Biomedical Research 2016; 27 (3): 758-761

ISSN 0970-938X
www.biomedres.info

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

Xudong Jiang[1,2,3], Weihao Yang[4], Chuanjian Zhou[4], Kailing Lu[4], Qingyun Qin[4], Cuiwu Lin[1,3*]

[1]School of Chemistry and Chemical Engineering, Guangxi University, Nanning, 530004, PR China

[2]School of Medicine, Guangxi University of Science and Technology, Liuzhou, 545005, PR China

[3]Guangxi Colleges and Universities Key Laboratory of Applied Chemistry Technology and Resource Development, Nanning, 530004, PR China

[4]Postdoctoral Workstation, Liuzhou LMZ Co., Ltd. Liuzhou, 545006, PR China

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

Accepted on February 25, 2016

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 aereus*, 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% N_2, 5% CO_2, 5% H_2) , then cultivated in Modified Scholtens'Broth (MSB) for 48 h (37°C, 90% N_2, 5% CO_2, 5% H_2). 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% N_2, 5% CO_2, 5% H_2) 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% N_2, 5% CO_2, 5% H_2) 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% N_2, 5% CO_2, 5% H_2), 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 l.

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.

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

Concentration	OD540 values	P-values (Compared with the control group)
50.0 mg/mL	-	-
25.0 mg/mL	-	-
12.5 mg/mL	-	-
6.25 mg/mL	0.59 ± 0.03	P<0.05
3.13 mg/mL	0.65 ± 0.03	P>0.05
Control group	0.73 ± 0.05	-

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).*

Concentration	OD540 values	P-values (Compared with the control group)
6.25 mg/mL	0.51 ± 0.03	P<0.01
3.13 mg/mL	0.57 ± 0.06	P<0.01
1.56 mg/mL	0.66 ± 0.03	P<0.05
0.78 mg/mL	0.73 ± 0.06	P<0.05
0.39 mg/mL	0.75 ± 0.08	P>0.05
Control group	0.81 ± 0.03	-

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).*

Concentration	ΔpH values	P-values (Compared with the control group)
6.25 mg/mL	0.51 ± 0.06	P<0.01
3.13 mg/mL	1.51 ± 0.08	P<0.01
1.56 mg/mL	3.43 ± 0.05	P<0.05
0.78 mg/mL	3.60 ± 0.07	P>0.05
0.39 mg/mL	3.75 ± 0.05	P>0.05
Control group	3.88 ± 0.08	-

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 anticaries 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.

Acknowledgement

Authors thank for Liuzhou LMZ Co., Ltd, China for financial and plant material support and Prof. Guangwei Huang for authentication of plant material.

References

1. Zhang YQ, Xu W, Li H, Chen LD, Zhu KD. Study progress in Bauhinia championi (Benth.) Benth of Chinese herbal medicine. Asia Pacific Traditional Med 2012; 8: 207-209.
2. Li HT, Li XH, Liu GZ, Chen JS, Weng XP, Liu FY, Xu HF, Liu XX, Ye HZ. Bauhinia championi (Benth.) Benth polysaccharides upregulate Wnt/β-catenin signaling in chondrocytes. Int J Mol Med 2013; 7: 1624-1630.
3. Cai LL, Ye HZ, Yu FR, Li HT, Chen JS, Liu XX. Effects of Bauhinia championii (Benth.) Benth polysaccharides on the proliferation and cell cycle of chondrocytes. Mol Med Rep 2013; 32: 1329-1336.
4. Zhang YQ, Chen LD, Xu W, Li H, Zhang X, Xia YF, Chu Kd. Therapeutic effects of total alkaloids of Tripterygium wilfordii Hook f. on collagen-induced arthritis in rats. J Ethnopharmacol 2013; 145: 699-705.

5. Yi JL, Zhang JJ, Zhou YS, Wang S, Zhao YH. Study on the anti-inflammatory and analgesic effects of Bauhinia championii Benth extract in mice. J Guangdong Pharm Univ 2012; 28: 647-651.
6. Xu W, Zheng HY, Hong ZF, Tang XS. RP-HPLC determination of four flavonols in Bauhinia championii Benth. Chin J Mod Appl Pharm 2009; 26: 763-766.
7. Xu W, Li H, Chu KD, Zhang YQ, Chen LD, Sha M. Studies on Chemical Constituents of Bauhinia championii (Benth.) Benth. Nat Prod Res Dev 2013; 25: 1209-1211.
8. Prabu GR, Gnanamani A, Sadulla S. Guaijaverin-a plant flavonoid as potential antiplaque agent against Streptococcus mutans. J Appl Microbiol 2006; 101: 487-495.
9. Kang MS, Oh JS, Kang IC, Hong SJ, Choi CH. Inhibitory effect of methyl gallate and gallic acid on oral bacteria. J Microbiol 2008; 46: 744-750.
10. Lin LW, Zhou YS, Zhou Z, Meng J, Sun ZS. Extraction, Separation and Determination of Total Flavonoids from Bauhinia chaampioni Based on Pharmacodynamics Tracking Test. Chinese J Exp Trad Med Formul 2015; 21: 103-106.
11. Batista MT, Souza RD, Ferreira EL, Robinette R, Crowley PJ, Rodrigues JF. Immunogenicity and in vitro and in vivo protective effects of antibodies targeting a recombinant form of the Streptococcus mutans P1 surface protein. Infect Immun 2014; 82: 4978-4988.
12. He JZ, Wang SD, Wu TX, Cao YP, Xu X, Zhou XD. Effects of ginkgoneolic acid on the growth, acidogenicity, adherence, and biofilm of Streptococcus mutans in vitro. Folia Microbiol 2013; 58: 147-153.
13. Rahim Z, Khan H. Comparative studies on the effect of Crude Aqueous (CA) and solvent (CM) extracts of clove on the cariogenic properties of Streptococcus mutans. J Oral Sci 2006; 48: 117-123.
14. Limsong J, Benjavongkulchai E, Kuvatanasuchati J. Inhibitory effect of some herbal extracts on adherence of Streptococcus mutans. J Ethnopharmacol 2004; 92: 281-289.
15. Xiao J, Liu Y, Zuo YL, Li JY, Ye L, Zhou XD. Effects of nidus vespae extract and chemical fractions on the growth and acidogenicity of oral microorganisms. Arch Oral Biol 2006; 51: 804-813.
16. Joycharat N, Thammavong S, Limsuwan S, Homlaead S, Voravuthikunchai SP, Yingyongnarongkul B. Antibacterial substances from albizia myriophylla wood against cariogenic Streptococcus mutans. Arch Pharm Res 2013; 36: 723-730.
17. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986; 50: 353-380.
18. Jin SH, MA B, Zhou JG, Li ZQ, Bao GJ. The research progress on Chinese herbs in preventing dental caries. Chinese J Microecol 2014; 26: 234-238.
19. Yang L, Lan H. Research Progress on Varieties of Natural Medicines for Common Oral Cariogenic Bacteria. Liaoning J Trad Chinese Med 2015; 42: 901-905.
20. Huang GW, Hu YZ, Huang LY. Feasibility Study of Chinese herbs in preventing dental caries. Oral Care Industry 2014; 24: 42-44.

***Correspondence to:**

Cuiwu Lin

School of Chemistry and Chemical Engineering

Guangxi University

PR China