

# Anticariogenic effect of *Sambucus williamsii* var. *Coreana* NAKAI (*S. Williamsii*) Extract on Against *Streptococcus Mutans*

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## Abstract

**Background/Objectives:** The purpose of the present study was to assess the anticariogenic effects of *Sambucus williamsii* var. *coreana* NAKAI (*S. williamsii*) extract against *Streptococcus mutans* (*S. mutans*).

**Method/Statistical Analysis:** *S. williamsii* purchased from Foodsynergy Co., Ltd. (Seoul, South Korea). Dried *S. williamsii* were then crushed and percolated in 70% ethanol, and concentrated *S. williamsii* extract was applied to *S. mutans* diluted to  $5 \times 10^5$  CFU/ml at the concentrations of 0, 2.5, 5, 10, 20, and 30 mg/ml. Then the colony-forming units (CFUs) were evaluated at 6 and 24 h.

**Findings:** The *S. williamsii* extract against *S. mutans* had an minimal inhibitory concentration (MIC) of 20 mg/ml and an minimum bactericidal concentration (MBC) of 30 mg/ml at 6 h, and an MIC of 10 mg/ml and an MBC of 20 mg/ml at 24 h. As the concentration of *S. williamsii* extract increased, the *S. mutans* was more clearly killed over time. The application of *S. williamsii* extract to *S. mutans* has an anti-dental-caries effect.

**Improvements/Applications:** *S. williamsii* extract promotes oral health promotion by inhibiting the growth of *S. mtans*. It could be used as an effective oral anticariogenic agent.

**Keywords:** *Sambucus williamsii* var. *coreana* NAKAI (*S. williamsii*), Anticariogenic agent, *Streptococcus mutans*, Anti-dental caries, Oral health.

## Introduction

Bacteria stay in the teeth, palates, gingiva, tongue, and buccal mucosa, which have suitable conditions for the bacterial characteristics<sup>[1]</sup>. In the world, dental caries (tooth decay) and periodontal (gum) diseases are the most common oral disease, known as cause of tooth loss among adults<sup>[2]</sup>. Dental caries is one of the most common and costly diseases. Caries are caused by of the bacteria *Streptococcus mutans* (*S. mutans*). It is most

closely related to dental caries and is mainly found in dental plaque<sup>[3]</sup>. *S. mutans* is characterized by high acid resistance compared with other bacteria, and maintains its metabolism continuously even at a low pH<sup>[4]</sup>.

It is said that oral disease causes difficulty of food intake, pain, discomfort, and dissatisfaction with appearance<sup>[5]</sup>. Tooth loss has been reported to reduce the chewing capacity, narrowing the range of foods that one can consume, and to decrease the quantity and quality of meals, making it difficult to maintain one's health and fitness<sup>[6]</sup>. Therefore, oral health refers to the state of the oral-related tissues that allow people to eat, speak, and socialize without discomfort, thereby contributing to systemic health<sup>[7]</sup>. For this reason, health promotion refers to healthcare that changes the individual's environment or health habits and brings about a higher level of well-being<sup>[8]</sup>.

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Resistant bacteria were found in most antibiotics commonly used in the treatment of infectious diseases, making it difficult to maintain oral health<sup>[9]</sup>. Therefore, research on substitutes is underway, and research on natural products to search for new materials with pharmacological activity has been actively conducted worldwide for plants and the like<sup>[10]</sup>.

*Sambucuswilliamsii* var. *coreana* NAKAI (*S. williamsii*) is a deciduous shrub belonging to Caprifoliaceaesambucus, which grows in the marshes and valleys of mountains<sup>[11]</sup>. *S. williamsii* is a medicinal substance widely used in folk remedies that is known to alleviate swelling and pain and improve blood circulation, and to have anti-inflammatory action<sup>[12]</sup>. Therefore, the purpose of this study was to apply *S. williamsii* to the typical dental-caries-inducing bacteria, *S. mutans*, to confirm the possibility of its use as an anti-caries material and to promote oral health.

## Method

**S. williamsii preparation:** The dried *S. williamsii* used in this study was purchased from Foodsynergy Co., Ltd. (Seoul, South Korea). After adding 70% ethanol to crushed *S. williamsii*, an extract was obtained at 60°C for 12 h. The extract was filtered using filter paper (Whatman No. 2, Tokyo, Japan), and the *S. williamsii* extract was concentrated using a rotary vacuum evaporator (N-1300E.V.S. EYELA Co., Tokyo, Japan). The concentrated *S. williamsii* was lyophilized using a freeze dryer (Ilshin Lab Co., South Korea). The sample was stored at -20°C after dilution.

**Bacterial Strains:** In this experiment, *S. mutans* (KCTC 3065/ATCC 25175) was used after the subculture in brain heart infusion (BHI) broth. *S. mutans* was incubated in BHI at 37°C for 24 h, and was diluted at a  $5 \times 10^5$  ratio.

**Antimicrobial activity by S. williamsii extract concentration:** 100 µL of cultured *S. mutans* ( $5 \times 10^5$  colony-forming units; CFUs/ml) was inoculated into a 24-microwell plate containing BHI broth in which *S. williamsii* extract was added at each concentration (0, 2.5, 5, 10, 20, and 30 mg/ml). The total volume of each mixture was 1 ml. The microplate was incubated anaerobically for 6 and 24 h at 37°C, and then the mixture in each well was uniformly smeared in an agar medium and then cultured at 37°C for 24 h to check the number of CFUs.

**Statistical Analysis:** Student's t-test was conducted to identify the changes that could have occurred after 6 and 24 hours. The difference in each concentration was evaluated through one-way analysis of variance (ANOVA), followed by the Tukey test at  $\alpha = 0.05$ . Significance analysis of the inhibitory effect was carried out using Ver. 21.0 (SPSS Inc., Chicago, IL, USA)

## Result and Discussion

In modern times, the concept of health has evolved into the concept of physical, mental, and social well-being, not merely the absence of physical illness, and as the relationship between quality of life and health has been emphasized, the interest in the impact of oral health on quality of life has also increased<sup>[13]</sup>. Due to the increased interest in oral health, the prevention rate of oral diseases is higher than those of other diseases, but people in developed and developing countries still suffer from oral diseases<sup>[14]</sup>.

Pathogens usually exist as normal bacterial flora, but when the number of specific bacteria increases or decreases in the normal bacterial flora in the oral cavity, it causes diseases like dental caries and periodontal disease, leading to pain and eating disorders, and results in tooth loss. It is also a major cause of bad breath and may cause social problems for the people afflicted with such condition<sup>[15]</sup>. Today, there is a comprehensive understanding of the concept of health, and the health behavior with regard to the teeth or oral conditions and their consequences must be considered together. Thus, general bodily health with poor oral health cannot be considered a healthy state<sup>[16]</sup>.

The widespread use of antibiotics has led to a dramatic decrease in the incidence of bacterial disease, but it has led to an increase in disease due to the bacteria that have become resistant to antibiotics<sup>[17]</sup>. For this reason, studies have been actively conducted on the separation and application of natural antimicrobials with superior antimicrobial activity and safety due to the side effects of synthetic antifungal agents, to prevent dental caries and to promote the avoidance of chemical substances<sup>[18]</sup>. Therefore, this study was conducted to apply *S. williamsii* extract as a substitute for chemical substances in the treatment and prevention of dental caries induced by *S. mutans*.

The antibacterial effect of *S. williamsii* extract against *S. mutans* was evident with increasing concentration and over time [Figure 1]. Figure 1(a) presents the change

in CFUs according to the application of *S. williamsii* extract after 6 h, and Figure 1(b), after 24 h. As shown in Figure 2, the death rate of *S. mutans* at 6 h depending on the concentration of *S. williamsii* extract was  $2.90 \times 10^7$  at 0 mg/ml,  $1.48 \times 10^5$  at 2.5 mg/ml,  $4.34 \times 10^4$  at 5 mg/ml,

$8.73 \times 10^2$  at 10 mg/ml,  $4.90 \times 10^1$  at 20 mg/ml, and 0 at 30 mg/ml. On the other hand, the death rate at 24 h was  $1.02 \times 10^{11}$  at 0 mg/ml,  $5.67 \times 10^7$  at 2.5 mg/ml,  $3.80 \times 10^7$  at 5 mg/ml,  $4.33 \times 10^6$  at 10 mg/ml, 0 at 20 mg/ml, and 0 at 30 mg/ml.

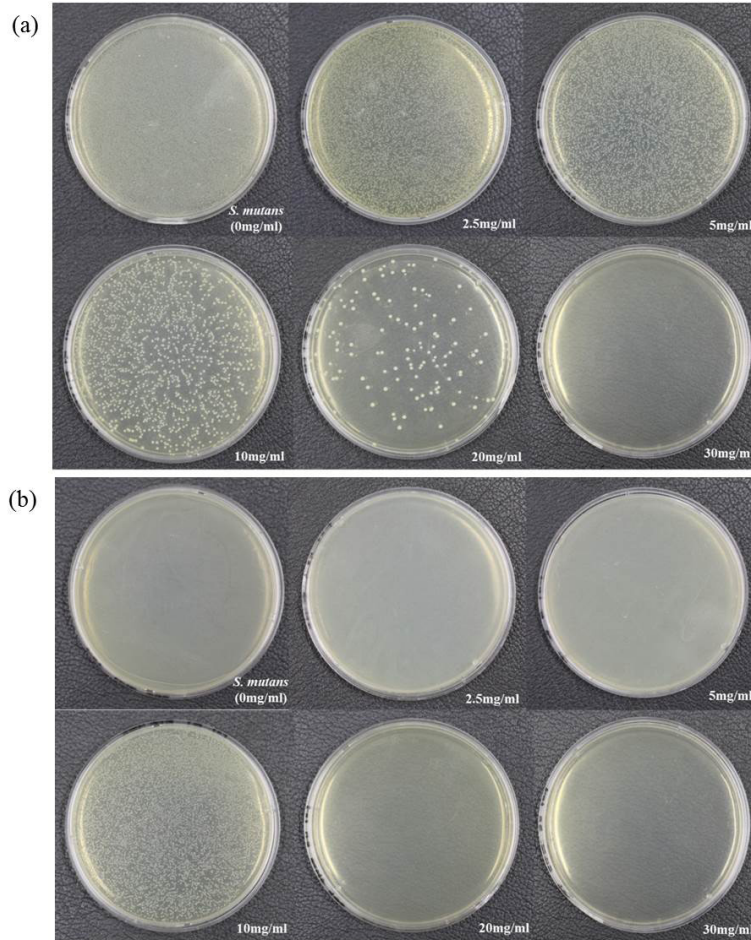


Figure 1. Anticariogenic activity of *S. williamsii* extract against *S. mutans* (A) after 6 h and (B)24 h

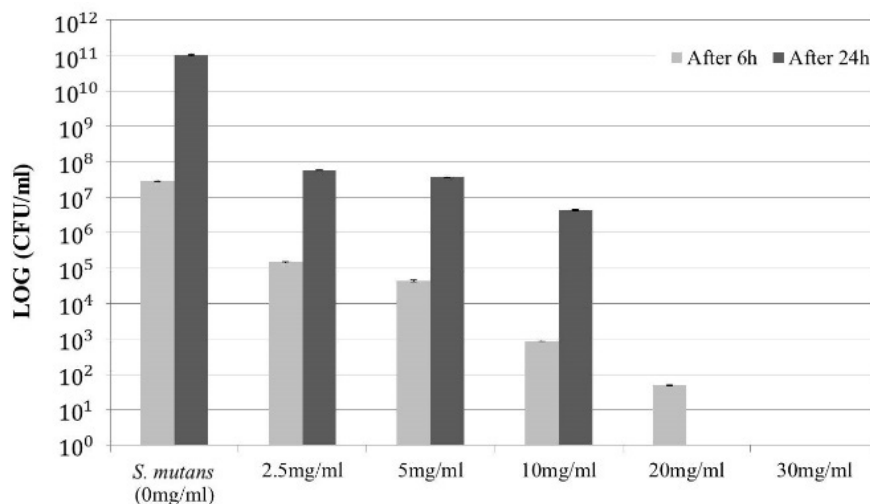


Figure 2. The survival rate of *S. mutans* by *S. williamsii* extract

According to statistical test for the significance of the difference between the death rates at 6 and 24 h, respectively, there was no significant difference from 20 mg/ml ( $P>0.05$ ). This means that the time did not affect the killing of the bacteria at 20 mg/ml. In the change

according to the concentration, there was a statistically significant evidence ( $P<0.05$ ), but in between groups, except for *S. mutans* (0 mg/ml), for which *S. williamsii* extract was not treated, there was no difference depending on the concentration [Table 1].

**Table 1. The mean±SD number of CFUs and P-value of the antibacterial activity by *S. williamsii* extract**

Group	S. mutans (0mg/ml)		2.5mg/ml		5mg/ml		10mg/ml		20mg/ml		30mg/ml		ANOVA P-Value
	Mean ±SD	t-test P-Value	Mean±SD	t-test P-Value	Mean ±SD	t-test P-Value	Mean ±SD	t-test P-Value	Mean ±SD	t-test P-Value	Mean±SD	t-test P-Value	
6h	2.90±0.1 10 <sup>7a</sup>	0.000*	1.48±0.4 10 <sup>5b</sup>	0.004*	4.34±0.8 10 <sup>4b</sup>	0.008*	8.73±0.9 10 <sup>2b</sup>	0.004*	4.90±0.1 10 <sup>1b</sup>	0.149	0 <sup>b</sup>	-	0.000*
24h	1.02±0.8 10 <sup>11a</sup>		5.67±0.3 10 <sup>7b</sup>		3.80±0.1 10 <sup>7b</sup>		4.33±0.2 10 <sup>6b</sup>		0 <sup>b</sup>		0.000*		

\*The significant difference for 6h and 24h comparison by student t-test ( $P<0.05$ )., \* Different letters (a and b) by the presented statistically significant result of the one-way ANOVA and post hoc Tukey HSD ( $P<0.05$ )

The results of this study proved that the application of *S. williamsii* extract to *S. mutans* has an anti-dental-caries effect. As the concentration of *S. williamsii* extract increased, the *S. mutans* was more clearly killed, showing a distinct antimicrobial activity over time. The minimal inhibitory concentration (MIC), which is the minimum concentration of bacteria that cannot be visibly detected, and the minimum bactericidal concentration (MBC), which the concentration at which the bacteria are completely killed, were 20 and 30 mg/ml, respectively, at 6 h. At 24 h, MIC and MBC were 10 and 20 mg/ml, respectively. The concentration at which the antimicrobial activity actively occurred, and at which the bacteria were completely killed, was confirmed to be 20 mg/ml. The statistical analysis showed no significant difference between the changes that occurred at 6 and 24 h at 20 mg/ml, which means that there was a difference in CFUs but there was no statistical difference between the antibacterial effects that occurred at 6 and 24 h.

As for the anti-dental-caries effect of natural extracts, a previous study reported that the MIC and MBC values of *Cyperusrotundus* extract on *S. mutans* in the study was 225 and 450 mg/ml<sup>[19]</sup>. When the fact that an antibacterial effect was shown in such study by applying an extract at a higher concentration compared to the present study is considered, the inhibitory effect against *S. mutans* was low compared to *S. williamsii* extract, and *S. williamsii* can be said to have a higher antibacterial effect. According to the study of Antonio et al.<sup>[20]</sup>, the antibacterial activities of *Coffeacaneophora* extract MIC and MBC for *S. mutans* were 7±2 and 160±0

mg/ml. This result showed that *S. williamsii* extract has a higher antimicrobial activity than *Coffeacaneophora* extract. Therefore, it can be concluded that *S. williamsii* extract has an excellent effect as an anticariogenic agent against *S. mutans*, and can thus be employed as a natural antibacterial agent in oral care products.

## Conclusion

In this study, the application of *S. williamsii* extract to *S. mutans* was shown to be effective in preventing dental caries. The *S. williamsii* extract against *S. mutans* had an MIC of 20 mg/ml and an MBC of 30 mg/ml at 6 h, and an MIC of 10 mg/ml and an MBC of 20 mg/ml at 24 h. In the near future, *S. williamsii* extract may conceivably be used as an effective anti-dental-caries substance for dental caries prevention as for natural medicines.

**Ethical Clearance:** Not required

**Source of Funding:** This study was supported by Basic Science Research Program through a National Research Foundation of Korea (NRF) grant funded by the Ministry of Science, ICT, and Future Planning (2017R1C1B5074410).

**Conflict of Interest:** Nil

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