# INHIBITIONS OF ACTIVITIES AND GROWTH OF *SALIX GRACILISTYLA* AGAINST DENTAL CARIES

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## ABSTRACT

The degree of inhibition of activities and growth on five strains of dental caries were estimated by the leaf extracts of *Salix gracilistyla*. The strains were *Streptococcus mutans, Streptococcus mitis, Streptococcus sobrinus, Lactobacillius acidophilius,* and *Actinomyces* spp. which were the main causal bacteria for dental caries. The extraction solvent was ethanol. Various concentrations of leaf extract were prepared (0 ㎎/㎖, 1.0 ㎎/㎖, 2.0 ㎎/㎖, 4.0 ㎎/㎖, 6.0 ㎎/㎖, 8.0㎎/㎖, 10.0 ㎎/㎖). Among these strains, *S. sobrinus* was most effective inhibited by leaf extract and next followed by *Actinomyces* spp. *S. mitis* strain was most resistant to the extracts followed by *L. acidophilius.* The minimal inhibitory concentration (MIC) values against five strains were varied from 4.0 mg/ml to 8.0 mg/ml against antimicrobial activity. *S. mitis* has high MIC value with 8.0 mg/ml. As the concentration increased the inhibition effect was also increased. *S. mutans, S. sobrinus,* and *Actinomyces* spp. showed a highest inhibition effect growth, whereas *S. mitis* and *L. acidophilius* showed a lesser inhibition effect at 50% level. The findings from this work may add to the overall value of the medicinal potential ethanol extract of leaf extract of *S. gracilistyla*.

**Keywords:** *Salix gracilistyla,* dental caries, *Streptococcus mutans*.

## INTRODUCTION

Dental caries is a demineralization of the tooth surface caused by bacteria. Dental caries is a multifactorial disease that results from the interaction between the bacterial biofilm (i.e., dental plaque), the environment (e.g., diet, saliva composition and flow rate, fluoride exposure), and the tooth structure. There is a long history of dental caries. Over a million years ago, hominids such as Australopithecus suffered from cavities (Suddick and Harris, 1990). Dental caries usually occurs in children and young adults but can affect any person. According to the WHO report, dental caries remains a major public health problem in most industrialized countries, affecting 60–90% of school children and the vast majority of adults (Petersen and Lennon, 2004). It is also the most prevalent oral disease in many African, Asian, and Latin American countries.

The mouth contains a wide variety of oral bacteria. The principal causative agents are a group of *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus* species among them (Balakrishnan et al., 2000). 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. Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen, is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes, obesity, and dental caries (Sales et al., 2012). Dental caries and periodontal diseases are the most prevalent oral infectious diseases that cause significantly

impact a person's overall health, having considerable economic impact, if not adequate treated (Touger-Decker and Loveren, 2003). The potential role of the medicinal plants as inhibitors of α-amylase has been reviewed by several authors (Sales et al., 2012).

*Salix gracilistyla* Miq. is one of two species of aquatic plant in the family Salicaceae and low spreading shrub, not over 2 m high (Lee, 2007). *S. gracilistyla* is a species of willow native to Japan, Korea, and China known in English as rose-gold pussy willow. *S. gracilistyla* is easily recognized by its greyish elliptic leaves which have numerous prominent lateral veins at an angle of less than 45 degrees to the midrib, and large persistent stipules **(**Lee, 2007). This species was distributed near the streams and local rivers. The objective of the research is to investigate that the leaf extract should have become help in prevention of main etiological agents of dental caries.

## METHODOLOGY

*Salix gracilistyla* was collected from the wetlands of Weonsa, district of Sacheon-ci, Gyeongsangnam-do. Leaves (500 g) were ground with pestles and liquid nitrogen at –70℃ and homogenized prior to beginning extraction experiments. The extraction solvent was ethanol. Extracted sample was filtered and the filtrate diluted to 5000 mL with ethanol in a volumetric flask. The ultrasound extraction was carried out using a ultrasonic bath (5510, Branson, USA). 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 (N-1001S-W, Eyela, Tokyo, Japan) until evaporation.

Five strains with dental caries, *Streptococcus mutans, Streptococcus mitis, Streptococcus sobrinus, Lactobacillius acidophilius,* and *Actinomyces* spp. were obtained from the Korean Collection for Type Cultures, KCTC. Antibacterial assay was performed using agar well diffusion method (Choi et al., 2007; Huh, 2013). 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℃. $10^5$ CFU/ml of isolates were inoculated on nutrient agar. Growth was monitored by measuring turbidity at 490 nm (Microplate Reader, Germany).

Various concentrations of leaf extract were prepared (0 ㎎/㎖, 1.0 ㎎/㎖, 2.0 ㎎/㎖, 4.0 ㎎/㎖, 6.0 ㎎/㎖, 8.0㎎/㎖, 10.0 ㎎/㎖). The MIC was determined using a standard susceptibility broth dilution technique. Overnight cultures of oral bacteria were diluted to $1\times10^7$ CFU/㎖and inoculated into 96-microwell plate containing BHI. The cultures were incubated overnight at 37℃ 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.

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 (Zar, 1984). In some cases the paired t-test was used for comparisons.

## RESULTS AND DISCUSSION

Leaf extracts by hot water inhibited the growth of *Streptococcus mutans, Streptococcus mitis, Streptococcus sobrinus, Lactobacillius acidophilius,* and *Actinomyces* spp. Among these strains, *S. sobrinus* was most effective inhibited by leaf extract next followed by *Actinomyces* spp. *S. mitis* strain was most resistant to the extracts followed by *L. acidophilius* (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 4.0 mg/ml. Next strains were *S. mutans* and *S. sobrinus* (5.0 mg/ml). *S. mitis* has high MIC value with 8.0 mg/ml. The activities of leaf extracts were half-fold than those of the standard antibiotics. As the concentration increased the inhibition effect was also increased. *S. mutans, S. sobrinus,* and *Actinomyces* spp. showed a highest inhibition effect growth, whereas *S. mitis* and *L. acidophilius* showed a lesser inhibition effect at 50% level (Figure 1).

In this study, although we did not examined wide range of human pathogenic microorganisms, this might partly indicate that the leaf extracts of leaf had inhibitory activities to pathogenic microorganisms and are promising to act as potential antibacterial agents from natural plant sources. 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 anticariogenic role in food products, oral products and medicines. Many studies have demonstrated the antibacterial effect of plant extracts against oral bacteria (Ross et al., 1980; Tichy and Novak, 1998; Morgan et al., 2001). In this study, the plant extract of leaf was investigated antimicrobial effects on growth in five strains with dental caries. The results show that the leaf extracts shown to inhibit growth on all strains with five studied dental caries.

Shapiro et al. (1994) showed that *Rosmarinus officianalis and Smullen officianalis* essential oils inhibited the growth of extracts of *R. officianalis* leaves had an MIC of 24 mg/ml against *S. mutans* ATCC 25175 (Larsen et al., 1996). This was 4.8-fold higher than that determined in the present study (5.0 mg/ml), possibly because an aqueous infusion of *R. officianalis* leaves in buffer was used compared to the ethanol extract used here. Willow based herbal products containing salicin and its derivatives can be used to alleviate aches and fevers (Paunonen et al., 2009). Willow (genus *Salix*) with its high salicylate content is a promising source of herbal drugs.

Willow bark was traditionally used in folk medicine as cure for headache, fever and pain by pre-industrial cultures (Ruuhola, 2001). Leroux isolated salicin in crystalline form as far back as 1829, and thus salicin was the first recorded glycoside (Pierpoint, 1994). The discovery of salicin eventually led to the invention of its synthetic derivative, acetyl salicylic acid (aspirin) by Hoffman and Dreser, in 1897 (Pierpoint, 1994). In addition, the main degradation products of salicylates were salicin, 6-HCH (6-hydroxy-2-cyclohexenone) and catechol, both in enzymatic and in pHmediated decomposition.

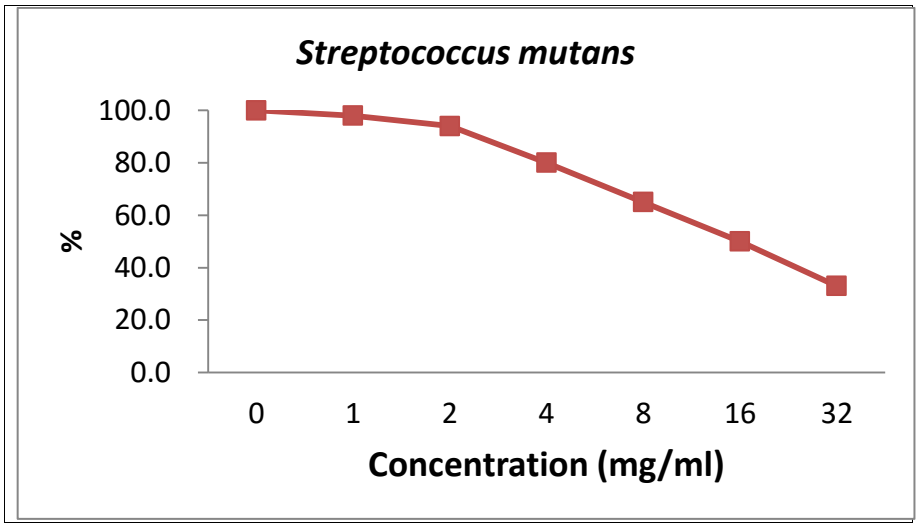
Leaves of *S. gracilistyla* have antimicrobial activity against cariogenic bacteria. However, the extracts used in this study are natural ingredients and may be acceptable for use in food products as well as dental preparations. The starting materials for these extracts were readily available all year round, were relatively inexpensive and preparation of the extracts was relatively easy.

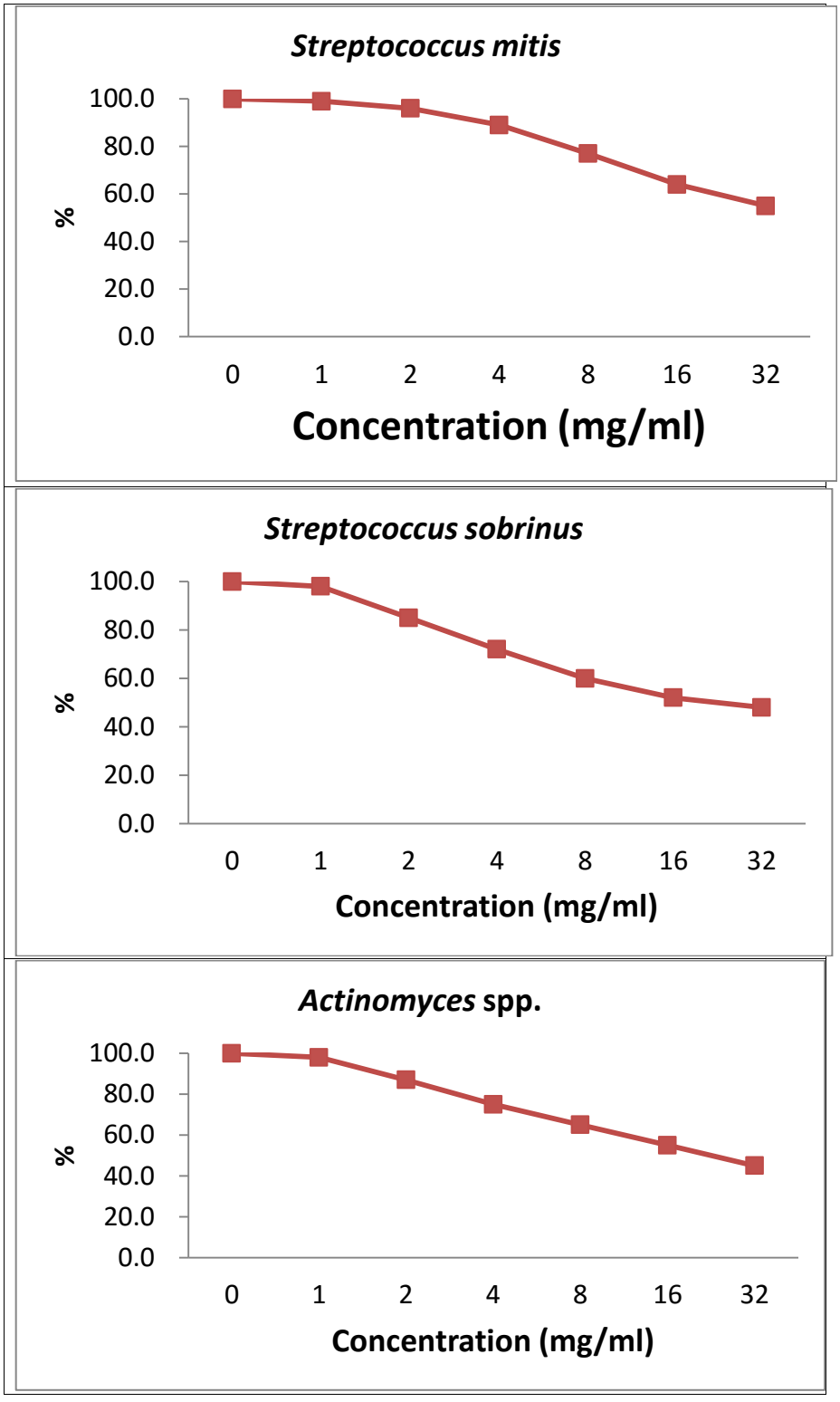
**Table 1.** Antibacterial activity of *Salix gracilistyla* against dental caries. Zones of inhibition in mm ± standard deviation.

| Strains | Concentration (mg/Mℓ) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1.0 | 2.0 | 4.0 | 8.0 | 16.0 |
| *Streptococcus mutans* | - | - | - | 1.7±0.6 | 3.8±0.7 | 5.2±1.1 |
| *Streptococcus mitis* | - | - | - | - | 2.1±0.5 | 3.3±0.8 |
| *Streptococcus sobrinus* | - | - | 1.6±0.4 | 2.0±0.8 | 4.2±1.2 | 6.8±1.3 |
| *Actinomyces* spp. | - | - | 1.1±0.2 | 2.0±0.6 | 3.7±0.9 | 5.4±0.8 |
| *Lactobacillius acidophilius* | - | - | - | 1.1±0.3 | 2.3±0.8 | 4.5±1.5 |

**Table 2.** MIC values on leaf extracts of *Salix gracilistyla* against dental caries.

| Strains | Concentration (mg/Mℓ) | | | | | | | | | | | MIC (mg/Mℓ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 | |
| *S. mutans* | + | + | + | + | + | + | - | - | - | - | - | 5.0 |
| *S. mitis* | + | + | + | + | + | + | + | + | - | - | - | 8.0 |
| *S. sobrinus* | + | + | + | + | + | - | - | - | - | - | - | 5.0 |
| *Actinomyces* spp. | + | + | + | + | - | - | - | - | - | - | - | 4.0 |
| *L. acidophilius* | + | + | + | + | + | + | - | - | - | - | - | 6.0 |




Streptococcus mitis
100.0
80.0
60.0
40.0
20.0
0.0
%
0
1
2
4
8
16
32
Concentration (mg/ml)
Streptococcus sobrinus
100.0
80.0
60.0
40.0
20.0
0.0
%
0
1
2
4
8
16
32
Concentration (mg/ml)
Actinomyces spp.
100.0
80.0
60.0
40.0
20.0
0.0
%
0
1
2
4
8
16
32
Concentration (mg/ml)

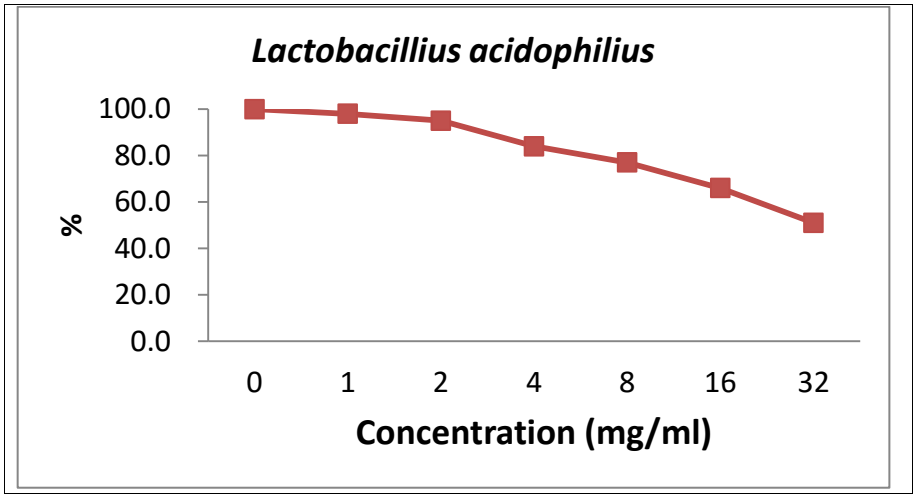



**Figure 1.** Relative growth ratio of dental caries on leaf extracts of *Salix gracilistyla*.Unit: mg/ml.

## CONCLUSIONS

The leaf extract of *S. gracilistyla* tested for the first time here was most active against five strains. Although growth was inhibited, there was rapid decrease in five species which would be expected with bactericidal activity.

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