

Antibacterial effect of *Forsythiae fructus* extract in dental caries.

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Abstract

Oral-resident bacteria cause various diseases in the oral cavity, and studies have been conducted to inhibit the activities of many of such bacteria. This study was conducted to investigate the antibacterial effect of the natural extract *Forsythiae fructus* against the oral-resident bacteria *Streptococcus intermedius* (*S. intermedius*) and *Streptococcus gordonii* (*S. gordonii*), characterized by anti-inflammation and antimicrobial activity. The clearzone confirmation method was used to test the antimicrobial activity, and the number of colony-forming units (CFUs) was checked to measure the bacterial proliferation. For the experiment, 5, 10 and 20 mg/mL of *Forsythiae fructus* extract were set as the experimental groups, and the group without any treatment was set as the control group. For the results of the antimicrobial activity testing, the size of the clear zone became larger as the concentration increased from 5 to 10 and 20 mg/mL compared to the control group. As for the results of the CFU measurement, many bacteria were observed in the control group while no bacteria appeared in all the experimental groups. Therefore, the present study confirmed the antibacterial effect of *Forsythiae fructus* extract in the oral cavity, which could be used as basic data for the development of antimicrobial agents using natural extracts against oral-resident bacteria.

Keywords: Antibacterial effect, Dental caries, Oral bacteria, *Forsythiae fructus*, Oral hygiene.

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Introduction

The oral cavity has an environment suitable for the proliferation of bacteria, thus always having resident bacterial communities where various bacteria settle. It is also a place where invasion of microorganisms occurs through direct contact with the outside [1]. In the oral cavities of ordinary people, more than 30 species of bacteria are usually isolated, and they appear in various forms depending on the age, health level, dietary level, and hygiene level [2].

The pathological mechanisms that can be caused by oral microorganisms include dental caries, periodontal disease, and missing teeth [3]. These bacterial diseases are developed through the following mechanism. The acid is generated in the process where large amounts of Viridans streptococci and lactobacilli break down the sugars remaining in the oral cavity, and the acid produced is attached to the tooth surface, dissolving or demineralizing the inorganic substance, and eventually destroying the dental tissue [4]. Many studies have attempted to classify Viridans streptococci, and they are known as consisting of four groups: Anginosus (*S. anginosus*, *S. constellatus*, *S. intermedius*), Mitis (*S. oralis*, *S. mitis*, *S. gordonii*, *S. sanguinis*, *S. parasanguis*), Salivarius (*S.*

vestibularis, *S. salivarius*), and Mutans (*S. mutans*, *S. sorbinus*) [5]. These bacteria have been reported to be present as resident bacteria in the oral cavity, gastrointestinal tract, and female genital organ, and to cause bacteremia and endocarditis through opportunistic infection [6,7].

Forsythiae fructus is a forsythia fruit of the forsythia plant belonging to Oleaceae, and is known to mainly contain lignans, such as phillyrin (forsythin), phillygenin, (+)-pinoselinol, and (+) pinoselinol- β -d-glycosides [8]. The pharmacological actions of *Forsythiae fructus* have been known as anti-inflammation, antipyretic action, liver function protection, and vasodilation. Previous studies have been conducted on the antimicrobial activity, whitening function, suppression of production of inflammatory mediators, and antioxidant-related antiviral agents of *Forsythiae fructus* extract [9-11] but the studies on its effects against the causative bacteria of dental caries and periodontal diseases have been limited.

Therefore, this study was conducted to investigate the antimicrobial activity of *Forsythiae fructus* ethanol extract against the causative bacteria of dental caries and periodontal diseases to identify its efficacy and determine its usability for

the development of various oral hygiene products for the prevention of oral diseases [12,13].

Materials and Methods

Experiment materials

Forsythiae fructus was purchased from Food synergy Co., Ltd. (Seoul, South Korea). After adding 80% ethanol to 100 g of crushed *Coptis Chinensis*, the extraction was done at 65°C for 12 hours. The extract was filtered and was concentrated using a rotary vacuum evaporator (N-1300E.V.S EYELA Co., Japan). The concentrated *Forsythiae fructus* extract was again lyophilized using a freeze dryer (FD5508, Ilshin Lab, Yangjukun, Kyunggi-do, South Korea), and was diluted in distilled water to form 5, 10 and 20 mg/mL concentrations. For the control group, only brain heart infusion (BHI; Sigma-Aldrich, St. Louis, MO, USA) was used.

Experimental bacteria

S. intermedius (ATCC 9895) and *S. gordonii* (ATCC 10558) were purchased from Korean Culture Center of Microorganisms (KCCM). Each microorganism was activated at the BHI and was diluted at a 1×10^6 ratio.

Experiment Method

Antimicrobial activity test

To test the antimicrobial activity of *Forsythiae fructus* extract, 100 μ L (1×10^6) each of *S. intermedius* and *S. gordonii* was applied on a solid medium, respectively, and after the bacteria were absorbed, 100 μ L of each experimental group was dropped onto the paper disc carefully placed on the solid medium. After keeping such paper disc in a 37°C bacterial incubator for 24 hours, the diameter of the clear zone was measured. The average value and standard deviation were obtained after three repeated experiments to measure the diameter of the clear zone, where the bacterial activity was inhibited. Only the average value was recorded, however, because the degree of deviation was insignificant.

Measurement of bacterial proliferation

To measure the bacterial proliferation, the media for the bacteria (1×10^5) were mixed at a ratio of 9 (BHI):1 (bacteria). Then the resulting mixture was mixed with the extracts prepared at 5, 10 and 20 mg/mL concentrations, and 100 μ L of each mixture was inoculated into the solid medium. After keeping the final mixture in a 37°C bacterial incubator for 24 hours, the number of colony forming units (CFUs) was checked.

Results

Measurement of inhibition zone

For the results of the antimicrobial activity test of *Forsythiae fructus* extract against *S. gordonii*, a 15 mm clear zone was observed in the 5 mg/mL group, a 20 mm clear zone in the 10 mg/mL group, and a 30 mm clear zone in the 20 mg/mL group. As for the results of *S. intermedius*, a 13 mm clear zone was observed in the 5 mg/mL group, an 18 mm clear zone in the 10 mg/mL group, and a 30 mm clear zone in the 20 mg/mL group.

Antimicrobial activity test

The results of the antimicrobial activity test of *Forsythiae fructus* extract against *S. gordonii* and *S. intermedius* showed that many bacteria survived in the control group, which had no treatment, but no bacteria were observed overall in the 5, 10 and 20 mg/mL experimental groups (Figures 1-4).



Figure 1. Results of the antimicrobial activity test of *Forsythiae fructus* extract against *S. gordonii*.

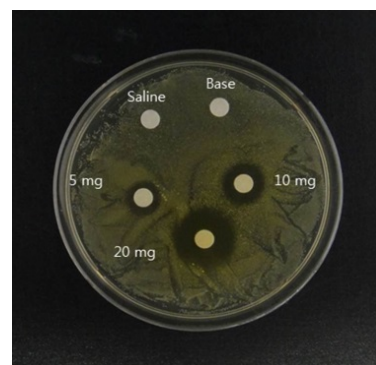


Figure 2. Results of the antimicrobial activity test of *Forsythiae fructus* extract against *S. intermedius*.

Discussion

In relation to the antimicrobial activity of a natural product against oral microorganisms, funoran, a substance extracted from algae, has been reported to have an excellent antibacterial effect on dental-caries inducing bacteria [14]. Several natural extracts, such as green tea, propolis, flavonoids, chitosan, *Akebia quinata*, cinnamon, *Ulmus davidiana*, and *Schisandra chinensis*, have been reported to inhibit the development of

dental plaque disease [15,16]. In addition, grapefruit seed, cassia seed, and Korean angelica root showed an anti-caries effect [17]. Among the 105 kinds of herbal medicines, Korean Aristolochia fruit, buckler fern, dried clove buds, *Hemerocallis fulva*, pinellia, *Adonis amurensis*, and Scrophularia root have been reported to have an excellent growth inhibitory effect against dental-caries-inducing bacteria [18]. There have been few studies, however, on the anti-caries effect of *Forsythiae fructus*, the natural substance that was used in this study.

With regard to *Forsythiae fructus*, various physiological activities, such as the anticancer, antimicrobial, and antiviral activities, have been reported [19]. In particular, it has been reported to have antimicrobial activity against *Staphylococcus aureus*, *Dysentery bacillus*, *Streptococcus hemolytic*, *Diplococcus pneumoniae*, and eruptive typhus, and has also been reported to have a therapeutic effect on tuberculosis because it significantly inhibits the tubercular bacillus [20]. According to the results of the present study, the inhibition zone against *S. intermedius* measured 13 mm in the 5 mg/mL group, 18 mm in the 10 mg/mL group, and 30 mm in the 20 mg/mL group. The inhibition zone against *S. gordonii*, on the other hand, measured 15 mm in the 5 mg/mL group, 20 mm in the 10 mg/mL group, and 30 mm in the 20 mg/mL group. As the concentration increased, a more pronounced and larger inhibition zone appeared. For the CFU results of each *Forsythiae fructus* extract sample, there was an antibacterial effect at 5 mg/mL against *S. intermedius* and *S. gordonii*. This result was consistent with the clear-zone results: a definite clear zone was shown at 5 mg/mL, and the same antibacterial effect was also seen in the CFU result.

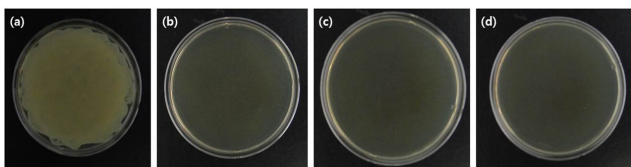


Figure 3. CFU results of *Forsythiae fructus* extract for *S. gordonii*: (a) Control, (b) 5 mg/mL, (c) 10 mg/mL, (d) 20 mg/mL.

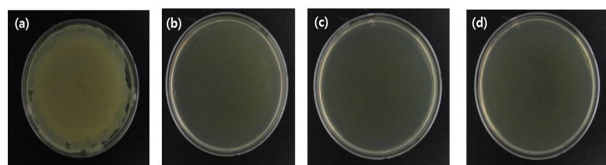


Figure 4. CFU results of *Forsythiae fructus* extract for *S. intermedius*: (a) Control, (b) 5 mg/mL, (c) 10 mg/mL, (d) 20 mg/mL.

Therefore, *Forsythiae fructus* extract showed a remarkable antibacterial effect against the dental-caries inducing bacteria *S. intermedius* and *S. gordonii*, suggesting that it could have an anti-caries effect, preventing or alleviating dental caries.

Conclusion

In this study, *Forsythiae fructus* ethanol extract, which is known to have antimicrobial, antiviral, and anticancer effects, was proven to be effective through the measurement of the clear zone and colony-forming units (CFUs) to confirm its antimicrobial activity against the oral-resident bacteria *Streptococcus intermedius* (*S. intermedius*) and *Streptococcus gordonii* (*S. gordonii*). *Forsythiae fructus* extract revealed the largest clear zone when 20 mg/mL of it was applied to the two aforementioned bacteria, and the death rate of the said bacteria was improved according to the concentration. If these study results lead to the development of an oral product that can prevent and treat dental caries, one of the typical oral diseases, it can be used as an alternative to maintain and promote oral health. The development of complex extracts containing *Forsythiae fructus* rather than single extracts through continuous research will promote the growth of oral hygiene products.

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