IN VITRO ANTIMICROBIAL EFFECTS OF AQUEOUS EXTRACTS OF CAESALPINIA SAPPAN LINN. DERIVATIVES AGAINST ORAL PATHOGENS

DR. HAMED KERAMAT\textsuperscript{a1}, DR. ALI MOADDABI\textsuperscript{b} AND DR. ARSALAN RANJBARI\textsuperscript{c}

\textsuperscript{a}\textsuperscript{b}\textsuperscript{c}DDS Resident of Oral Medicine of Shahed University

ABSTRACT

Dental caries is a biofilm-related oral 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, Sappan Lignum (the dried heartwood of Caesalpinia sappan L.) were evaluated for antimicrobial activity against five common oral bacteria as a screen for potential candidates for the development of natural antibiotics. Aqueous extracts of Sappan Lignum 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. The aqueous extracts of Sappan Lignum demonstrated antimicrobial activity against the five types of pathogenic oral bacteria. The present study is confirmed the antimicrobial activity of the extract of Sappan Lignum against all five species of oral bacteria strains. These results suggest that certain herbal medicines with proven antimicrobial effects, such as Sappan Lignum, may be useful for the treatment of dental diseases.

KEYWORDS: antimicrobial activity, dental caries, Sappan Lignum, oral bacteria

Major dental diseases in the world are dental caries and periodontal disease, both of which are caused by various bacteria in the oral cavity. Periodontal disease is one of the most common health problems in the human communities (1) and dental caries is still a common disease among children and adolescents (2). Many developed countries have shown a marked decrease in the prevalence of dental caries in children over the past decades, however, in many other developing countries caries prevalence has increased (3). Gingivitis is one of the most common forms of periodontal disease (4) and around 100\% of people aged 17 to 22 have gingivitis in different degrees. Dental plaque, a biofilm of microorganisms on tooth surface, plays an important role in the development of caries and periodontal disease (5). The accumulation and metabolism of bacteria on teeth and implants surfaces are considered the primary cause of caries, gingivitis, periodontitis, periimplantitis and breathe (6). The accumulation of plaque on teeth is a highly organized and ordered sequence of events (7). Cariogenic bacteria and periodontopathic bacteria are present in dental plaque as biofilms (8). Essentially, all oral bacteria possess surface molecules that foster some type of cell-to-cell interaction (6). Only a few specialized organisms, primarily streptococci are able to adhere to oral surfaces such as the mucosa and tooth structure (7). Mutans streptococci can colonize the tooth surface and initiate plaque formation by their ability to synthesize extracellular polysaccharides from sucrose, using glucosyltransferase (9, 10). This sucrose dependent adherence and accumulation of cariogenic streptococci is critical to the development of pathogenic plaque (10). All Streptococcus mutans serotypes such as Streptococcus sobrinus (serotypes d, g and h) have been shown to have significant potential to cause caries, but because of their significant genetic and biochemical differences, they should not be referred as simply as S. mutans. S. mutans and lactobacilli are acidogenic and acid uric bacteria and seem to be the primary organisms associated with caries in humans. S. mutans are most strongly associated with the onset of caries, whereas lactobacilli are associated with active progression of cavitated lesions (7). Bacterial attachment to preexisting plaque is studied by examining the adherence between different bacterial strains (co-aggregation). One of the best characterized interactions is the adherence of Actinomyces viscosus through surface fimberiae to polysaccharide receptor on cells of Streptococcus sanguis (6). These types of interactions are thought to be of primary importance in the colonization of the periodontal environment (6). The further accumulation of plaque around the gingival and subgingival region may lead to a shift in its microbial composition from streptococcus-dominated to a larger number of Actinomyces spp. and an increased number of capnophilic and obligatory anaerobic bacteria, such as Porphyromonas

\textsuperscript{1}Corresponding author
gingivalis (11). Both streptococci and actinomycetes which are facultative anaerobes and seem to be involved in root caries and periodontal disease, respectively (12,13), and doubling times for microbial populations during the first 4 h of development are less than 1 h (6). Consequently, these two groups of primary colonizers are taught to prepare a favorable environment for secondary colonizers, which have more fastidious growth requirements. The microorganisms primarily considered secondary colonizers fell into the green (includes Eikenella corrodens), orange, or red complexes (6). E. corrodens, a fastidious, slow growing, gram negative and rod shaped bacteria that is part of the normal human oral flora, has been isolated from a variety of infections associated with human oral flora (14). This organism is implicated as a human periodonto-pathogen and may also cause extra oral infections (15). Recent advances in microbiology and host defense studies allow clinicians to couple conventional mechanical therapy with locally and systemically delivered antimicrobial and host modulation agents (6). Mechanical procedures such as root planning is hard and takes usually more than one visit schedule and can cause wearing of enamel (16); moreover, improved understanding of the infectious nature of dental disease has dramatically increased interest in chemical methods of plaque control and holds great promise for advances in disease control and prevention. Chemical plaque control has been shown to be effective for both plaque reduction and improved wound healing after periodontal surgery; moreover it can augment mechanical plaque control procedures (6). Antimicrobial agents against oral microorganisms, especially those contributing to sub and supra gingival biofilm formation, play an important role in the prevention of dental caries, and periodontal disease (17). Since some chemical materials including Chlorhexidine can cause brown staining of the teeth (16,6), tongue and silicate and resin restorations transient impairment of taste perception, toxic effects on connective tissues, dryness and soreness of oral cavity (16), allergic reactions in patients (18) and oral desquamation in children, use of herbal agents can be a useful alteration. Herbal products have been used since ancient times in folk medicine, involving both eastern and western medical traditions (17). Many plants and plant-derived antimicrobial components are used in folklore therapeutics for the treatment of periodontal disorders and for the purposes of oral hygiene (18). Some have been evaluated for possible use in modern medicine, while thousands of other potentially useful plants have not been tested (18). During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has lead to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the foregoing disadvantages (19-21). A wide range of antimicrobial agents and herbal products are added to dentifrice and mouth rinsing solutions with the aim of preventing carries or biofilms formation (17). Traditionally herbal medicines have been used to treat infectious diseases since ancient times (22). 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 (23). 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 (24). Screening for herbal medicines effective against oral bacteria is the required first step in the identification of natural phyto-chemicals that could be used as antimicrobial substances (22). Caesalpinia sappan Linn, a traditional plant used widely in oriental medicine. The plant extracts were found to be a good source of secondary metabolites, vitamins and metals. The extracts were further tested against certain human pathogenic microbes. The methanol and ethyl acetate extracts of the heartwood was found to be effective against certain pathogenic microbes. In recent years, the extract of Sappan Lignum (the dried heartwood of Caesalpinia sappan L.) has been found to be a potential immunosuppressive agent. The reported main phenolic com- pounds in Sappan Lignum were divided into to four structural sub-types: i.e. brazilin, chalcone, protosappanin and homisoflavonoid. Among the protosappanin derivatives, such as protosappanin B and isoprotosappanin B, 10-O-methylprotosappanin B and 10-O-methylisoprotosappanin B, as well as protosappanin E1 and protosappanin E2 occur as pairs of epimers. Meanwhile, the homisoflavonoid epimers sappanol and epsipananol, 4'-O-methylsappanol and 4-O-methylepsipananol, 3'-O-methylsappanol and 3'-O-methylepsipananol were successively isolated along with a new compound, a 3-benzylchroman derivative 3'-deoxy-4'-O-methylepsipananol (25). Considering the medicinal importance of C. sappan, a study on the efficacy of C. sappan extracts on human pathogenic bacteria and fungus
has been made anticipating to develop antibiotic in future. Considering that only a few studies have been reported on the in vitro effect of Sappan Lignum extracts against oral pathogens and a great demand in dentistry for new and better substances to inhibit or suppress bacteria and biofilm formation, improve the quality of dental treatment, and facilitate some dental procedures; this study have been designed to evaluate the in vitro antimicrobial activity of aqueous extract of Sappan Lignum on some oral micro-organisms.

METHODS

Identification and preparation of medicinal herbs extracts

Sappan Lignum used in this study were purchased from the Medicinal Herbs Association. Identification of all Sappan Lignum was verified by Department of Pharmacy, Tehran University of Medical Sciences. Sappan Lignum was extracted by heating in water of 8 to 10 times of the herb weight for 3 hours at 115 °C. After boiling, the extract was filtered using standard testing sieves (150 m) and freeze-dried to a powder. A 50 mg sample of powdered herbal medicine was dissolved in 1 mL of distilled water and stored at −20 °C before use.

Microorganisms and growth conditions

Enterococcus faecalis, Actinomyces viscosus, Streptococcus salivarius, Streptococcus sanguis and Streptococcus mutans were purchased from the Pasture Institute of Iran. Five types of strains were incubated in brain heart infusion (BHI) broth and BHI agar at 37 °C in the presence of 5% CO2.

Determination of MIC and MBC of aqueous extract of Sappan Lignum

Plate dilution method was used for determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Sappan Lignum extracts on mixture of five kinds of oral bacterial strains. For MIC and MBC, the bacteria were incubated for 20 hours at 37 °C in 5% CO2. To determine MIC, the extract concentration was diluted two-fold from 5000 g/mL to 80 g/mL and the inoculums of 1 × 104 CFU/mL were used. The lowest concentration of the herbal medicine extracts that inhibited the growth of the organism, corresponding to an inhibition of 99% of the inoculums, 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 3L of bacterial culture broth on BHI agar plate. The lowest concentration of herbal medicine extract that yielded no growth on the agar (99.9% killed) was defined as MBC.

Agar diffusion assay

The antibiotic sensitivity profile of five kinds of oral bacteria was determined on assay plate including inoculums of 1 × 104 CFU/mL on the top layer of the BHI agar plate. A sterile paper disk (8 mm) was soaked with extracts of herbal medicines, so that each disk was impregnated with 312-5000 g of Sappan Lignum extract per disk. The plates were then incubated for 20 hours at 37 °C in 5% CO2. The antibacterial activity was evaluated by measuring the diameter (mm) of the inhibition zone.

RESULTS

Sappan Lignum has strong activity against oral bacteria

Aqueous extract of Sappan Lignum demonstrated the strong antimicrobial activity, inhibiting the growth of all oral bacteria examined. Agar plates spread with oral bacteria were treated with a series of dilutions, including 312, 625, 1250, 2500, and 5000g/mL of Sappan Lignum. Most concentrations of Sappan Lignum produced inhibition zones in all tested oral bacteria, with the highest concentration, 5000g/mL, of Sappan Lignum producing inhibition zones for mixture culture of E. faecalis, S. salivarius, A. viscosus, and S. sanguis.
Figure 1: Disk diffusion assay of Sappan Lignum extract against oral bacteria. Inhibition zone formed according to the concentration of the Sappan Lignum extract was indicated in mm. Control, 312g/mL, 625g/mL, 1250g/mL, 2500 g/mL and 5000g/mL (clockwise) respectively

**DISCUSSION**

The result of this study showed that aqueous extract of Sappan Lignum, had strong antibacterial activity against E. faecalis, S. salivarius, A. viscosus, and S. sanguis, respectively. Dental disease is one of the most prevalent public health concerns. The problems caused by dental caries affect all age groups, and treatment is both expensive and labor-intensive (26). Dental caries and periodontal diseases are infectious diseases caused by common oral bacteria; therefore, controlling or even reducing the levels of these causative pathogens, such as S. mutans and E. faecalis, is a key step in the prevention and treatment of these diseases (27) Dental caries is a common oral disease caused by many cariogenic microbes, including Lactobacillus spp., Streptococcus spp., and Actinomyces spp., which usually form plaque biofilms on the tooth surfaces (26). Dental plaque is initially synthesized by the glucosyltransferase from S. mutans, and oral microorganisms then colonize and accumulate in this water-insoluble glucan layer. The viridans streptococci S. salivarius, S. sanguis, and S. mutans were the most representative human cariogenic bacteria included in the present study; however, these species are also moderately resistant to antibiotics (24). Actinomyces spp. is involved in early plaque development on tooth surfaces and contributes to root caries and periodontal infections. E. faecalis is an opportunistic pathogen that is frequently isolated from asymptomatic and persistent endodontic infections, especially from the failed root canals undergoing retreatment (28). E. faecalis is a better survivor than other root canal microbes, being able to resist various harsh conditions such as bile salts and starvation as well as many antibacterial agents (29). The characteristics of these oral bacteria determine the antimicrobial agents that can be used for the prevention and treatment of dental diseases. In our study, aqueous extracts of Sappan Lignum were prepared and evaluated for their antimicrobial activities against five species of oral bacteria. Sappan Lignum has traditionally been used as a red dyestuff and also as a herbal medicine to treat inflammation or improve blood circulation (30,31). The antimicrobial activity of Sappan Lignum against S. mutans has been reported by scientist (32). However, we widely confirmed the antimicrobial activity of Sappan Lignum about periodontal diseases as well as dental caries. In our study, the aqueous extract of Sappan Lignum showed strong antimicrobial activity against all five species of oral bacteria. The MIC values of Sappan Lignum against E.
faecalis and A. viscosus were especially low relative to those of the other herbal medicines. Further, Sappan Lignum extract had strong activity against oral bacteria in the agar diffusion assay; this activity was more pronounced for Streptococcus spp. than for other species such as E. faecalis and A. viscosus. Therefore, we have provided primary data showing that Sappan Lignum extract could be a potential treatment for both dental caries and periodontal diseases. In conclusion, we evaluated the antimicrobial activities of aqueous extract of Sappan Lignum against oral bacteria. Aqueous extract of Sappan Lignum showed the strong antimicrobial activity against all of the bacteria tested. Therefore, these results suggest that herbal medicines with proven antimicrobial effects, such as Sappan Lignum, may be useful for the treatment of dental diseases.

REFERENCES


