Antibacterial actions of acacetin against oral pathogens

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Abbreviations:  
MICs: Minimum inhibitory concentrations, MBCs: Minimum bactericidal concentrations, CFU: Colony Forming Unit, FIC index: Fractional Inhibitory Concentration, FBC index: Fractional Bactericidal Concentration index

Keywords:  
Acacetin, antibacterial activity, oral pathogen bacteria, Synergistic effect, Minimum inhibitory concentrations (MICs), Minimum bactericidal concentrations (MBCs)

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Abstract  
Acacetin (5,7-dihydroxy-4’-methoxyflavone), a flavonoid compound, has anti-peroxidative and anti-inflammatory effects. Flavonoids have been reported to possess many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity, antiallergic activity, antioxidant activity, vascular activity and cytotoxic antitumour activity. In this study, the combination effect of acacetin was evaluated against oral bacteria, either alone or with antibiotics, via broth dilution method and checkerboard and time kill assay. In this results, MIC/MBC values for acacetin against all the tested bacteria ranged between 50-200/100-800 microg/ml, for ampicillin 0.0156-8/0.0625-16 microg/ml and for gentamicin 1-256/4-512 microg/ml respectively. Furthermore, the MIC and MBC were reduced to one half-eighth as a result of the combination of acacetin or/and apigenin with antibiotics. 1-2 hours of treatment with 1/2 MIC of acacetin 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 acacetin is important in the antibacterial actions of oral pathogen agents.

Citation:  
1. Introduction

Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases (Marsh, 2006; Grossner-Schreiber et al., 2006). Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases (Chi et al., 2010; Sheiham et al., 2001). The association between oral diseases and the oral microbiota is well established (Collins, Dawes, 1987; Marsh, 2006; Sbordone, Bortolaia, 2003). 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 (Kleinberg, 2002; Marsh, 2004). 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. (Palombo, 2011; Ardila et al., 2011). 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 (Lagdive et al., 2013; Graves et al., 2011). 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) (Graves et al., 2011; Van Strydonck et al., 2012).

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 (Mishra, Tiwari, 2011; Choi et al., 2012; Bag et al., 2012; Vermani et al., 2009; Cha et al., 2007). 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 (Radulović et al., 2012; Fu et al., 2012; Prasad et al., 2012). Acacetin (5,7-dihydroxy-4’-methoxyflavone), a flavone compound found in several plants, has been reported to show anti-peroxidative, anti-mutagenic, anti-cancer, anti-inflammatory, antibacterial, and anti-plasmodial activities (Ha et al., 2012; Fong et al., 2010; Cholbi et al., 1991; Navarro-Navarro et al., 2013). Although a broad range of biological and pharmacological activities of acacetin have been reported, the mechanism(s) behind its antibacterial effects are not fully understood.

2. Objective Research

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

3. Experimental

3.1 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 Porphyromonas 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.

3.2 Minimum inhibitory concentrations/minimum bactericidal concentrations assay

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined for acacetin (Sigma) by the broth dilution method (Cha et al., 2007), and were carried out in triplicate. The antibacterial activities were examined after incubation at 37° c 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. MIC90s and MBC90s, 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 acacetin that kills 99.9% of the test bacteria by plating out onto each appropriate agar plate. Ampicillin (Sigma) and gentamicin (Sigma) were used as standard antibiotics in order to compare the sensitivity of acacetin against oral bacteria.

3.3 Checker-board dilution test
The antibacterial effects of a combination of acacetin, which exhibited the highest antimicrobial activity, and antibiotics were assessed by the checkerboard test as previously described (Cha et al., 2007). The antimicrobial combinations assayed included acacetin with ampicillin or gentamicin. 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 acacetin 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/FBCA + FIC/FBCB = (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.

3.4 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 as concentrations of the MIC50 were inoculated with a suspension of the test strain, giving a final bacterial count between 5–6.6×10^6 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.

4. Results and Discussion
Acacetin was evaluated for their antimicrobial activities against eleven common bacterial species present in the oral cavity. The results of the antimicrobial activity showed that acacetin exhibited antimicrobial activities against cariogenic bacteria (MICs, 25 to 200 µg/ml; MBCs, 100 to 800 µg/ml), against periodontopathogenic bacteria (MICs, 25 to 100 µg/ml; MBCs, 50 to 200 µg/ml) and for ampicillin, either 0.0313/16 or 0.125/32 µg/ml; for gentamicin, either 2/4 or 256/512 µg/ml (Table 1). The range of MIC50 and MIC90 were from 6.25 to 12.5 µg/ml and 25 to 200 µg/ml, respectively. The acacetin 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 MIC50 and MIC90 were 6.25 µg/ml and 25 µg/ml.

Natural products are a major source of chemical diversity and have provided important therapeutic agents for many bacterial diseases (Mishra, Tiwari, 2011; Yamanoto, Ogawa, 2002; Hemaiswarya et al., 2008). Combinations of some herbal materials and different antibiotics might affect the inhibitory effect of these antibiotics (Hemaiswarya et al., 2008; Cha et al., 2007; Ma et al., 2009).

The synergistic effects of acacetin alone or with antibiotics were evaluated in oral bacteria (Table 2 and 3). In combination with acacetin, the MIC for ampicillin was reduced 4-fold in S. mutans, S. sanguinis, S. ratti, S. anginosus, S. gordonii, F. nucleatum, P. intermedia, and P. gingivalis, producing a synergistic effect as defined by FICI ≤ 0.5. The MBC for ampicillin was shown synergistic effects in S. ratti, S. anginosus, S. gordonii, and F. nucleatum by FBCI ≤ 0.5 (Table 2). In combination with acacetin, the MIC for gentamicin was reduced...
≥4-8-fold in all tested bacteria except S. sanguinis, S. ratti, A. actinomycetemcomitans, and P. intermedia by FICI ≥ 0.75 and MBC in S. mutans, S. sobrinus, S. criceti, S. anginosus, F. nucleatum, P. gingivalis by FBCI ≤ 0.5 (Table 3).

Table 1: Antibacterial activity of acacetin and antibiotics in oral bacteria

<table>
<thead>
<tr>
<th>Samples</th>
<th>Acacetin (µg/ml)</th>
<th>Ampicillin</th>
<th>Gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;≤ MIC&gt;</td>
<td>MIC/MBC</td>
<td>MIC/MBC</td>
</tr>
<tr>
<td>S. mutans ATCC 25175¹</td>
<td>12.5  50</td>
<td>50/100</td>
<td>0.0625/0.25/8/16</td>
</tr>
<tr>
<td>S. sanguinis ATCC 10556</td>
<td>25 100</td>
<td>100/200</td>
<td>0.25/0.5 16/32</td>
</tr>
<tr>
<td>S. sobrinus ATCC 27607</td>
<td>12.5 50</td>
<td>50/100</td>
<td>0.0313/0.125/16/32</td>
</tr>
<tr>
<td>S. ratti KCTC 3294²</td>
<td>6.25 25</td>
<td>25/100</td>
<td>0.125/0.5 8/16</td>
</tr>
<tr>
<td>S. criceti KCTC 3292</td>
<td>12.5 50</td>
<td>50/100</td>
<td>0.0313/0.125/8/16</td>
</tr>
<tr>
<td>S. anginosus ATCC 31412</td>
<td>25 200</td>
<td>200/800</td>
<td>0.0625/0.25/8/16</td>
</tr>
<tr>
<td>S. gordonii ATCC 10558</td>
<td>6.25 25</td>
<td>25/50</td>
<td>0.0625/0.25/16/32</td>
</tr>
<tr>
<td>A. actinomycetemcomitans ATCC 43717</td>
<td>25 100</td>
<td>100/200</td>
<td>16/32     8/16</td>
</tr>
<tr>
<td>F. nucleatum ATCC 51190</td>
<td>12.5 50</td>
<td>50/100</td>
<td>8/16      2/4</td>
</tr>
<tr>
<td>P. intermedia ATCC 49049</td>
<td>6.25 25</td>
<td>25/100</td>
<td>1/2       32/32</td>
</tr>
<tr>
<td>P. gingivalis ATCC 33277</td>
<td>6.25 50</td>
<td>50/100</td>
<td>0.5/0.5   256/512</td>
</tr>
</tbody>
</table>

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

Table 2: Synergistic effects of acacetin with ampicillin against oral bacteria

<table>
<thead>
<tr>
<th>Strains</th>
<th>Agent</th>
<th>MIC/MBC (µg/ml)</th>
<th>FIC/FBC¹</th>
<th>FICI/FBCI²</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Alone</td>
<td>Combination³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. mutans ATCC 25175¹</td>
<td>Acacetin</td>
<td>50/100</td>
<td>12.5/25</td>
<td>0.25/0.25</td>
<td>0.5/0.75</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>0.0625/0.25</td>
<td>0.0156/0.125</td>
<td>0.25/0.5</td>
<td></td>
</tr>
<tr>
<td>S. sanguinis ATCC 10556</td>
<td>Acacetin</td>
<td>100/200</td>
<td>25/50</td>
<td>0.25/0.25</td>
<td>0.5/0.75</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>0.25/0.5</td>
<td>0.0625/0.25</td>
<td>0.25/0.5</td>
<td></td>
</tr>
<tr>
<td>S. sobrinus ATCC 27607</td>
<td>Acacetin</td>
<td>50/100</td>
<td>25/50</td>
<td>0.5/0.5</td>
<td>1.0/0.75</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>0.0313/0.125</td>
<td>0.0156/0.0313</td>
<td>0.5/0.25</td>
<td></td>
</tr>
<tr>
<td>S. ratti KCTC 3294²</td>
<td>Acacetin</td>
<td>25/100</td>
<td>6.25/25</td>
<td>0.25/0.25</td>
<td>0.5/0.5</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>0.125/0.5</td>
<td>0.0313/0.125</td>
<td>0.25/0.25</td>
<td></td>
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<tr>
<td>S. criceti KCTC 3292</td>
<td>Acacetin</td>
<td>50/100</td>
<td>12.5/25</td>
<td>0.25/0.25</td>
<td>0.75/0.75</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>0.0313/0.125</td>
<td>0.0156/0.0625</td>
<td>0.5/0.5</td>
<td></td>
</tr>
<tr>
<td>S. anginosus ATCC 31412</td>
<td>Acacetin</td>
<td>200/800</td>
<td>50/200</td>
<td>0.25/0.25</td>
<td>0.5/0.5</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>0.0625/0.25</td>
<td>0.0156/0.0625</td>
<td>0.25/0.25</td>
<td></td>
</tr>
<tr>
<td>S. gordonii ATCC 10558</td>
<td>Acacetin</td>
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<td>6.25/12.5</td>
<td>0.25/0.25</td>
<td>0.5/0.5</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>0.0625/0.25</td>
<td>0.0156/0.0625</td>
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<td></td>
</tr>
<tr>
<td>A. actinomycetemcomitans ATCC 43717</td>
<td>Acacetin</td>
<td>100/200</td>
<td>50/100</td>
<td>0.5/0.5</td>
<td>1.0/1.0</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>16/32</td>
<td>8/16</td>
<td>0.5/0.5</td>
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</tr>
<tr>
<td>F. nucleatum ATCC 51190</td>
<td>Acacetin</td>
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<td>12.5/25</td>
<td>0.25/0.25</td>
<td>0.5/0.5</td>
</tr>
<tr>
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<td>Ampicillin</td>
<td>8/16</td>
<td>2/4</td>
<td>0.25/0.25</td>
<td></td>
</tr>
<tr>
<td>P. intermedia ATCC 49049</td>
<td>Acacetin</td>
<td>25/100</td>
<td>6.25/50</td>
<td>0.25/0.25</td>
<td>0.5/1.0</td>
</tr>
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<td>0.25/1</td>
<td>0.5/0.5</td>
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<tr>
<td>P. gingivalis ATCC 33277</td>
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<td>12.5/25</td>
<td>0.25/0.25</td>
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<td></td>
<td>Ampicillin</td>
<td>0.5/0.5</td>
<td>0.125/0.25</td>
<td>0.25/0.5</td>
<td></td>
</tr>
</tbody>
</table>

¹The MIC and MBC of acacetin with ampicillin ²The fractional inhibitory concentration (FIC)/ the fractional bactericidal concentration (FBC) ³The fractional inhibitory concentration index (FICI)/ the fractional bactericidal concentration index (FBCI)

¹American Type Culture Collection (ATCC) ²Korean collection for type cultures (KCTC)
Table 3: Synergistic effects of acacetin with gentamicin against oral bacteria

<table>
<thead>
<tr>
<th>Strains</th>
<th>Agent</th>
<th>MIC/MBC (µg/ml)</th>
<th>FIC/FBC</th>
<th>FICI/FBC</th>
<th>Outcome</th>
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<td>Alone Combination¹</td>
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<td></td>
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<td>50/100</td>
<td>6.25/25</td>
<td>0.125/0.25</td>
<td>0.375/0.5</td>
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<tr>
<td></td>
<td>Gentamicin</td>
<td>8/16</td>
<td>2/4</td>
<td>0.25/0.25</td>
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<tr>
<td>S. sanguinis ATCC 10556</td>
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<td>100/200</td>
<td>50/50</td>
<td>0.5/0.25</td>
<td>0.75/0.75</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>16/32</td>
<td>4/16</td>
<td>0.25/0.5</td>
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<tr>
<td>S. sobrinus ATCC 27607</td>
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<td>0.25/0.5</td>
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<td>2/4</td>
<td>0.25/0.25</td>
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<tr>
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<td>2/4</td>
<td>0.25/0.25</td>
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<td>4/8</td>
<td>0.5/0.5</td>
<td></td>
</tr>
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<td>F. nucleatum ATCC 51190</td>
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<td>12.5/25</td>
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<td>Acacetin</td>
<td>25/100</td>
<td>12.5/50</td>
<td>0.5/0.5</td>
<td>0.75/1.0</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>32/32</td>
<td>8/16</td>
<td>0.25/0.5</td>
<td></td>
</tr>
<tr>
<td>P. gingivalis ATCC 33277</td>
<td>Acacetin</td>
<td>50/100</td>
<td>12.5/25</td>
<td>0.25/0.25</td>
<td>0.5/0.5</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>256/512</td>
<td>68/128</td>
<td>0.25/0.25</td>
<td></td>
</tr>
</tbody>
</table>

¹The MIC and MBC of acacetin with gentamicin
²The fractional inhibitory concentration (FIC)/the fractional bactericidal concentration (FBC)
³The fractional inhibitory concentration index (FIC index)/the fractional bactericidal concentration index (FBC index)
⁴American Type Culture Collection (ATCC)
⁵Korean collection for type cultures (KCTC)

Figure 1: Time-kill curves of MIC of acacetin 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 acacetin (●), acacetin + Amp (○), and acacetin + Gen (▼) over time. Data points are the mean values±S.E.M. of six experiments. CFU, colony-forming units
Figure 2: Time-kill curves of MIC of acacetin alone and its combination with MIC of Amp or Gen against S. gordonii, A. actinomycetemcomitans, F. nucleatum, P. intermedia, and P. gingivalis. Bacteria were incubated with acacetin (●), acacetin + Amp (○), and acacetin + Gen (▼) over time. Data points are the mean values±S.E.M. of six experiments. CFU, colony-forming units.

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defence mechanism against prediction by many microorganisms, insects and other herbivores (Politeo et al., 2012; Madhumitha, Saral, 2011; de Paula et al., 2012; Sukumaran et al., 2011). Flavonoid complexes attach with extracellular soluble protein and with bacterial cell wall. Thus they exhibit antibacterial activity (Harborne, Williams, 1992; Mavri et al., 2012). Therefore may have a significant clinical value in treatment of resistant microbial strains (Sukumaran et al., 2011). In this study, acacetin, a flavone compound found in several plants also shows susceptibility on gram-positive bacteria as well as gram-negative bacteria. The compounds in the flavonol, flavan-3-ol and flavone classes have been shown to inhibit energy metabolism (through ATP synthase inhibition) (Crasoto et al., 2013; Wright et al., 2013). The bacterial effect of acacetin with ampicillin or gentamicin against oral bacteria was confirmed by time-kill curve experiments. The acacetin (MIC or MIC\textsubscript{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 acacetin (MIC\textsubscript{50}) with ampicillin (MIC\textsubscript{50}) or gentamicin (MIC\textsubscript{50}) (Fig 1, 2). A strong bactericidal effect was exerted in drug combinations.

Conclusion

In conclusion, these findings suggest that acacetin, 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.

Competing Interest

The authors declare that they have no competing interests.

Authors’ Contribution

Jeong MR, Choi MR, Hwang SM have substantial contributions to conception and design and drafting and revising it. Ko ES, Bang MA, Lim JY, Kang JR have substantial contributions to acquisition and analysis of data. Cha JD overall supervision and coordination of the study. Jeong US, Bu HO, Song WS, Choi KM helped in refining concept and designing of the study with critical reading of the manuscript.

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There is no conflict of interest related to this research.

**Research Highlights**

Highlight the main points of your research in 3-5. It shows how this review article is excellent compared to other available reviews.

- The acacetin 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 MIC50 and MIC90 were 6.25 µg/ml and 25 µg/ml.
- In this study, acacetin, a flavone compound found in several plants also shows susceptibility on gram-positive bacteria as well as gram-negative bacteria.
- A strong bactericidal effect was exerted in drug combinations.

**Limitations**

Please declare here. What according to you are major lacunae in this research report? That has not been analysed yet by other researchers.

Although a broad range of biological and pharmacological activities of acacetin have been reported, the mechanism(s) behind its antibacterial effects are not fully understood.

**Recommendations**

Please declare here. What are further research potentials, which need to be researched?

For medicinal purposes, the safety and toxicity of acacetin need to be addressed.

**Justification of Research**

Please declare here. It shows how this review article is excellent compared to other available reviews.

These findings suggest that acacetin, 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.

**References**


