Efficacy of Nelumbo Nucifera Extracts Against Dental Caries Causing Microbes

Man Kyu Huh

ABSTRACT

Aim: To assess the inhibitory effect of leaf extracts of lotus (Nelumbo nucifera) against dental caries causing organisms. Material and method: The leaf extracts of lotus were assessed for its degree of inhibition of activities, growth and glucosyl transferase (GTA) on six bacterial strains i.e., Streptococcus mutans, Streptococcus mitis, Streptococcus sobrinus, Lactobacillus acidophilus, Actinomyces spp., and Nocardia spp., the main causal bacteria for dental caries. Results: N. Nucifera extracts possessed antimicrobial activity on all test bacterial strains. For example, 4mg/ml extract of lotus showed growth inhibition of S. mutans. The minimal inhibitory concentration (MIC) values against six species were varied from 2mg/ml to 16 mg/ml against antimicrobial activity. The relative growth ratio (RGR) against of N. nucifera extracts was determined as 50% in concentration of 4.0mg/ml. Conclusion: The extract of N. nucifera was effective in reducing the GTA activity of six strains of dental caries in vitro.

Key Words: Lotus; Streptococcus mutans; Glucosyltransferase

Introduction

Dental caries is usually common in children and young adults. In India, nearly 60-70% of the child populations is affected by dental caries.1-3 There is no the exception in Africa, Asia, American, and Europe.1 Carbohydrates (starches) such as sucrose, fructose, and glucose increase the risk of tooth decay. Sticky foods are more harmful than non-sticky foods because they remain on the teeth. Frequent snacking increases the time that acids are in contact with the surface of the tooth.4 Korean usually eat the sticky rice (japonica type of Orysa sativa L.). They also eat the noodles, the buckwheat and the bread, which are starch food. Thus, Korean have been exposed to the risk of tooth decay.4 Undoubtedly, medicinal plants are the prime source of drugs in both developing and developed nations, as drugs or herbal extracts for various chemotherapeutic purposes.5-9 There are about 2000+ plant species known to possess medicinal value in the traditional Asian system of medicine.6-9 The use of plant derived natural compounds used as alternative sources of medicine continues to play major roles in the general wellness of people all over the world.4,8,9 Nelumbo nucifera is one of two species of aquatic plant in the family Nelumbonaceae and well known by a number of names including Indian lotus, sacred lotus, bean of India, or simply lotus.6,7,10 In Asia, the petals are sometimes used for garnish, while the large leaves are used as a wrap for food, not frequently eaten.11 In Korea, the leaves and petals are used as a tisane.11,12 Young lotus stems and the roots are also used in traditional Asian herbal medicine.6,13 The main objective of this research was assess the inhibitory effect of leaf extracts of lotus (Nelumbo Nucifera) against dental caries etiological factors.

Materials and Method

Leaves of lotus were collected from the pond of Songjeng-ri, district of Gyeongsangnam-do. Leaves (500 g) were ground with pestles in liquid nitrogen at ~70°C and homogenized prior to extraction experiments. Hot water was used as extraction solvent. Extract was filtered and the filtrate was diluted to 5000 mL with hot water in a volumetric flask. The ultrasound extraction was carried out using an ultrasonic bath. The sample was treated with ultrasound at 65°C for a given duration. The sample was evaporated to dryness under reduced pressure and controlled temperature by using rotary vacuum evaporator until evaporation. Six strains of dental caries causative organisms i.e., Streptococcus mutans, Streptococcus mitis, Streptococcus sobrinus, Lactobacillus acidophilus, Actinomyces spp., and Nocardia spp. were obtained from the Korean Collection for Type Cultures, (KCTC).

Antibacterial assay was performed using agar well diffusion method.14 Plates were prepared and 0.1 ml of Brain Heart Infusion (BHI) was added spread with a sterile spreader. A well was made in the centre of plate with the help of a cork borer. 100 ul test compound was introduced into the well and the plates were kept in a refrigerator for diffusion for 30 min and then incubated overnight at 37°C. 105 CFU/ml of isolates were inoculated on nutrient agar. Growth was monitored by measuring turbidity at 490 nm (Microplate Reader, Germany).

Various concentrations of lotus extract were prepared (0 mg/ml, 1.0 mg/ml, 2.0 mg/ml, 4.0 mg/ml, 6.0 mg/ml, 8.0 mg/ml, 10.0 mg/ml). The MIC was determined using a standard susceptibility broth dilution technique. Overnight cultures of oral bacteria were diluted to 1×10^7 CFU/ml and inoculated into 96-microwell plate containing BHI. The cultures were incubated overnight at 37°C and the MIC recorded as the lowest concentration inhibiting growth. The antibacterial activity was interpreted by measuring the diameter of clear zone of inhibition in mm. To analyze GTA, each strain inoculated with an overnight culture in Todd Hewitt (Oxoid) at 37°C for 24 hours. Then it was transferred to 20 ml of BHI broth (2% w/v) and left to incubate at 37°C for 24 hours. The culture was centrifuged at 10,000 g for 20 min at 4°C. The supernatant fluid was removed and protein precipitated with 700 ml methanol overnight at 4°C. The supernatant was discarded and the precipitate dissolved in 0.6 M potassium phosphate buffer pH 6.8 (PB) and dialysed at 4°C with continuous gentle stirring against PB for 24 h. After centrifuging at 10000 g for 5 minutes, the supernatant was removed and the precipitate extracted again under
Table 1. Antibacterial activity of Nelumbonucifera against dental caries and adult periodontists. Zones of inhibition in mm

<table>
<thead>
<tr>
<th>Strains</th>
<th>Concentration mg/ml</th>
<th>0</th>
<th>1.0</th>
<th>2.0</th>
<th>4.0</th>
<th>8.0</th>
<th>16.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus mutans</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Streptococcus mitis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Streptococcus sobrinus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Actinomyces spp.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Nocardia spp.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. MIC values of Nelumbo nucifera against dental caries and adult periodontists

<table>
<thead>
<tr>
<th>Strains</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus mutans</td>
<td>0.112±0.018</td>
<td>0.102±0.007</td>
<td>0.097±0.007</td>
<td>0.086±0.007</td>
<td>0.079±0.012</td>
<td>0.070±0.009</td>
</tr>
<tr>
<td>Streptococcus mitis</td>
<td>0.113±0.016</td>
<td>0.099±0.004</td>
<td>0.097±0.007</td>
<td>0.086±0.007</td>
<td>0.078±0.013</td>
<td>0.063±0.009</td>
</tr>
<tr>
<td>Actinomyces spp.</td>
<td>0.109±0.018</td>
<td>0.099±0.004</td>
<td>0.097±0.007</td>
<td>0.086±0.007</td>
<td>0.076±0.011</td>
<td>0.062±0.008</td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
<td>0.102±0.007</td>
<td>0.095±0.004</td>
<td>0.092±0.006</td>
<td>0.086±0.006</td>
<td>0.076±0.011</td>
<td>0.062±0.006</td>
</tr>
<tr>
<td>Nocardia spp.</td>
<td>0.108±0.013</td>
<td>0.098±0.005</td>
<td>0.094±0.002</td>
<td>0.086±0.005</td>
<td>0.078±0.009</td>
<td>0.068±0.009</td>
</tr>
</tbody>
</table>

the same conditions. The supernatant fluid (=crude enzymes) stored at –20°C. GTA was assayed indirectly by measuring the formation of fructose from sucrose with a fructose assay kit (kit no. F A-20, Sigma-Aldrich, UK) used as in the manufacturer’s instructions. Lotus extracts were dissolved in 0.6 M potassium phosphate buffer pH 6.8 (PB). Glucan was measured at 1 min intervals for 15 min with a spectrophotometer. Controls contained PB alone.

Control and repeat tests were analyzed by a one-sample t test with values above the 95% confidence interval considered significant (P <0.05). The difference in group mean values among in vivo treated groups were analyzed by one way analysis of variance followed by Student Newman Keuls (SNK) multiple comparisons test. In some cases the paired t-test was used for comparisons.

Results
Lotus extracts by hot water inhibited the growth of Streptococcus mutans, Streptococcus mitis, Streptococcus sobrinus, Lactobacillus acidophilus, Actinomyces spp and Nocardia spp. Among these strains, Actinomyces spp mg/ml was most effective followed by Nocardia spp. Streptococcus mitis strains were most resistant to the extracts followed by Nocardia spp. The activities of lotus extracts were half-fold than those of the standard antibiotics. As the concentration increased the inhi-
bition effect was also increased. Actinomyces spp. and Nocaridia spp. showed a highest inhibition effect of 3.0 and 3.5 respectively where as S. mitis and L. acidophilus showed a lesser inhibition effect at 50% level (Figure 1).

The antimicrobial activity of hot water extract of lotus leaves against strains causing human dental caries (S. mutans, S. mitis, S. sobrinus, L. acidophilus, Actinomyces spp., and Nocardia spp.) were measured by measuring the zone of inhibition in disc diffusion method (Table 1). To find out more accurate concentration of inhibition effect, MIC value for leaf extract against the bacterial strains were done by serial dilution method (Table 2). Actinomyces spp. was a highest inhibition with a 2 mg/ml and next was Nocardia spp (5mg/ml). In this study, a wide range of human pathogenic microorganisms were examined, including gram-negative bacteria. This may partly indicate that the leaf extracts of lotus have broad inhibitory activities to pathogenic microorganisms and are promising to act as potential antibacterial agents from natural plant sources. Active compounds present in the crude hot water extracts show the antibacterial activity with the dose dependant manner. If the active principle is present in high quantities, there could be other constituents exerting antagonistic effects of the bioactive compounds. So, use of the crude hot water extract of this plant as an agent to control microbial pathogens needs further extensive research for their better economic and therapeutic utilization. The findings from this work may add to the overall value of the medicinal potential hot water extract of leaf extract of lotus. Further phytochemical studies are required to determine the purified fractions/bioactive compounds responsible for the antibacterial activities of these species, which could serve as useful sources for new antimicrobial agents. From the above studies it can be concluded that the hot water extracts of Leaf extract of lotus exhibit significant antibacterial activity against pathogenic bacteria. Therefore this lotus leaf may be act as another source of natural antibiotic. This study reaffirms the methano-medicinal property of lotus. The inhibitory effects of leaf extracts of lotus on glucan synthesis by crude GTA obtained from six dental cariess were examined as a function of its concentrations were shown in Table 3. The inhibitory effects were gradually expressed as the relative inhibition rate produced at a certain sample concentration as compared to the amount produced in the control. All of the tested stains showed the inhibitory activity ranging 6.5% at 1.0 mg/ml for Actinomyces spp. to 44.2% 16.0 mg/ml for S. mitis.

Discussion
Plant products are of interest as a source of safer or more effective substitutes for synthetically produced antimicrobial agents and, as such, could have an anti-cariogenic role in food products, oral products and medicines. Many studies have demonstrated the antibacterial effect of plant extracts against oral bacteria. In this study, the plant extract of lotus was investigated antimicrobial effects on growth and GTA in six strains with dental caries. The results show that the lotus extracts shown to inhibit growth and inhibited GTA by six strains with dental caries including.

The extract of lotus tested for the first time here was most active against six strains. Although growth was inhibited, there was no rapid decrease in six species, which would be expected with bactericidal activity. Shapiro et al showed that Rosmarinus officinalis and Smullen officinalis essential oils inhibited the growth. The extracts of R. officinalis leaves had an MIC of 24 mg/ml against S. mutans ATCC 25175. This was 32-fold lower than that determined in the present study (8 mg/ml), possibly because an aqueous infusion of R. officinalis leaves in buffer was used compared to the hot water extract used here. The Buddhists usually eat meal with the rice for boiling which wrapped the leaf of lotus. So the author avoided methanol, ethanol, or propanol as a leaf extraction solvent, but used hot water instead. Some species, Terminalia bellirica, Emblica officinalis, Syzygium aromaticum, in India possess antimicrobial activity against pathogens causing dental caries. They reported that their plant extracts are 2-3 folds more effective than common antibiotics and these plants are very safe and have acceptable taste. The extract of lotus was less active against Gram-negative bacteria. However, antimicrobial activity of lotus is similar to that of antimicrobial and anti-oral malodor efficacy of Schisandra chinensis extracts against oral pathogens.

Glucon formation in dental plaque mediates binding of S. mutans and S. sanguis to one another as well as to other bacteria. The results showed that the combined inhibition of growth and GTA inhibited plaque formation in-vitro. The inhibition of growth and GTA in extract of lotus was similar to those seen by extracts of other plants. For example, the degrees of GTA inhibition of Streptococcus mutans by Dioscorea batatas and Prunella vulgaris extract were similar to the result by lotus. Leaves of Nelumbo nucifera contain flavonol micquelanin (Quercetin 3-O-glucuronide), alkaloids (+)-1(R)-coclaurine, and (-)-1(S)-norcoclaurine which have antimicrobial activity against cariogenic bacteria. Water extract from Nelumbo nucifera has a possible functional cosmetic agent such as tyrosinase inhibition, DOPA-oxidase inhibition, and anti-wrinkle effect.

Conclusion
In conclusion, the extract of N. nucifera was effective in reducing on the glucosyl transferase activity of microbial strains causing dental caries in-vitro.

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References


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