopathogenic bacteria (MICs, 100 to 200 μg/mL; MBCs, 200 to 400 μg/mL) and for ampicillin, either 0.0313/0.125 or 16/32 μg/mL; for gentamicin, either 2/4 or 256/512 μg/mL; for erythromycin, either 0.008/0.016 or 32/64; for vancomycin, either 0.25/1 or 64/128 on tested all bacteria (Table 1). The MIC_{50} and MIC_{90} ranges of apigenin were from 6.25 to 12.5 μg/mL and 25 to 200 μg/mL, respectively. The apigenin showed stronger antimicrobial activity against *S. ratti*, *S. gordonii*, and *P. intermedia* than another bacteria (MIC/MBC, 25/50-100 μg/mL) and the range of MIC_{50} and MIC_{90} were 6.25 μg/mL and 25 μg/mL.

TABLE 1
ANTIBACTERIAL ACTIVITY OF APIGENIN AND ANTIBIOTICS IN ORAL BACTERIA

Samples	Apigenin			Ampicillin	Gentamicin	Erythromycin	Vancomycin
	$MIC_{50<}$	$MIC_{90<}$	MIC/MBC	MIC/MBC			
S. mutans **ATCC 25175[1]**	**25**	**100**	**100/200**	**0.125/0.25**	**8/16**	**0.063/0.125**	**1/2**
S. sanguinis **ATCC 10556**	**12.5**	**50**	**50/200**	**0.25/0.5**	**16/32**	**0.016/0.031**	**0.25/1**
S. sobrinus **ATCC 27607**	**25**	**100**	**100/200**	**0.0313/0.125**	**16/32**	**0.031/0.063**	**1/2**
S. ratti **KCTC 3294[2]**	**12.5**	**50**	**50/100**	**0.125/0.5**	**8/16**	**0.008/0.016**	**0.5/1**
S. criceti **KCTC 3292**	**6.25**	**25**	**25/100**	**0.0313/0.125**	**8/16**	**0.125/0.25**	**1/4**
S. anginosus **ATCC 31412**	**50**	**200**	**200/800**	**0.0625/0.25**	**8/16**	**0.125/0.5**	**1/4**
S. gordonii **ATCC 10558**	**12.5**	**50**	**50/100**	**0.0625/0.25**	**16/32**	**0.031/0.063**	**0.5/1**
A. actinomycetemcomitans **ATCC 43717**	**50**	**200**	**200/400**	**16/32**	**8/16**	**0.125/0.25**	**2/4**
F. nucleatum **ATCC 51190**	**25**	**100**	**100/200**	**8/16**	**2/4**	**32/64**	**64/128**
P. intermedia **ATCC 49049**	**50**	**200**	**200/400**	**1/2**	**32/32**	**16/32**	**16/36**
P. gingivalis **ATCC 33277**	**25**	**200**	**200/400**	**0.5/0.5**	**256/512**	**2/8**	**8/16**

[1]American Type Culture Collection (ATCC)
[2]Korean collection for type cultures (KCTC)

Natural products, polyphenols are a major source of chemical diversity and have provided important therapeutic agents for many oral bacterial diseases [36-38]. Combinations of some herbal materials and different antibiotics might affect the inhibitory effect of these antibiotics [18, 39, 40]. The synergistic effects of apigenin alone or with antibiotics were evaluted in oral bacteria (Table 2 and 3). In combination with apigenin, the MIC for ampicillin was reduced ≥4-fold in tested bacteria, except *S. anginosus*, producing a synergistic effect as defined by FICI ≤ 0.5. The MBC for ampicillin was shown synergistic effects in *S. mutans*, *S. sanguinis*, *S. ratti*, *S. gordonii*, *F. nucleatum*, *P. intermedia*, and *P. gingivalis* by FBCI ≤ 0.5 (Table 2). In combination with apigenin, the MIC for gentamicin was reduced ≥4-8-fold in all tested bacteria expect *S. ratti* by FICI ≤ 0.5 and MBC in *S. mutans*, *S. sobrinus*, *S. criceti*, *S. anginosus*, *S. gordonii*, *F. nucleatum*, *P. intermedia*, and *P. gingivalis* by FBCI ≤ 0.5 (Table 3). Moreover, the MIC for erythromycin with apigenin was reduced ≥4-fold in tested bacteria, except *S. sanguinis*, *S. rattii*, and *S. anginosus*, producing a synergistic effect as defined by FICI ≤ 0.5 and the MBC for erythromycin was shown synergistic effects in *S. mutans*, *S. criceti*, *S. anginosus*, *S. gordonii*, *A. actinomycetemcomitans*, and *P. gingivalis* by FBCI ≤ 0.5 (Table 2).

TABLE 2
SYNERGISTIC EFFECTS OF APIGENIN WITH AMPICILLIN AGAINST ORAL BACTERIA.

Strains	Agent	MIC/MBC (µg/ml)		FIC/FBC	FICI/FBCI[2]	Outcome
		Alone	Combination[1]			
S. mutans **ATCC 25175[3]**	Apigenin	100/200	12.5/50	0.125/0.125	0.375/0.375	Synergistic/ Synergistic
	Ampicillin	0.125/0.25	0.0313/0.0625	0.25/0.25		
S. sanguinis **ATCC 10556**	Apigenin	50/200	12.5/50	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Ampicillin	0.25/0.5	0.0625/0.125	0.25/0.25		
S. sobrinus **ATCC 27607**	Apigenin	100/200	25/50	0.25/0.25	0.5/0.75	Synergistic/ Additive
	Ampicillin	0.0625/0.125	0.0156/0.0625	0.25/0.5		
S. ratti **KCTC 3294[4]**	Apigenin	50/100	12.5/25	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Ampicillin	0.25/0.5	0.0625/0.125	0.25/0.25		
S. criceti **KCTC 3292**	Apigenin	25/100	6.25/12.5	0.25/0.125	0.5/0.625	Synergistic/ Additive
	Ampicillin	0.0625/0.125	0.0156/0.0625	0.25/0.5		
S. anginosus **ATCC 31412**	Apigenin	200/800	50/200	0.25/0.25	0.75/0.75	Additive/ Additive
	Ampicillin	0.125/0.25	0.0625/0.125	0.5/0.5		
S. gordonii **ATCC 10558**	Apigenin	50/100	12.5/12.5	0.25/0.125	0.5/0.375	Synergistic/ Synergistic
	Ampicillin	0.0625/0.25	0.0156/0.0625	0.25/0.25		
A. actinomycetemcomitans **ATCC 43717**	Apigenin	200/400	25/100	0.125/0.25	0.25/0.75	Synergistic/ Additive
	Ampicillin	16/32	2/16	0.125/0.5		
F. nucleatum **ATCC 51190**	Apigenin	100/200	25/50	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Ampicillin	16/32	4/8	0.25/0.25		
P. intermedia **ATCC 49049**	Apigenin	200/400	50/100	0.25/0.25	0.375/0.5	Synergistic/ Synergistic
	Ampicillin	2/4	0.25/1	0.125/0.25		
P. gingivalis **ATCC 33277**	Apigenin	200/400	50/100	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Ampicillin	0.5/1	0.125/0.25	0.25/0.25		

[1]The MIC and MBC of apigenin with ampicillin
[2] The fractional inhibitory concentration index (FIC index)
[3]American Type Culture Collection (ATCC)
[4]Korean collection for type cultures (KCTC)

TABLE 3
SYNERGISTIC EFFECTS OF APIGENIN WITH GENTAMICIN AGAINST ORAL BACTERIA.

Strains	Agent	MIC/MBC (µg/ml)		FIC	FICI[2]	Outcome
		Alone	Combination[1]			
S. mutans **ATCC 25175[3]**	Apigenin	100/200	12.5/50	0.125/0.25	0.375/0.5	Synergistic/ Synergistic
	Gentamicin	8/16	2/4	0.25/0.25		
S. sanguinis **ATCC 10556**	Apigenin	50/200	12.5/50	0.25/0.25	0.5/0.75	Synergistic/ Additive
	Gentamicin	16/32	4/16	0.25/0.5		
S. sobrinus **ATCC 27607**	Apigenin	100/200	12.5/25	0.125/0.125	0.375/0.375	Synergistic/ Synergistic
	Gentamicin	16/32	4/16	0.25/0.5		
S. ratti **KCTC 3294[4]**	Apigenin	50/100	12.5/25	0.5/0.25	0.75/0.75	Additive/ Additive
	Gentamicin	16/16	4/8	0.25/0.5		
S. criceti **KCTC 3292**	Apigenin	25/100	6.25/12.5	0.25/0.125	0.5/0.375	Synergistic/ Synergistic
	Gentamicin	8/16	2/4	0.25/0.25		
S. anginosus **ATCC 31412**	Apigenin	200/800	50/200	0.25/0.25	0.375/0.5	Synergistic/ Synergistic
	Gentamicin	16/32	2/8	0.125/0.25		
S. gordonii **ATCC 10558**	Apigenin	50/100	6.25/25	0.125/0.25	0.375/0.5	Synergistic/ Synergistic
	Gentamicin	16/32	4/8	0.25/0.25		
A. actinomycetemcomitans **ATCC 43717**	Apigenin	200/400	50/100	0.25/0.25	0.5/0.75	Synergistic/ Additive
	Gentamicin	8/16	2/8	0.25/0.5		
F. nucleatum **ATCC 51190**	Apigenin	100/200	12.5/25	0.125/0.125	0.375/0.375	Synergistic/ Synergistic
	Gentamicin	4/8	1/2	0.25/0.25		
P. intermedia **ATCC 25611**	Apigenin	200/400	50/100	0.25/0.25	0.375/0.75	Synergistic/ Additive
	Gentamicin	32/32	4/16	0.125/0.5		
P. gingivalis **ATCC 33277**	Apigenin	200/400	50/100	0.25/0.25	0.375/0.5	Synergistic/ Synergistic
	Gentamicin	256/512	32/128	0.125/0.25		

[1]The MIC and MBC of apigenin with gentamicin

[2] The fractional inhibitory concentration index (FIC index)

[3]American Type Culture Collection (ATCC)

[4]Korean collection for type cultures (KCTC)

TABLE 4
SYNERGISTIC EFFECTS OF APIGENIN WITH ERYTHROMYCIN AGAINST ORAL BACTERIA

Strains	Agent	MIC/MBC (μg/ml)		FIC	FICI[2]	Outcome
		Alone	Combination[1]			
S. mutans **ATCC 25175[3]**	Apigenin	100/200	25/50	0.25/0.25	0.375/0.5	Synergistic/ Synergistic
	Erythromycin	0.063/0.125	0.008/0.031	0.125/0.25		
S. sanguinis **ATCC 10556**	Apigenin	50/200	12.5/50	0.25/0.25	0.75/0.75	Additive/ Additive
	Erythromycin	0.016/0.031	0.008/0.016	0.5/0.5		
S. sobrinus **ATCC 27607**	Apigenin	100/200	25/100	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Erythromycin	0.031/0.063	0.008/0.016	0.25/0.25		
S. ratti **KCTC 3294[4]**	Apigenin	50/100	12.5/25	0.25/0.25	0.75/0.75	Additive/ Additive
	Erythromycin	0.008/0.016	0.004/0.008	0.5/0.5		
S. criceti **KCTC 3292**	Apigenin	25/100	6.25/25	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Erythromycin	0.125/0.25	0.031/0.063	0.25/0.25		
S. anginosus **ATCC 31412**	Apigenin	200/800	50/100	0.25/0.125	0.75/0.375	Additive/ Synergistic
	Erythromycin	0.125/0.5	0.063/0.0125	0.5/0.25		
S. gordonii **ATCC 10558**	Apigenin	50/100	12.5/25	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Erythromycin	0.031/0.063	0.008/0.016	0.25/0.25		
A. actinomycetemcomitans **ATCC 43717**	Apigenin	200/400	50/100	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Erythromycin	0.125/0.25	0.031/0.063	0.25/0.25		
F. nucleatum **ATCC 51190**	Apigenin	100/200	25/50	0.25/0.25	0.5/0.75	Synergistic/ Additive
	Erythromycin	32/64	8/32	0.25/0.5		
P. intermedia **ATCC 25611**	Apigenin	200/400	50/200	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Erythromycin	16/32	4/8	0.25/0.25		
P. gingivalis **ATCC 33277**	Apigenin	200/400	50/100	0.25/0.25	0.375/0.5	Synergistic/ Synergistic
	Erythromycin	2/8	0.25/2	0.125/0.25		

[1]***The MIC and MBC of apigenin with erythromycin***

[2] ***The fractional inhibitory concentration index (FIC index)***

[3]***American Type Culture Collection (ATCC)***

[4]***Korean collection for type cultures (KCTC)***

TABLE 5
SYNERGISTIC EFFECTS OF APIGENIN WITH VANCOMYCIN AGAINST ORAL BACTERIA.

Strains	Agent	MIC/MBC (µg/ml)		FIC	FICI[2]	Outcome
		Alone	Combination[1]			
S. mutans **ATCC 25175[3]**	Apigenin	100/200	25/100	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Vancomycin	1/2	0.25/0.5	0.25/0.25		
S. sanguinis **ATCC 10556**	Apigenin	50/200	12.5/50	0.25/0.25	0.5/0.375	Synergistic/ Synergistic
	Vancomycin	0.25/1	0.063/0.125	0.25/0.125		
S. sobrinus **ATCC 27607**	Apigenin	100/200	25/50	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Vancomycin	1/2	0.25/0.5	0.25/0.25		
S. ratti **KCTC 3294[4]**	Apigenin	50/100	12.5/50	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Vancomycin	0.5/1	0.125/0.25	0.25/0.25		
S. criceti **KCTC 3292**	Apigenin	25/100	6.25/25	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Vancomycin	1/4	0.25/1	0.25/0.25		
S. anginosus **ATCC 31412**	Apigenin	200/800	50/100	0.25/0.125	0.5/0.25	Synergistic/ Synergistic
	Vancomycin	1/4	0.25/0.5	0.25/0.125		
S. gordonii **ATCC 10558**	Apigenin	50/100	12.5/25	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Vancomycin	0.5/1	0.125/0.25	0.25/0.25		
A. actinomycetemcomitans **ATCC 43717**	Apigenin	200/400	50/100	0.25/0.25	0.5/0.75	Synergistic/ Additive
	Vancomycin	2/4	0.5/2	0.25/0.5		
F. nucleatum **ATCC 51190**	Apigenin	100/200	25/100	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Vancomycin	64/128	16/32	0.25/0.25		
P. intermedia **ATCC 25611**	Apigenin	200/400	50/100	0.25/0.25	0.5/0.75	Synergistic/ Additive
	Vancomycin	16/36	4/16	0.25/0.5		
P. gingivalis **ATCC 33277**	Apigenin	200/400	50/100	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Vancomycin	8/16	2/4	0.25/0.25		

[1]The MIC and MBC of apigenin with vancomycin

[2] The fractional inhibitory concentration index (FIC index)

[3]American Type Culture Collection (ATCC)

[4]Korean collection for type cultures (KCTC)

Flavonoid complexes attach with extra cellular soluble protein and with bacterial cell wall. Thus they exhibit antibacterial activity [41, 42]. Naturally derived apigenin was reported to have potential antimicrobial activity [26, 27, 43]. Trihydroxyflavone or apigenin is a naturally occurring bioflavonoid abundantly present in fruits and vegetables whose antibacterial activity against certain strains of Gram-negative and Gram-positive bacteria like *Escherichia coli, Staphylococcus aureus, Bacillus cereus, and Pseudomonas aeruginosa* has been reported recently [26,27,43,44].

In this study, apigenin, a flavone compound found in several plants also shows susceptibility on gram-positive bacteria as well as gram-negative bacteria. The bacterial effect of apigenin with ampicillin or gentamicin against oral bacteria was confirmed by time-kill curve experiments. The apigenin (MIC or MIC_{50}) alone resulted rate of killing increasing or not changing in CFU/ml at time dependent manner, with a more rapid rate of killing by apigenin (MIC_{50}) with ampicillin or/and gentamicin (MIC_{50}) (Fig 1-2). A strong bactericidal effect was exerted in drug combinations.

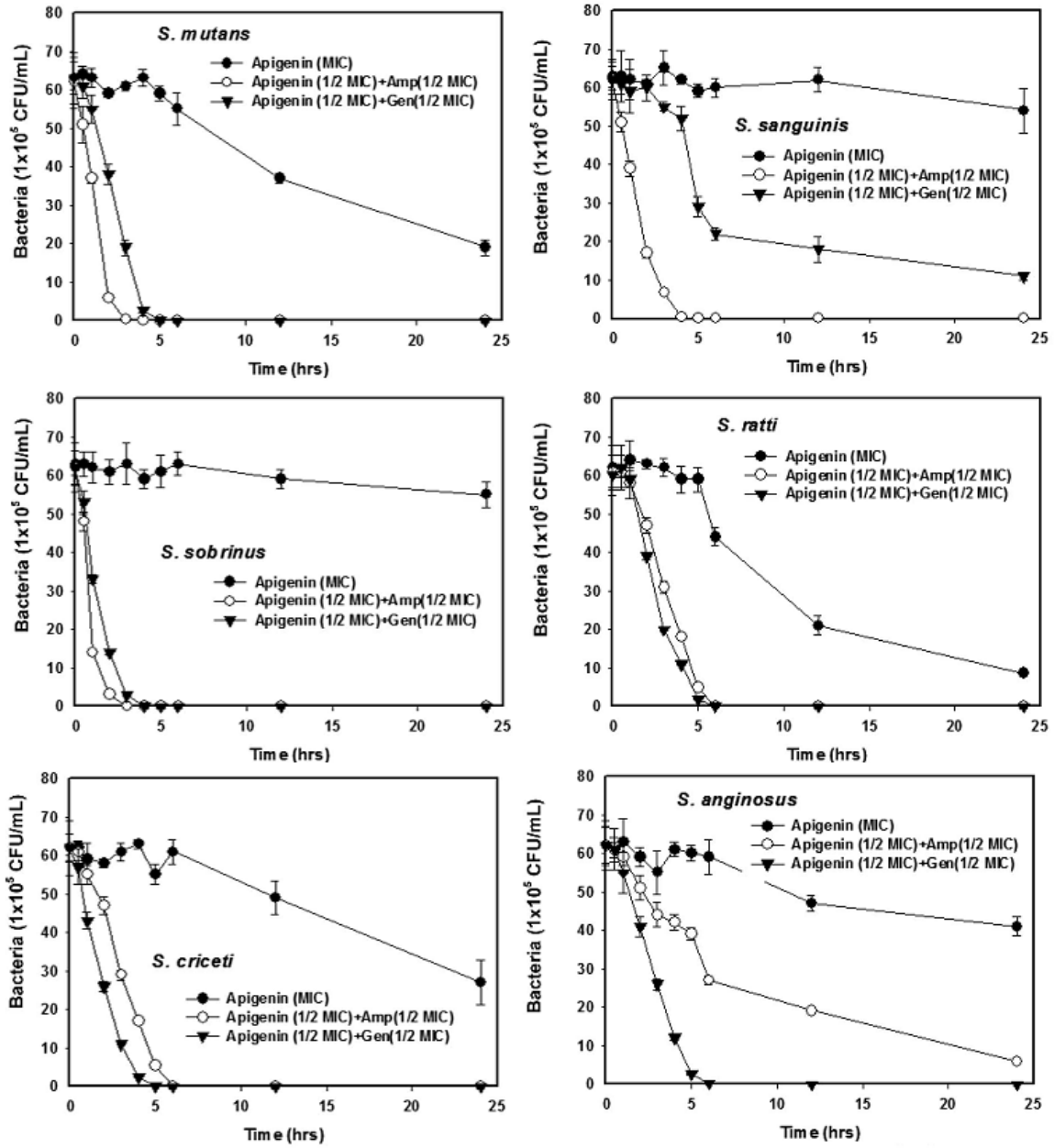

FIG. 1. TIME-KILL CURVES OF MIC OF APIGENIN ALONE AND ITS COMBINATION WITH MIC50 OF AMP OR GEN AGAINST. S. MUTANS, S. SANGUINIS, S. SOBRINUS, S. ANGINOSUS, S. CRICETI, AND S. RATTI. BACTERIA WERE INCUBATED WITH APIGENIN (●), APIGENIN + AMP (○), AND APIGENIN + GEN (▼) OVER TIME. DATA POINTS ARE THE MEAN VALUES±S.E.M. OF SIX EXPERIMENTS. CFU, COLONY-FORMING UNITS.

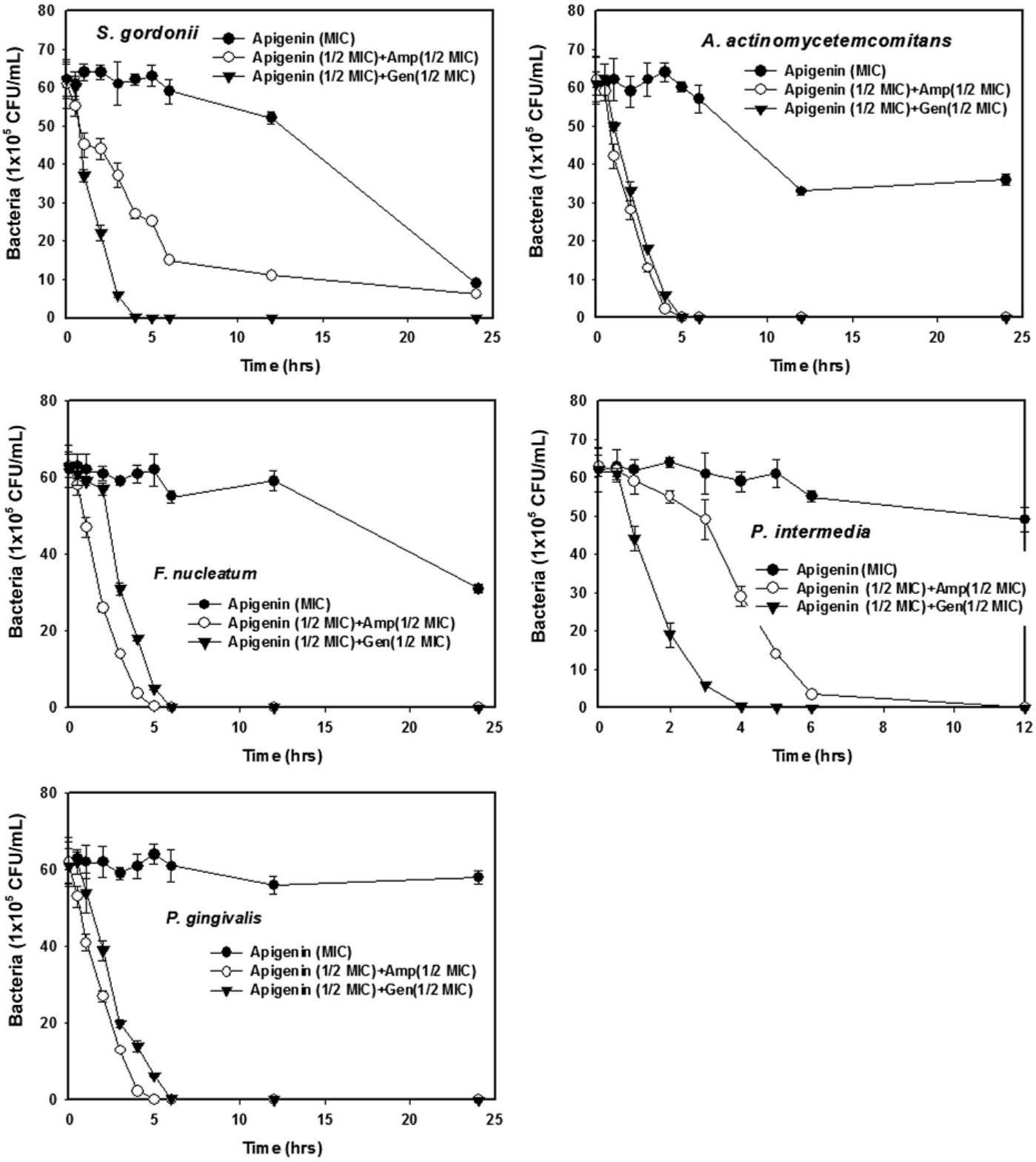

FIG. 2. TIME-KILL CURVES OF MIC OF APIGENIN ALONE AND ITS COMBINATION WITH MIC50 OF AMP OR GEN AGAINST S. GORDONII, A. ACTINOMYCETEMCOMITANS, F. NUCLEATUM, P. INTERMEDIA, AND P. GINGIVALIS. BACTERIA WERE INCUBATED WITH APIGENIN (●), APIGENIN + AMP (○), AND APIGENIN + GEN (▼) OVER TIME. DATA POINTS ARE THE MEAN VALUES±S.E.M. OF SIX EXPERIMENTS. CFU, COLONY-FORMING UNITS.

IV. CONCLUSION

In conclusion, these findings suggest that apigenin, a flavone compound found in several plants fulfills the conditions required of a novel cariogenic bacteria and periodontal pathogens, particularly bacteroides species drug and may be useful in the future in the treatment of oral bacteria.

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.

AUTHORS' CONTRIBUTION

Jeong-Dan Cha has substantial contributions to conception and design and drafting and revising it. Su-Mi Cha and Gi-Ug Kim have substantial contributions to acquisition and analysis of data.

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