

Antibacterial Effect of Flavonoids from *Lophatherum Gracile Brongn* on *Streptococcus*, the Main Component of Dental Plaque

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Abstract

To study the inhibitory activity of flavonoids extracts from *Lophatherum Gracile Brongn* against the microorganisms mainly composed of dental plaque. Ethanol reflux was used to extract flavonoids from *Lophatherum Gracile Brongn*. *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus saliva* and β -hemolytic *Streptococcus* were determined by microbroth dilution method Minimum inhibitory concentration (MIC) of major dental plaque associated microorganisms. Flavonoids from *Lophatherum Gracile Brongn* had antibacterial effects on four kinds of bacteria, among which the inhibitory activities against *Streptococcus mutans* and *Streptococcus salivarius* were significantly stronger than those against *Streptococcus sanguis* and β -hemolytic *Streptococcus*. The inhibitory activity of flavonoids from *Lophatherum Gracile Brongn* against *Streptococcus mutans* and *Streptococcus salivary* was significant, which could be used to improve oral health.

Keywords

Lophatherum Gracile Brongn; Flavonoids; Oral streptococcus; Bacteriostatic effect.

1. Introduction

Dental caries and periodontitis are common oral diseases. Dental plaque is the initiating factor of dental caries and periodontitis. Dental plaque is a bacterial biofilm attached to the tooth surface, dental space or surface of the repair body, containing a variety of aerobic bacteria, anaerobic bacteria. *Streptococcus mutans*, *Streptococcus salivary*, *streptococcus minor* and *Streptococcus haematae* accounted for a large proportion of anaerobic streptococcus in human dental plaque, and *Streptococcus mutans* was the dominant bacterium causing caries. Therefore, inhibition of dental plaque mainly composed of streptococcus has the opportunity to inhibit the formation of dental plaque, thus effectively preventing caries and periodontal disease [1-3].

As a kind of traditional Chinese medicine, *Lophatherum Gracile Brongn* are often used to prevent and treat oral diseases in clinic. *Lophatherum Gracile Brongn* have good anti-inflammatory, analgesic and detoxifying effects [4-6]. China is rich in traditional Chinese medicine resources, and the toxic and side effects of traditional Chinese medicine are small. It is a hot research topic to find safe and effective anti-caries drugs from traditional Chinese medicine.

At present, there are a lot of researches on oral pathogenic bacteria caused by Traditional Chinese medicine, most of which have definite antibacterial effect. However, as a traditional Chinese medicine that can treat oral diseases, the researches on oral pathogenic bacteria caused by *Lophatherum Gracile Brongn* are very limited. The combined use of *Lophatherum Gracile Brongn* and other Chinese herbs can inhibit streptococcus mutans and so on. Therefore, this study will study the in vitro inhibitory activity of *Lophatherum Gracile Brongn* against streptococcus, the main component of four kinds of dental plaque, in order to further study

whether *Lophatherum Gracile Brongn* have the inhibitory activity of dental plaque formation, so as to provide scientific basis for the prevention of dental caries or periodontal disease.

2. Materials and Methodst

2.1. Cells, Media and Drugs

Streptococcus mutans (ATCC 25175), *Streptococcus sanguinis* (ATCC 49295), *Streptococcus salivarius* (ATCC 7073) and β -hemolytic *Streptococcus* (ATCC 21059) were purchased from ATCC. BHI liquid medium purchased from Qingdao Haibo Biotechnology Co., LTD. *Lophatherum Gracile Brongn* purchased from Beijing Tongren Tang Pharmacy.

2.2. Drug Preparation

Lophatherum Gracile Brongn were extracted by solvent method. The dried *Lophatherum Gracile Brongn* were crushed, and 50g *Lophatherum Gracile Brongn* were ground with an electronic mill. The 50g *Lophatherum Gracile Brongn* were extracted with 50% ethanol, 10 times the drug weight and volume, at reflux for 2 hours each time. The extracted solution was combined and condensed into 150ml extract under reduced pressure. Finally, the dried extract is stored in the refrigerator for further use. The stock solution is divided equally and frozen at -20°C . Avoid the freeze/thaw cycle.

2.3. Preparation of Bacterial Suspension

After resuscitating for 48h, each strain was inoculated in BHI agar medium and incubated in 37°C constant temperature incubator for 24h. Bacteria were inoculated in BHI liquid medium and prepared into 0.5 MCS of liquid bacteria (1.5×10^8 / mL), and diluted 1 000 times with BHI liquid medium to form suspension.

2.4. Drug Sensitive Test

Drug sensitivity was assessed using disk diffusion method. In short, bacterial suspensions from agarose solution were inoculated on BHI AGAR plates and the gel was allowed to cure completely at room temperature. The drug-infused Whatman filter tray was placed on a tablet and cultured for 24 hours. Drug sensitivity was assessed using a linear fit of the square radius of the inhibition zone.

2.5. Determination of Minimum Inhibitory Concentration

Take 96-well plates and label streptococcus on the left of each row. Take the first row as an example, the 11th well from left to right was used as "BHI liquid medium + positive control", labeled as "+", and the 12th well was labeled as "BHI liquid medium negative control", labeled as "-". Add tohosh extract (10mg /mL) 200 μL to the first well, add BHI liquid medium 100 μL to each well from the 2nd to the 12th well. Use a pipette gun to take 100 μL extract from the 1st well to the 2nd well, mix well, then proceed to the 10th well, discard 100 μL mixture, achieve the purpose of double dilution of drug extract, make the drug concentration from the 1st to the 10th well 5 mg/ml, 2.5mg /mL, 1.25 mg/mL, 0.625 mg/mL, 0.312 mg/mL, 0.156 mg/mL, 0.078 mg/mL, 0.039 mg/mL, 0.0195 mg/mL, 0.001 mg/mL. And so on, each bacterium was tested with four rows of holes. Add 100 μL bacterial suspension corresponding to the labeled bacteria in each row to the 1st to 11th Wells of each row. Finally, the 96-well plate is cultured in 37°C constant temperature incubator for 18-24h. Repeat the experiment 3 times to observe the phenomenon. The MIC of the antibacterial drug to the tested bacteria is the lowest drug concentration that can completely inhibit bacterial growth in the micropore.

3. Results

After 3 independent repeated tests, the MIC and mean values of flavonoids from *Lophatherum Gracile Brongn* to 4 kinds of microorganisms that are the main components of dental plaque were measured, as shown in Table 1. It can be seen that the flavonoids from *Lophatherum Gracile Brongn* have antibacterial effects on several main streptococcus components of dental plaque, among which, the inhibitory effect on streptococcus mutans and streptococcus salivary is the most obvious, and the MIC can reach about 0.2mg/mL, which is significantly better than the effect on *Streptococcus sanguis* and β -hemolytic *Streptococcus*, and the MIC of the latter two is greater than 1mg/mL.

Table 1. MIC of flavonoids from *Lophatherum Gracile Brongn* on four main streptococcus species in dental plaque

Strain	MIC (mg/mL)
<i>Streptococcus mutans</i>	0.19 $\pm$ 0.03
<i>Streptococcus sanguinis</i>	0.21 $\pm$ 0.02
<i>Streptococcus salivarius</i>	1.23 $\pm$ 0.13
Beta hemolytic <i>Streptococcus</i>	1.09 $\pm$ 0.11

4. Conclusion

Dental plaque biofilm is the initiating factor of dental caries and periodontal disease, which are the two most common oral diseases. Four kinds of streptococcus in this study are common streptococcus in dental plaque biofilm species, among which *Streptococcus mutans* and *Streptococcus haematae* play a very important role in maintaining the ecological balance of dental plaque. *Streptococcus mutans* adhere to the smooth tooth surface by producing extracellular polysaccharide, and its acid production and

Acid resistance makes it an important caries-causing bacterium. Beta hemolytic streptococcus is highly pathogenic and often causes many diseases in humans and animals. Therefore, it is of practical significance to use these four streptococcus species as experimental strains for the study of anti-dental plaque formation or anti-caries drugs.

In the early stage, antibiotics were used to prevent or treat caries and periodontal diseases, but the long-term use of antibiotics can cause the imbalance of oral flora, resulting in other diseases. In recent years, it has been found that drug resistance and bacteria detection rate of oral bacteria are increasing year by year. Therefore, it is very necessary to find new drugs with less toxic and side effects. A lot of Chinese traditional herbal medicines have the functions of clearing heat and detoxifying, bacteriostatic and detoxifying, and the side effects are small. If we can select a Chinese herbal medicine that can inhibit the four main streptococcus species of dental plaque, it will provide an important way to delay or inhibit the formation of dental plaque.

Lophatherum Gracile Brongn have the functions of clearing heat and detoxifying, anti-cancer and anti-oxidation, and the flavonoid extract *Lophatherum Gracile Brongn* has good bactericidal activity. As a common Chinese herbal medicine, *Lophatherum Gracile Brongn* have been used to treat oral diseases since ancient times. It can be seen that *Lophatherum Gracile Brongn* have good antibacterial properties and have good inhibitory activity against some oral pathogens. In this study, flavonoids extract from *Lophatherum Gracile Brongn* was used as experimental reagent to study the inhibitory activity of 4 kinds of streptococcus that constitute dental plaque. The results showed that flavonoids from *Lophatherum Gracile Brongn* had significant inhibitory effect on *Streptococcus mutans* and *Streptococcus salivary*, and the minimum inhibitory concentration was 0.19 mg/mL. It has good inhibitory concentration to

streptococcus haematae and β -hemolytic streptococcus, and the minimum inhibitory concentration can reach 1.09 mg /mL. *Lophatherum gracile Brongn* are widely used in China, and have the advantages of small toxicity and side effects, abundant resources and low cost. The results of this study showed that the flavonoids from *Lophatherum gracile Brongn* had good inhibitory activity against streptococcus, which is mainly composed of four kinds of dental plaque, and it has great development potential as a drug for the prevention and treatment of dental caries or periodontal disease. However, the formation of dental plaque is a complex process, and dental caries and periodontal disease are also multi-factor diseases. Only studying the inhibitory activity of drugs against some specific bacteria can not prove their value as drugs. The influence of different extraction methods on the flavonoid extract of *Lophatherum gracile Brongn*, the influence of long-term use of flavonoids from *Lophatherum gracile Brongn* on the ecological balance of oral flora, and whether the inhibition of these four streptococcus has an inhibitory effect on the formation of dental plaque, all these issues need further exploration and research.

References

- [1] Tinanoff, N. (2019). Dental Caries.
- [2] Golubovi, L. , Selimovi-Draga, M. , Kobalija, S. , & Huseinbegovi, A. . (2020). The role of the pediatricians in dental caries prevention in montenegro-the knowledge, attitude and practice. *Balkan Journal of Dental Medicine*, 24(1), 29-37.
- [3] Xiao, Guo, Shiyu, Liu, Xuedong, & Zhou, et al. (2019). Effect of d-cysteine on dual-species biofilms of streptococcus mutans and streptococcus sanguinis. *Scientific reports*.
- [4] Xu, Y. , Tan, L. , Sun, B. , Lei, M. , & Zhao, Y. . (2020). Memristive effect with non-zero-crossing current-voltage hysteresis behavior based on ag doped lophatherum gracile brongn. *Current Applied Physics*, 20(4), 545-549.
- [5] Huang, S. J. , Yin, A. W. , Gong, D. , & Li, T. F. . (2015). Anti-aging effects of polysaccharides from lophatherum gracile brongn. *Modern Food ence and Technology*.
- [6] Lai, K. H. , Chen, P. J. , Chen, C. C. , Yang, S. H. , & Hwang, T. L. . (2020). *Lophatherum gracile brongn*. attenuates neutrophilic inflammation through inhibition of jnk and calcium. *Journal of Ethnopharmacology*, 264, 113224.