Effect of *Bauhinia championii* (Benth.) Benth extract on *Streptococcus mutans* in vitro.

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Abstract

The *in vitro* antibacterial activity of ethanol extract from *Bauhinia championii* (Benth.) Benth growing in Guangxi, China, was evaluated against *S. mutans* using twofold agar dilution method. The present study showed that minimal inhibitory concentration was 12.5 mg/mL and there have significant inhibitory activity of adherence and acidogenicity against *S. mutans* when the extract concentration was greater than or equal to 3.13 mg/mL. These results indicate that *Bauhinia championii* (Benth.) Benth extract can significantly inhibit the growth, adherence and acidogenicity of *S. mutans* and might be used in the area of dental caries prevention and treatment in the future.

Keywords: *Bauhinia championii* (Benth.) Benth extract, *Streptococcus mutans*, Inhibit

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Introduction

The use of medicine plants and their preparations to treat diseases is an age-old practice and in the past possibly the only method available. *Bauhinia championii* (Benth.) Benth is a Lianas woody of Bauhinia, Leguminosae family, as a morphologically variable species, its medicinal part is the dry stem [1]. *Bauhinia championii* (Benth.) Benth is used traditionally for various medicinal purposes which show wide pharmacological effects [2,3]. It has been used in rheumatoid arthritis, the pains in waist and legs, bruises, stomachache and so on as a traditional medicine of ethnic minorities in China [4,5].

Extract from this plant has demonstrated antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Acinetobacter baumannii* [1]. Phytochemical studies have shown that its active components include volatile oil, quercetin, polysaccharide, alkaloids, gallic acid and flavones, etc [6,7]. Among these, alkaloids, gallic acid and flavones have been shown to the most potent to inhibit *Streptococcus mutans* (*S. mutans*) [8,9].

However, there are no reports of biological investigations to *S. mutans* carried out on the extract. *S. mutans* is the main cariogenic bacteria, dental caries is fundamentally a microbial disease which involves the destruction of hard tissue of tooth. The present study therefore, attempts to evaluate the effects of the extract from *Bauhinia championii* (Benth.) Benth growing in Guangxi, China on growth, adherence and acidogenicity of *S. mutans*, explore its cariostatie effect. Aim is to prove that *Bauhinia championii* (Benth.) Benth may be employed as a new caries-preventing natural medicine plants.

Materials and Methods

Plant materials

*Bauhinia championii* (Benth.) Benth was collected during the month of October 2015 from Jinxiu, Guangxi, China. Then the plant sample was shade dried at temperature 23-26°C and ground to a fine powder (60 meshes) with a grinder for herbal medicine and stored in airtight container.

Preparation of extracts

The powdered plant material (50 g) were macerated with 1000 ml 70% ethanol at 23-26°C temperature standing for 24 h, and then subjected to stirring at 65°C for 100 min according to the literature [10]. The extract filtered and evaporated to dryness under reduced pressure (at 50°C temperature) to obtain dry extract. The dry extract was stored in refrigerator at -18°C until use.
Bacterial strains and culture conditions

Bacterial strains used in this study were *S. mutans* ATCC 25175 (Guangdong Microbiology Culture Center, China). The bacterial were dissolved according to instruction, were cultivated in Trypticase Soy Broth (TSB) for 3 h (37°C, 90% N₂, 5% CO₂, 5% H₂), then cultivated in Modified Scholtens’Broth (MSB) for 48 h (37°C, 90% N₂, 5% CO₂, 5% H₂). Colonies picked in normal sodium and centrifuged 15 min, washed twice with sterilized normal sodium [11]. At last adapted the Optical Density (OD) value at 540 nm to 1.0, and then used bacteria count board to calculate the concentration of bacteria was approximately 0.9 × 10⁸ Colony Forming Units (CFU)/ml.

Evaluation of the effect of extract on growth of *S. mutans*

Minimal Inhibitory Concentration (MIC) was determined by twofold dilution method. A series of two fold dilutions of different concentrations extracts ranging from 50.0 mg/ml to 1.56 mg/ml were done in TPY liquid medium, *S. mutans* (0.9 × 10⁸ CFU.mL⁻¹, 0.1 mL) were added to each culture tube containing serially diluted test extract TPY liquid medium (2.0 ml) cultivated for 48 h (37°C, 90% N₂, 5% CO₂, 5% H₂) respectively [12]. The Minimal inhibitory concentration of the extract that produced no visible *S. mutans* growth with visual inspection and OD540 values was recorded as the MIC. As the control group, there is no extract in TPY liquid medium.

Evaluation of the effect of extract on adherence of *S. mutans*

According to the MIC and to avoid false-positive, extract at concentrations of 1/2 MIC, 1/4 MIC, 1/8 MIC, 1/16 MIC and 1/32 MIC were investigated respectively [13,14] with some modifications. A total of 9.3 g of concentrated crude ethanol extract was obtained from 50 g of *Bauhinia championi* (Benth.) Benth., showing a yield of 18.6% from dried fine powder.

Influence of the extract on growth of *S. mutans*

In this study, the growth inhibition test of *S. mutans* for the extract was investigated and the results are shown in table 1. There is no *S. mutans* growth when the concentration was greater than or equal to 12.5 mg/mL and an increased extract concentration can obviously improve the inhibitory ability. As the control group, there is no extract in TPY liquid medium.

**Table 1. Inhibition of different concentration extract against *S. mutans*, values expressed as means ± SD (n=3).**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>OD540 values</th>
<th>P-values (Compared with the control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.0 mg/mL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25.0 mg/mL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.5 mg/mL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.25 mg/mL</td>
<td>0.59 ± 0.03</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>3.13 mg/mL</td>
<td>0.65 ± 0.03</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Control group</td>
<td>0.73 ± 0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

Influence of the extract on adherence of *S. mutans*

The results of evaluating the effect of extract on *S. mutans* adherence to glass surface are shown in table 2. We found in this study that with the extract concentration increases, the OD value of *S. mutans* suspension becomes small. *S. mutans* adhesion to glass surface significantly lower compared with the
Effects of Bauhinia championii (Benth.) benth extract on Streptococcus mutans in vitro

control group (p<0.01) when the concentration of the extract was greater than or equal to 3.13 mg/mL.

Table 2. Effect of different concentration extract on adherence of S. mutans, values expressed as means ± SD (n=3).

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>OD540 values</th>
<th>P-values (Compared with the control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>0.51 ± 0.03</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>3.13</td>
<td>0.57 ± 0.06</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>1.56</td>
<td>0.66 ± 0.03</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>0.78</td>
<td>0.73 ± 0.06</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>0.39</td>
<td>0.75 ± 0.08</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Control group</td>
<td>0.81 ± 0.03</td>
<td>-</td>
</tr>
</tbody>
</table>

However, there was no significant difference in adherence compared with the control group when the concentration of the extract was less than or equal to 0.39 mg/mL (p>0.05). Results of the present study show the extract was able to significantly inhibit S. mutans adherence and the inhibit S. mutans adherence is more significantly with the extract concentration increased.

Influence of the extract on acidogenicity of S. mutans

The results of examining the inhibitory effects of extract on production of acid by S. mutans are shown in table 3.

Table 3. Effect of different concentration extract on acidogenicity of S. mutans, values expressed as means ± SD (n=3).

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>ΔpH values</th>
<th>P-values (Compared with the control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>0.51 ± 0.06</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>3.13</td>
<td>1.51 ± 0.08</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>1.56</td>
<td>3.43 ± 0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>0.78</td>
<td>3.60 ± 0.07</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>0.39</td>
<td>3.75 ± 0.05</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Control group</td>
<td>3.88 ± 0.08</td>
<td>-</td>
</tr>
</tbody>
</table>

In the present investigation, all the tested extract samples exhibited inhibitory effects on the acidogenicity of S. mutans. The terminal pH of the TPY liquid medium in test extract group dropped to significant differences compared with the control group after 48 h incubation when the concentration of the extract was greater than or equal to 3.13 mg/mL (p<0.01). However, there was no significant difference in statistically that the pH reduction compared with the control group when the concentration of the extract was less than or equal to 0.78 mg/mL (p>0.05), although the reduction of the pH in the tested extract groups were marginally lower than the control group. We found in this study that the extract inhibited the pH reductions was more significantly with the extract concentration increased too.

Discussion

Dental caries is the common microbial disease, which involves the hard tissue of tooth. S. mutans is one of the dominating and resident germs of oral, also is the dominating germ factor of the occurrence of caries [17]. S. mutans can growth, adherence and acidogenicity at tooth surfaces and further to lead to demineralization. Plant extracts possess a series of bioactive compounds that use of plants and their preparations to treat diseases has been extensively studied based on their biological activities. The recent researchers have discovered some natural medicine plants, such as Mangnolia officinalis, Galla chinesis, etc., also can inhibit the growth, adherence and acidogenicity of S. mutans, and have significant anticiaries effects in vitro [18-20]. The MIC of the plant extracts ranged from 3.13 mg/mL to 100.00 mg/mL. Due to the significantly inhibit S. mutans and their stability character, some plants were development as oral health care agent for the prevention of dental caries.

We evaluated the antibacterial activity of Bauhinia championii (Benth.) Benth extract on one of important oral bacteria S. mutans in our study with the aim to prevent dental caries. The present study showed that minimal inhibitory concentration was 12.5 mg/mL and there have good inhibitory activity of adherence and acidogenicity against S. mutans when the extract concentration was greater than or equal to 3.13 mg/mL. Based on these findings, showed that Bauhinia championii (Benth.) Benth extract have good antibacterial activity, significantly inhibited the growth of S. mutans as well as the adherence and acidogenicity of the bacteria. The significant anti-S. mutans activity of the extract suggests that it is a good candidate for further development as a valuable and economic oral care agent for the prevention of dental caries.

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References


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