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Effect of Polyphenol Extract of Artemisia Selengensis on Acid Production of Streptococcus Mutans

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Abstract

Using two different methods, such as water extraction and alcohol extraction, the antibacterial active component of Artemisia Selengensis polyphenols was extracted. Through MIC value, cell growth curve and other methods, the antibacterial properties of Artemisia Selengensis polyphenols extracts on gingival porphyria and Streptococococcus gingivalis and their impact on the biological membrane of pathogenic bacteria were analyzed, researched. The results showed that Artemisia extract showed certain antibacterial activity on Porphyria gingival Porphyria monas and Streptococcus morphosis, inhibiting acid production and inhibiting its bacterial adhesion.

Keywords

Artemisia Selengensis, Polyphenol; Streptococcus Mutans.

1. Introduction

According to statistics, the oral prevalence rate of the Chinese population reaches more than 80%, and seriously threatens the population of all ages, oral care is imminent. Common oral problems include dental caries and periodontal disease [1-3]. According to relevant studies, dental caries is an oral bacterial disease caused by multiple factors, manifested as enamel demineralization. Cariogenic bacteria, diet, host susceptibility and time accumulation are four important factors leading to the development of dental caries. In particular, cariogenic bacteria metabolize carbohydrates and promote biofilm formation, acid production and adaptation to complex environmental stresses, which lead to the development and progression of caries. The characteristics of Streptococcus mutans group make it recognized as the main cariogenic bacteria. The existence of dental caries will further destroy the dental hard tissue and its surrounding soft tissues, not only affecting the mastication, language and aesthetic function, but also causing social disorders and even psychological diseases; in addition, if it is ignored, these diseases lead to or exacerbate systemic diseases such as coronary heart disease, diabetes, etc., endangering systemic health and affecting the quality of life.

The simplest way to prevent oral diseases is to pay attention to oral hygiene, and conventional methods include mechanical methods (toothbrushes and dental floss) and the use of oral cleansers with antibacterial function. Clinical oral care often gives patients normal saline and other mouthwashes to clean the oral cavity, but when the body's low resistance of germs will multiply, causing halitosis; become a secondary/complication of infection, aggravate the condition, or even become the cause of cross infection in hospitalized patients. At present, the oral cleanser chemical agents reported in the literature, or a few mixed prescriptions of traditional Chinese and western medicine, have more toxic and side effects and should not be used for a long time. A large number of research works have shown that plant polyphenols have good effects in many aspects such as anti-mutagenesis, anti-tumor, antiviral, anti-microbial, and

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anti-aging. The physiological activity of plant polyphenols is often a comprehensive embodiment of their chemical activity. These physiological activities are not only related to the complex reactions of polyphenols with proteins (enzymes), alkaloids, biological macromolecules and polysaccharides, but also closely related to the free radical scavenging and antioxidant properties of polyphenols [4]. Plant polyphenols are an important source of natural medicines in traditional medicine, which are safer than chemical agents, have less toxic side effects and irritation, and are not easy to cause human microecosystem disorders [5]. Artemisia Selengensis is a special vegetable resource rich in southern China. Studies have shown that it has antioxidant, antibacterial, hemostatic, anti-inflammatory and other effects. Therefore, the cariogenic effect of Artemisia Selengensis polyphenol on Streptococcus mutans was determined by analyzing its MIC value, MBC value and inhibitory effect on bacterial cell growth curve.

2. Material and Method

2.1. Preparation of Artemisia Multiphenols

Fresh Artemisia Selengensis leaves were dried and ground into powder, and 10 g of powder from Artemisia Selengensis leaves was weighed and added with 90% ethanol at a ratio of 1:30, extracted with ultrasound at 160W and 50 for 80 min, and then centrifuged at 4000 r/min for 5 min to collect the supernatant. The extraction was repeated three times, and finally the supernatant was combined and concentrated under vacuum at 50 °C, and finally the volume was made to 50mL for later use. To prevent chemical changes in the extracts, the preparation of the extracts was performed in a dark place. The extracts obtained were kept in sterilized vials at 4 °C.

2.2. Culture and Recovery of Strains

Separately inoculate streptococcus mutans and Porphyromonas gingivalis on nutrient agar slant, incubate at 37 °C for 24 h, pick out single colony, inoculate it to BHI, incubate at 37 °C and 200 r/min in a constant temperature shaker for 5 h, so as to obtain the bacterial solution with certain concentration; place 5 mL of bacterial solution in a centrifuge tube, centrifuge at 5000 r/min for 2 min, discard the supernatant, add 5 mL of bacterial solution with 0.85% NaCl solution adjusted to A630 nm 0.2 for later use.

2.3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Artemisia Selengensis polyphenol extract solution was diluted by broth microdilution method in BHI medium (sterilized treatment) to the following concentrations: 102.4, 51.2, 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2 mg/L. 1 mL of medicated medium and 1 mL of S. mutans bacterial solution were added to the 24-well plate, respectively; similarly, P. gingivalis was added. After overnight, observe the growth and development of colonies, and find the minimum effective value of inhibitory concentration, that is, MIC value, from the growth well. Two different concentration gradient cultures with a value greater than the MIC were smeared on BHI medium and then subcultured, and the test concentration with less than 5 CFU/mL of the resulting colony count was the desired MBC value.

2.4. Determination of antibacterial Effect of Polyphenol Extract of Artemisia Selengensis at Subinhibitory Concentration

The MIC was determined by mixing the bacterial solution with the extract, and the control solution was prepared without adding the drug. After sufficient bactericidal treatment of the culture medium, multiple concentrations of Artemisia Selengensis polyphenol extract solution (1 mL) were obtained from the experimental bacterial group and introduced into the test tube

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(sterile), and 1 mL of bacterial solution (1×106 CFU/mL) was introduced. Another control solution with the concentration of polyphenol extract solution of Artemisia quinoa was set, and the test tubes were placed in an incubator (temperature set at 37 °C) for anaerobic culture. Within 24 hours, 150 μL was obtained from different bacterial solutions and added to the 96-well-plate in sequence every 2 hours, with 4 parallel wells in each group. In order to avoid edge effects, peripheral wells were abandoned. Microplate reader A630 nm wavelength was used to monitor bacterial growth.

2.5. Acidogenic Experiments

According to the results of MIC determination of polyphenol extract of Artemisia Selengensis against Streptococcus mutans and Porphyromonas gingivalis, four concentration gradients below MIC and below MIC were prepared by doubling dilution method with BHI medium containing 1% sucrose, and a control group without liquid was set up. The initial pH was adjusted to 7.4, and the bacteria and liquid medicine were inoculated at a ratio of 1:10 and placed in an anaerobic chamber for culture. The supernatant medium was taken at 2-h intervals, and the pH value was measured using a pH meter as the pH value after growth of the strain, that is, pH1. ΔpH = pH0 − pH1 was calculated, and the inhibitory acid yield = (ΔpH control group − ΔpH experimental group)/ΔpH control group × 100%.

2.6. Statistical Analysis

SPSS 13.0 software was used to input data and complete the data analysis work. ANOVA was used for comparison between the control group and each group. $P < 0.05$ indicated statistical significance.

3. Results

3.1. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Polyphenol Extract of Artemisia Selengensis

The MIC and MBC of polyphenol extract of Artemisia Selengensis against S. mutans were detected by broth microdilution. Macroscopically, it was observed that the solution in the wells of 3.2 mg/L Artemisia Selengensis polyphenol extract was clear without bacterial growth, the medium liquid in the wells with Artemisia Selengensis polyphenol extract concentration > 3.2 mg/L was clear, and the medium in the wells with concentration 3.2 < mg/L was turbid. On the BHI solid medium of secondary culture, the colony count of 6.4 mg/L group was < 5 CFU/mL. According to the experimental results, the MIC of polyphenol extract of Artemisia Selengensis polyphenol against S. mutans was 3.2 mg/L and the MBC was 6.4 mg/L.

3.2. Determination of Antibacterial Effect of Polyphenol Extract of Artemisia Selengensis at Subinhibitory Concentration

A630 values were measured at different time points in Artemisia Selengensis polyphenol extracts with MIC concentrations of 1 to 1/16 (3.2, 1.6, 0.8, 0.4, and 0.2 mg/L) compared with the negative control group, all of which had antibacterial effects, and Artemisia Selengensis polyphenol extracts at concentrations of 0.2 mg/L and above were lower than those in the negative control group. At 6 hours, the bacteria entered the logarithmic growth phase, and the proliferation rate of S. mutans in the experimental group with MIC of 1 to 1/16 was significantly slower than that in the control group, with a flat growth curve, see Figure 1.

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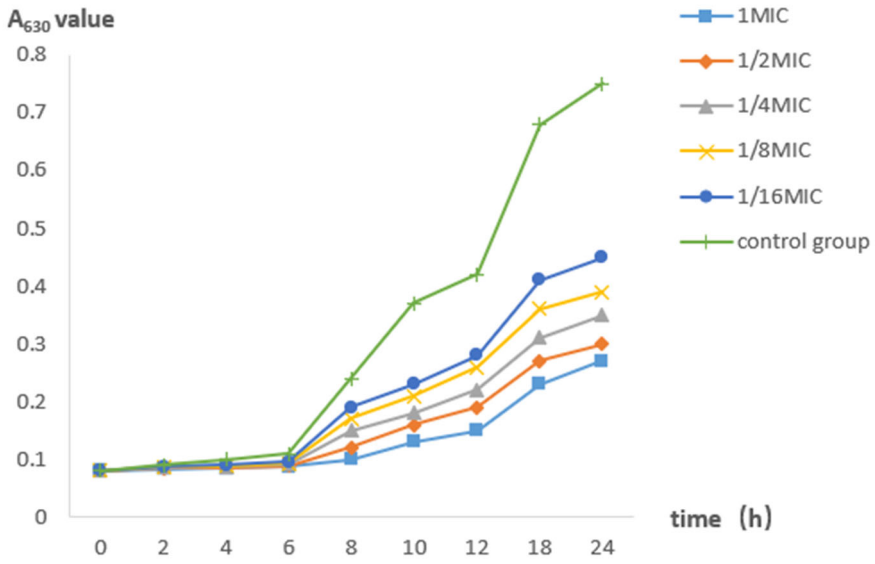

Figure 1. Different concentrations of artemisia selenifera polyphenol extract inhibit the effects of Alternaria

As the drug concentration decreased, the inhibitory effect of Artemisia Selengensis polyphenol extract on S. mutans gradually decreased, but the growth of bacteria was still lower than that of the control group (Fig. The inhibition rate of S. mutans ranged from 2.371% to 65.736% from 1 MIC to 1/16 MIC, and the antibacterial effect was exerted at 6 h. The inhibitory effect was most pronounced at 12 h, see Figure 2.

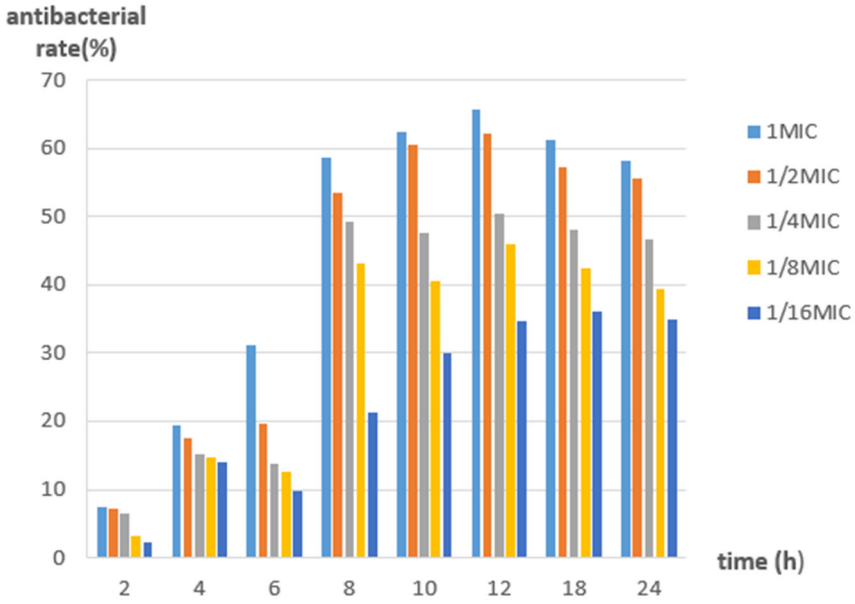

Figure 2. Antibacterial activity of polyphenol extracts from Artemisia selenifera at different concentrations in different time periods.

3.3. Effect of Polyphenol Extract of Artemisia Selengensis on Acid Production of Streptococcus Mutans

The effect of polyphenol extract of Artemisia Selengensis at drug concentrations ranging from 1 to 1/16 MIC, respectively, on acid production by S. mutans is shown in Figure 3. It can be seen that the pH value of the culture medium in the negative control group decreased dramatically after about 4 hours of culture, the pH value was below 5 at 6 hours, and the decrease tended to

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be flat after 12 hours. In the experimental group, the pH value of MIC did not change significantly during the 0 to 8 h culture stage and was maintained at about 7, and the pH decrease was finally stable above 6.0 after 8 h. The polyphenol extract of Artemisia Selengensis had a certain inhibitory effect on the acid production of Streptococcus mutans, and the inhibitory effect of the polyphenol extract of Artemisia Selengensis was gradually weakened at 12 hours in two experimental groups: 1 MIC and 1/2 MIC. The inhibitory effects of 1/4, 1/8, and 1/16 MIC were weaker than those of the first two groups, generally compared with the negative antibacterial trend, and the acid production at 4 hours was close to the trend of the control group. At 6 hours of culture, the acid production inhibition rates of polyphenol extracts at 1, 1/2, 1/4, 1/8, and 1/16 MICs against S. mutans were 82.257%, 72.619%, 58.9755, 50.143%, and 40.927%, respectively.

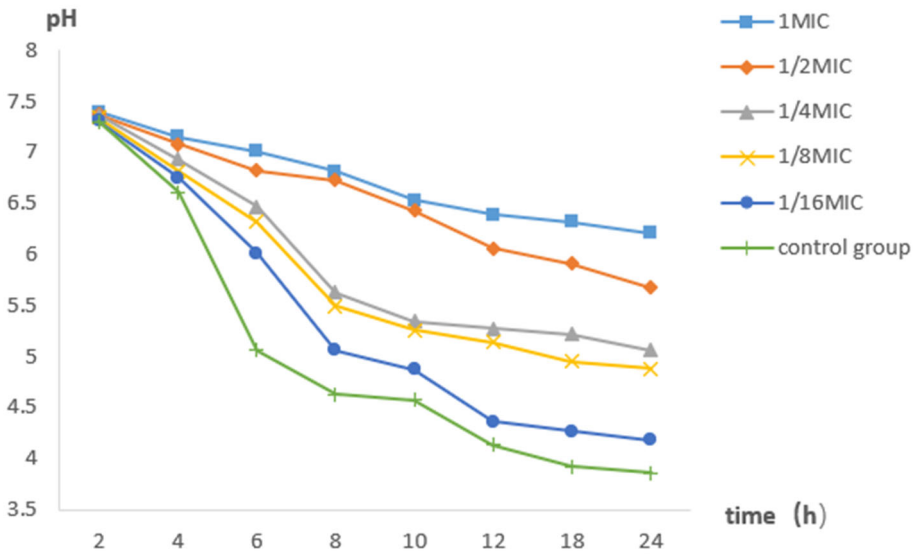

Figure 3. Effects of different concentrations of Artemisia selenifera polyphenol extracts on pH of Streptococcus mutans

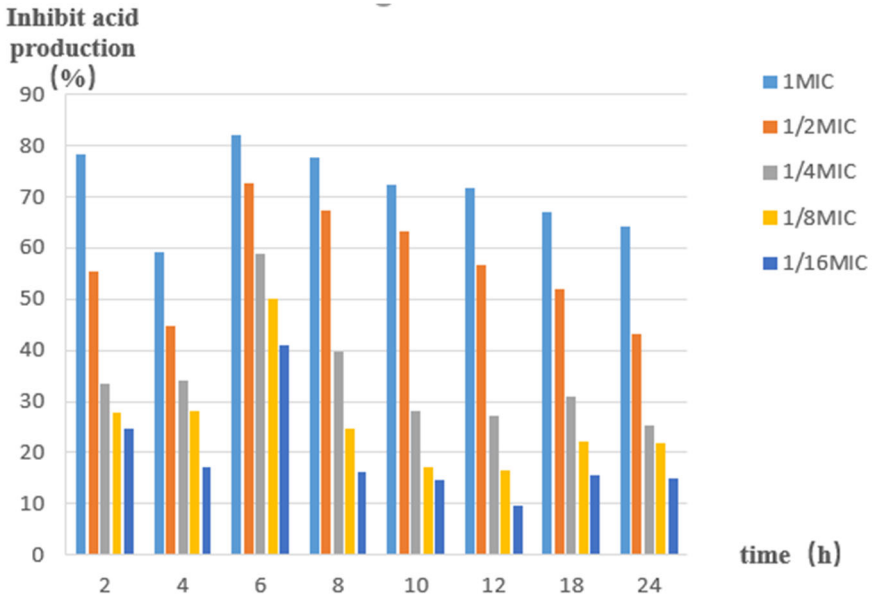

Figure 4. Inhibition of acid production of Streptococcus mutans by polyphenol extracts from Artemisia selenifera at different concentrations.

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4. Conclusion

In this study, the MIC, MBC, acid production rate, and inhibited acid production rate of polyphenols extracted from the Artemisia Selengensis with > 90% content were tested. The results showed that the MIC and MBC of Artemisia Selengensis polyphenols were 3.2 mg/L and 6.4 mg/L, respectively. The MIC and sub-inhibitory concentration antibacterial test showed that the antibacterial rate was 2.371% ~ 65.736%, and the antibacterial activity was positively correlated with the concentration gradient. The acid production test showed that Artemisia Selengensis polyphenols significantly inhibited bacterial acid production at 24 hours ($P < 0.01$), and the bacterial adhesion test results showed that Artemisia Selengensis polyphenols had the slowest biofilm production rate and the lowest total amount of biofilm at MIC ($P < 0.01$).

The limitations of the present study are the failure to determine the bactericidal time of the probiotics, and the efficacy of Artemisia Selengensis polyphenols in inhibiting bacterial adhesion to tooth surfaces, restorations, and dental applicants. Furthermore, this study was performed in vitro and could not show accurate inhibition of acid by Artemisia Selengensis polyphenols in saliva-containing media. Therefore, other studies are recommended to investigate the time-based bacterial growth curve, as well as to inhibit the adhesion of the aforementioned tooth structures and acid production in saliva.

Artemisia Selengensis polyphenols are therefore considered as a potential development direction for caries prevention therapy, which can be added to mouthwashes for caries prevention therapy.

Acknowledgments

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