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 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 cariostatic 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 bacteria 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 × 10^8 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 × 10^8 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. Glass surface adherence assay was evaluated according to the previously described method [13,14] with some modifications. S. mutans (0.9 × 10^8 CFU.mL^-1) were grown at an angle of 30°C in glass test tubes for 48 h (37°C, 90% N2, 5% CO2, 5% H2) with 10 mL of TPY liquid medium containing 1% sucrose and extract. After incubation, supernatants were removed from the test tube, which were then gently washed three times with distilled water. The adhered bacteria were resuspended into 0.1 mol/L NaOH solution by shocking with a sonic oscillator, the supernatant were removed by centrifuging at 3500 rpm for 15min, suspended in normal sodium and measured the OD value at 540 nm. As the control group, there is no extract in TPY liquid medium.

Evaluation of the effect of extract on acidogenicity of S. mutans

The effect of extract on acidogenicity of S. mutans (0.9 × 10^8 CFU.mL^-1) was evaluated by a pH drop. S. mutans was grown in TPY liquid medium containing 1% sucrose and test extract at concentrations of 1/2 MIC, 1/4 MIC, 1/8 MIC, 1/16 MIC and 1/32 MIC respectively [15,16]. The starting pH of the mixtures was adjusted to 7.4. After incubation 48 h (37°C, 90% N2, 5% CO2, 5% H2), measured terminal pH of supernatants and calculated pH reduction. As the control group, there is no extract in TPY liquid medium.

Statistical analysis

Data for antibacterial activity are expressed as mean ± SD for analysis performed in triplicate. The mean values and standard deviation were calculated with the Excel program from Microsoft Office 2003 package.

Results

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 the number of S. mutans significant increase with the concentration decrease, they were concentration dependent. Therefore, the MIC of extract on S. mutans was 12.5 mg/mL according to determining by tube dilution test. There was significant difference of OD value between the 6.25 mg/mL and control group (P<0.05), showed this concentration was able to significantly inhibit S. mutans. While the concentration was 3.13 mg/mL, the extract inhibited the growth of S. mutans but it is no significant (P>0.05). That is, the growth of S. mutans significantly inhibited when the extract concentration greater than or equal to 6.25 mg/mL and an increased extract concentration can obviously improve the inhibitory ability.

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
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</th>
<th>ODS540 values</th>
<th>P-values (Compared with the control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25 mg/mL</td>
<td>0.51 ± 0.03</td>
<td>P=0.01</td>
</tr>
<tr>
<td>3.13 mg/mL</td>
<td>0.57 ± 0.06</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>1.56 mg/mL</td>
<td>0.66 ± 0.03</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>0.78 mg/mL</td>
<td>0.73 ± 0.06</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>0.39 mg/mL</td>
<td>0.75 ± 0.08</td>
<td>P&lt;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</th>
<th>ΔpH values</th>
<th>P-values (Compared with the control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25 mg/mL</td>
<td>0.51 ± 0.06</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>3.13 mg/mL</td>
<td>1.51 ± 0.08</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>1.56 mg/mL</td>
<td>3.43 ± 0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>0.78 mg/mL</td>
<td>3.60 ± 0.07</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>0.39 mg/mL</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 Magnololia officinalis, Galla chinesis, etc., also can inhibit the growth, adherence and acidogenicity of S. mutans, and have significant antacaries 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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