Antimicrobial Effects of Flavone Analogues and Their Structure-Activity Relationships

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Abstract It has been well known that the use of Saccharomyces cerevisiae can cause fungemia in critically ill patients and flavone shows an antimicrobial effect on S. cerevisiae. Therefore, we have investigated the activities of thirteen flavone analogues on S. cerevisiae in our studies. Because flavonoids including flavones have antioxidative effects, we try to carry out the activity studies of flavone analogues in vitro and in vivo. In addition, the relationships between the structures of flavone analogues and their biological activities, such as antimicrobial and antioxidative effects, were elucidated using Comparative Molecular Field Analysis calculations. Of the flavone analogues tested here, 3,2′-dihydroxyflavone showed both good antimicrobial and antioxidative activities.

Keywords: Antimicrobial, flavonoid, Saccharomyces cerevisiae, Caenorhabditis elegans, antioxidative effect

From ancient times, Saccharomyces cerevisiae has been the most important yeast because of its use in baking and brewing, and one of the most studied eukaryotic organisms [4, 5, 9, 14, 17]. Furthermore, it was the first eukaryotic genome that was completely sequenced. Its genome is composed of about 13,000,000 base pairs and 6,275 genes, although only about 5,800 of these are believed to be true functional genes [3]. It is estimated that yeast shares about 23% of its genome with that of humans and reproduces by a division process known as budding. In addition, it is comparatively similar in structure to human cells, both being eukaryotic, in contrast to the prokaryotes. Apparently, many important proteins in human biology were first discovered by studying their homologues in yeast [4, 10, 11, 16].

S. cerevisiae can cause disease in humans. In particular, it is marketed as a probiotic supplement (Ultralevura) for the treatment of Clostridium difficile colitis [2]. Unfortunately, its use can cause fungemia in critically ill patients [8]. Because its usefulness is much greater than its harmfulness, however, it is still used in baking and brewing.

It has been reported that flavone, one of natural products originated from plants, showed antimicrobial effect on S. cerevisiae [13]. Flavone belongs to the flavonoids, which are found from various plant sources and composed of a C6-C3-C9 skeleton. According to the oxidation condition of the pyran ring placed at the center of flavonoids, flavonoid can be further subdivided into five major subclasses as follows: flavonols, flavanols, flavones, isoflavones, and anthocyanidins [7]. Flavonoids show many biological activities such as antioxidative effects and proliferative effects. In this study, fourteen flavone analogues were tested for their antimicrobial and antioxidative effects, and those analogues are as follows: flavone (1), 2′,3′-dihydroxyflavone (2), 2′,4′-dihydroxyflavone (3), 2′,4′-dimethoxyflavone (4), 2′-dihydroxyflavone (5), 3′,4′-dihydroxyflavone (6), 3′,4′-dimethoxyflavone (7), 3,2′-dihydroxyflavone (8), 3,2′-dimethoxyflavone (9), 3,4′-dimethoxyflavone (10), 3-hydroxy-2′-methoxyflavone (11), 3-hydroxy-3′-methoxyflavone (12), 5-hydroxyflavone (13), and 6-hydroxyflavone (14) (Fig. 1).

In order to test antimicrobial effects, Saccharomyces cerevisiae was cultured in YM broth (3 g yeast extract, 3 g malt extract, 10 g peptone, 10 g dextrose in 11 distilled water) at 30°C, 180 rpm for 24 h. One ml of cultured broth was spread on the square plate (245×245×20 mm) with Sabouraud dextrose agar. On the top of the cultured broth, paper discs (Ø 6 mm) were placed and flavone analogue samples were dropped. After 24 h at 30°C, inhibitory zones were measured. Because flavone analogues were dissolved in dimethyl sulfoxide (DMSO), it was used as a control, and kanamycin and hygromycin were tested to compare with known antimicrobial agents. The concentrations of samples and two antimicrobial agents were 100 mM and 50 mg/mL., respectively. Aliquots of 1 µl and 10 µl of each

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Sample were dropped on paper discs. All experiments were performed twice and their results were exactly consistent with each other. The results showing the inhibitory effects are listed in Table 1 and the results of their paper discs are shown in Fig. 2.

The analogue with the strongest effect was 3,2'-dihydroxy flavone (8), which showed the effect at 10 µl as well as 1 µl. In order to elucidate the relationships between the antimicrobial effects and the structures, structure-activity relationships (SAR) were carried out on a Linux Pentium 3.2 GHz PC using Comparative Molecular Field Analysis (CoMFA) calculations with a set of 14 analogues. The software for the calculation was Sybyl 7.2 (Tripos, St. Louis, MO, U.S.A.). The CoMFA contour map represents the steric field values that correlate with the biological activity and it is illustrated in Fig. 3, where the contours are shown in green (more bulk favored) and yellow (less

Table 1. The antimicrobial effects of flavone analogues.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Name</th>
<th>Antimicrobial effects (diameter/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 µl</td>
</tr>
<tr>
<td>1</td>
<td>Flavone</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>2',3'-Dihydroxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>2',4'-Dihydroxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>2',4'-Dimethoxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>2-Hydroxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>3',4'-Dihydroxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>3',4'-Dimethoxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>3,2'-Dihydroxyflavone</td>
<td>1 mm</td>
</tr>
<tr>
<td>9</td>
<td>3,2'-Dimethoxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>3,4'-Dimethoxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>3-Hydroxy-2'-methoxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>3-Hydroxy-3'-methoxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>5-Hydroxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>6-Hydroxyflavone</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Kanamycin</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Hygromycin</td>
<td>2 mm</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not detected.

Fig. 1. Structures and nomenclatures of flavone and its thirteen analogues.

Fig. 2. The antimicrobial effects of flavone analogues. The concentration of the samples was 100 mM. Aliquots of 1 µl and 10 µl of each sample were dropped on paper discs.

Fig. 3. The CoMFA contour map representing the steric field values by showing the contours in green (more bulk favored) and yellow (less bulk favored).
bulk favored). The analysis revealed two green colored contours and one yellow colored contour. The favorable green contour is seen near the C-3 position of the C-ring, so that the analogues with bulky C-3 substituents show good antimicrobial activity. Furthermore, those compounds that satisfy this requirement are as follows: 2',3'-dihydroxyflavone (8), 3,2'-dihydroxyflavone (9), 3,4'-dimethoxyflavone (10), 3-hydroxy-2'-methoxyflavone (11), and 3-hydroxy-3'-methoxyflavone (12). Another green contour is placed at the surface area of C-2', C-3', and C-4' of the B-ring. Likewise, analogues 8, 9, 10, 11, and 12 contain substituents at C-2', C-3', and C-4'. On the other hand, the yellow contour is seen near the center of B-ring, so that plane B-ring is favorable.

In order to investigate antioxidative effects of flavone analogues, 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical-scavenging effects were tested according to the methods previously reported by Ahn et al. [1]. The data are reported as the mean of three independent experiments, and were evaluated by Student's t-test. Values of \( P < 0.05 \) were considered to be statistically significant. The scavenging effect of vitamin C used as a reference was 88.0%, and the effects of 14 flavone analogues are listed in Table 2, where 2',3'-dihydroxyflavone (2) showed statistically significantly (\( P < 0.05 \)) good antioxidative effects. In order to elucidate structure-activity relationships, SAR was performed as the method mentioned above. The analysis of the electrostatic contours revealed three red contours (electronegative substituents favored), which were near the 3-hydroxyl plane B-ring is favorable. Likewise, analogues 2, 6, and 8 could exhibit good activity. Even though dihydroxylated flavone analogues showed good activities, the position of dihydroxyl groups was important. 2',4'-Dihydroxyflavone contains two hydroxyl groups, but it showed worse activity than 2, 6, and 8. Therefore, it seems that ortho dihydroxyl groups exhibit good activity, whereas meta dihydroxyl groups do not. Among 14 flavone analogues tested in this study, analogue 2, 2',3'-dihydroxyflavone satisfying the conditions mentioned above, showed the best scavenging effect for DPPH radicals (85.8%).

To evaluate in vivo antioxidative activities of flavone analogues, Caenorhabditis elegans was used. The experimental procedure followed the methods reported previously [12, 15]. Because flavone was dissolved in DMSO, worms were also grown on plates containing 0.5% DMSO. The activities of several flavone analogues tested in C. elegans were detected by examining survival rates of worms after treatment with paraquat (1,1'-dimethyl-4-4'-bipyridinium dichloride hydrate; Sigma-Aldrich, St. Louis, MO, U.S.A.) to generate oxidative stress. The survival rate of worms grown in normal plate treated in 0.5% DMSO was 26.1%. According to the analysis of the oxidative stress study, the survival rate was influenced by the nature and positions of substituents around the B-ring in flavone analogues and the results are as follows: analogue 2 with ortho dihydroxyl groups, 2,3-fold; analogue 3 with meta dihydroxyl groups, 1.5-fold; analogue 12 with one hydroxyl group and one methoxyl group, 1.4-fold; and analogue 4 with dimethoxyl groups, almost 0-fold increase in comparison with the survival rate in normal plates. As a result, the in vivo antioxidative activities are consistent with in vitro activities.

Consequently, the antimicrobial activity of 3,2'-dihydroxyflavone on S. cerevisiae can compete with that of hygromycin, and the DPPH radical-scavenging effect of 2',3'-dihydroxyflavone also can compete with that of vitamin C. Therefore, 3,2'-dihydroxyflavone has both good antimicrobial and antioxidative activities.

![Fig. 4. CoMFA contour specification showing electrostatic sites (Red contours: negative potential is favored).](image-url)
Acknowledgments

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