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Screening of aqueous extracts of medicinal herbs for antimicrobial activity against oral bacteria

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ABSTRACT

Background: Dental caries is considered to be a preventable disease, and various antimicrobial agents have been developed for the prevention of dental diseases; however, many bacteria show resistance to existing agents. In this study, 14 medicinal herbs were evaluated for antimicrobial activity against five common oral bacteria as a screen for potential candidates for the development of natural antibiotics.

Methods: Aqueous extracts of medicinal herbs were tested for activity against *Enterococcus faecalis*, *Actinomyces viscosus*, *Streptococcus salivarius*, *Streptococcus mutans*, and *Streptococcus sanguis* grown in brain heart infusion (BHI) broth. A broth microdilution assay was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A disk diffusion assay was performed by inoculating bacterial cultures on BHI agar plates with paper disks soaked in each of the medicinal herb extracts. Inhibition of the synthesis of water-insoluble glucans by *S. mutans* was also investigated.

Results: The aqueous extracts of many of the 14 medicinal herbs demonstrated antimicrobial activity against the five types of pathogenic oral bacteria. The extracts of Sappan Lignum, Coptidis Rhizoma, and Psoraleae Semen effectively inhibited the growth of oral bacteria and showed distinct bactericidal activity. The extracts of Notoginseng Radix, Perillae Herba, and Psoraleae Semen decreased the synthesis of water-insoluble glucans by the *S. mutans* enzyme glucosyltransferase (GTase). The present study is the first to confirm the antimicrobial activity of the extract of Sappan Lignum against all five species of oral bacteria strains.

Conclusion: These results suggest that certain herbal medicines with proven antimicrobial effects, such as Sappan Lignum and Psoraleae Semen, may be useful for the treatment of dental diseases.

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1. Introduction

Two major dental diseases in the world are dental caries and periodontal disease, both of which are caused by various bacteria in the oral cavity.[1] Dental caries is a common oral disease that usually develops secondary to the formation of plaque biofilms on the tooth surfaces; the causative agents are Gram-positive bacteria such as *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus* spp., and some non-mutans streptococci.[2,3] Specific bacterial species such as *Actinomyces* spp. and *Enterococcus faecalis* contribute to tooth root caries and periodontal infections.[4,5] Although dental disease is only slowly progressive, oral bacteria can also cause infections of the head and neck, such as periapical abscesses and infections of the jaw bones and fascia.[6] Therefore, the control of oral bacteria is key to the prevention and treatment of these oral diseases. Various antibiotics, including ampicillin, chlorhexidine, erythromycin, spiramycin, and vancomycin, have been very effective at preventing dental caries, but these agents can cause unexpected side effects such as microorganism resistance, vomiting, and diarrhea.[7,8] Furthermore, the use of antibiotics can promote the development of multidrug-resistant (MDR) strains of bacteria.[9] These problems have led to a search for new antibacterial substances that are safe for humans and specific for oral pathogens. Various sources such as microorganisms, animals, and plants,[10] have been examined for components with these properties.

In Asian countries, including Korea, China, and Japan, traditional herbal medicines have been used to treat infectious diseases since ancient times.[11] Most oral diseases are due to bacterial infections, and medicinal plants are well known to exert considerable antimicrobial activity against many microorganisms, including the bacteria responsible for dental caries.[12] Furthermore, the natural phytochemicals isolated from herbal medicines could offer effective alternatives to antibiotics and represent a promising approach to the prevention and treatment of dental caries and other oral infections.[13] Screening for herbal medicines effective against oral bacteria is the required first step in the identification of natural phytochemicals that could be used as antimicrobial substances.[11] Therefore, the present study was performed to show that extracts of medicinal herbs inhibit the growth of oral bacteria as well as the synthesis of water-insoluble glucans by *S. mutans*.

2. Methods

2.1. Identification and preparation of medicinal herbs extracts

Aqueous extracts of medicinal herbs used in this study were purchased from the Korea Medicine Herbs Association (Yeongcheon, Korea). Identification of all herbal medicines was verified by Professor Bae of the College of Pharmacy, Chungnam National University, and the specimens of these herbs were deposited at Korean Institute of Oriental Medicine (KIOM). Each herbal medicine was extracted by heating in water of 8 to 10 times of the herb weight for 3 hours at 115 °C (Gyeongseo Extractor Cosmos-600, Incheon, Korea). After boiling, the extract was filtered using standard testing sieves (150 μm) (Retsch, Haan, Germany) and freeze-dried to a powder. A 50 mg sample of each powdered herbal medicine was dissolved in 1 mL of distilled water and stored at −20 °C before use.

2.2. Microorganisms and growth conditions

Enterococcus faecalis (KTCT3206), *Actinomyces viscosus* (KTCT9146), *Streptococcus salivarius* (KTCT5091) and *Streptococcus mutans* (KCTC3065) were purchased from the Biological Resource Center (BRC) in Korea Research Institute of Bioscience and Biotechnology (KRIBB). *Streptococcus sanguis* (NCTC 9811) was purchased from the School of Dentistry Seoul National University. Five types of strains were incubated in brain heart infusion (BHI; Difco, BD, USA) broth and BHI agar (Difco, BD, USA) at 37 °C in the presence of 5% CO_2.

2.3. Determination of minimum inhibitory concentration and minimum bactericidal concentration of herbal medicine extracts on oral bacteria

Plate dilution method was used for determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of herbal medicine extracts on five kinds of oral bacterial strains. For MIC and MBC, the bacteria was incubated for 20 hours at 37 °C in 5% CO_2. To determine MIC, the extract concentration was diluted two-fold from 5000 μg/mL to 78 μg/mL and the inoculum of 1×10^4 CFU/mL was used. The lowest concentration of the herbal medicine extracts that inhibited the growth of the organism, corresponding to an inhibition of 99% of the inoculum, was considered as the MIC. To determine MBC, we used a variant on the agar dilution method. The inoculation spots with no visible growth were cut and top down 3 μL of bacterial culture broth on BHI agar plate. The lowest concentration of herbal medicine extract that yielded no growth on the agar (99.9% kill) was defined as MBC.

2.4. Inhibition of water-insoluble glucan synthesis

The assay and preparation of crude GTase were based on the method previously described by Koo et al.[14] The cell-free enzymes were precipitated from culture supernatant of *S. mutans*. After filtering the culture supernatant of *S. mutans* using a 0.2 μm membrane filter, the cell-free enzymes were extracted and precipitated by Amicon ultra centrifugal filter (MWCO 30 kDa, Millipore, USA). The crude enzymes were restored at -80 °C and used for synthesis of water-insoluble glucan. A reaction mixture consisting of 20 μL of crude enzyme, 180 μL of the diluted herbal medicine extract in 800 μL of 62.5 mM potassium phosphate buffer (pH 6.5) containing 12.5 g of sucrose and 0.25 g of sodium azide were incubated at 37 °C for 30 hours. After incubation, the fluid was removed, and the contents that stick to tube wall were washed with sterile water and dispersed by a sonicator (JAC-2010P, Kodo, Korea). The total amount of water-insoluble glucan was measured the absorbance by UV/VIS spectrophotometer (U-2900, Hitachi, Japan).