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# A Study on the Antibacterial Effect of *Chamaecyparis Obtusa* Extract on the Causative Agent of Dental Caries

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**ABSTRACT**

We conducted to evaluate the antibacterial effect of the *Chamaecyparisobtusa* extract on *Sreptococcusmutans* (*S. mutans*). The strains used in this experiment were investigated for *S. mutans*. The activity of *Chamaecyparisobtusa*extract was measured the diameter (mm) of the clear zone by the paper disk (Φ8 mm) diffusion method and colony forming units (CFU). The significant difference among groups in one-way ANOVA and Duncan test.As a result, the ethanol extract of *Chamaecyparisobtusa* showed the strongest antimicrobial activity against *S. mutans*. The clear zone of *S. mutans* was found to be 14 mm, 15.5 mm and 17 mm at 20 mg/mL,30 mg/mLand at 40 mg/mL, respectively. In addition, the *Chamaecyparisobtusa* extract exhibited concentration-dependent inhibition of CFU over 1.25 mg/mL, with 100% inhibition at a concentration of 40 mg/mL. These results show that *Chamaecyparisobtusa* extract has anticariogenic effect on *S. mutans*. Therefore, *Chamaecyparisobtusa* extract can be used effectively in the prevention of oral diseases.

**Keywords**
Antibacterial activity, Dental caries, Plant extract, *Chamaecyparisobtusa*, *Streptococcus mutans*

## Introduction

Oral health is essential to general health and well-being. Good oral health enables individuals to eat and enjoy a variety of foods, communicate effectively, and have self-esteem, social confidence, and a good overall quality of life [1]. In fact, oral health affects every aspect of people's lives. To achieve oral health, oral health care is important [2]. With the increasing interest in oral health care, the prevention of oral diseases is now more prevalent than the prevention of other diseases. However, people in developed and developing countries still suffer from oral diseases [3].

According to a recent report of the World Health Organization (WHO), 60% of adults around the world suffer from oral diseases related to dental caries. This indicates that suffering from dental caries persists worldwide [4, 5]. Dental caries is a disease caused by complex actions of bacteria, dietary factors, and host factors. The role of bacteria is especially essential. The bacterium *Streptococcus mutans* (*S. mutans*) is known to be a major causative agent of dental caries [6]. It induces dental caries through its adhesion, proliferation, and acid production on the tooth surface [7]. *S. mutans* secretes an enzyme called glucosyl transferase (GTase) that induces dental caries outside or on the surface of the microbial body and breaks down sucrose in food to form insoluble glucan on the tooth surface. Glucan also attaches to the tooth surface with other microorganisms in the oral cavity to create a biofilm [8]. Therefore, to prevent and reduce dental caries, the growth of *S. mutans* and the formation of biofilms in the oral cavity should be inhibited.

Antibiotics, which are substances that inhibit the growth of microorganisms, have been widely consumed as therapeutic medicines until now. In recent years, however, due to the emergence of resistant bacteria from long-term administration of antibiotics, various side effects have appeared,

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which are considered very serious problems [9]. Accordingly, much attention has been paid to the development of antibiotics with low resistance using microorganisms and through the study of the antibacterial activity of natural products [10]. In recent years, as a part of basic research to prevent dental caries, interest in microbial inhibition using natural extracts has increased, and various studies on it have been conducted [11]. Magnolol and hinokiol [12], gymnemic acid [13], Japanese green tea extract [14], and cardinol [15] have been reported to have an anticaries effect on the oral cavity. Gold [16] and plum extract have been found to have antibacterial effects on cariogenic bacteria, and green tea, mulberry, and mate leaves extracts have been found to have growth inhibition effects on *S. mutans* [17].

Plant extracts have long been known to have antibacterial and antioxidant activities. In particular, essential oils, which are volatile secondary metabolites, have anti-inflammatory, antibacterial, and anti-allergic functions. Thus, they are being studied as natural pharmaceutical supplements or food preservatives [18]. In addition, the antibacterial effects of phytoncide and aroma essential oil, which are essential oils extracted from plants, are widely known [19].

Phytoncide is a volatile aromatic component of plants that is obtained by distilling plants with water vapor. This and other volatile components are composed of compounds such as phenolics, terpenoid, alkaloid, phenylpropane, acetogenin, and steroid, which have dozens to 200 species [20, 21]. Phytoncide essential oil has been reported to have antibacterial and antifungal effects [22].

A large amount of phytoncide with a peculiar scent is produced on the stems of *Chamaecyparisobtusa*, an evergreen coniferous tree of the genus *Charmaecyparis* of the *Cupressaceae* family, generally known as “hinoki cypress,” which grows wild in Japan and Taiwan and has been successfully cultivated in the southern part of Korea. [23]. From ancient times, such tree has been believed to have a natural healing effect. Recently, the essential oil extracted from the phytoncide on the stems of the tree has been found to have antioxidant and antibacterial effects. Thus, *Chamaecyparisobtusa* is used as a medicinal plant and for forest bathing [24].

This study was conducted to find out if a natural product with well-known antibacterial and antifungal effects can be used for therapeutic purposes against resident oral flora. It investigated the antibacterial activity of *Chamaecyparisobtusa* extract against dental caries and evaluated the potential of such extract for the prevention and treatment of dental caries.

## Materialsand Methods

### Bacterial culture

To measure the antibacterial activity, *S. mutans* (KCTC 3065/ATCC 25175) was incubated in Brain Heart Infusion (BHI; BD Bioscience, Franklin Lakes, NJ) at 37°C under microaerophilic conditions for 24 hours. The *S. mutans* was diluted to a concentration of $1\times10^5$ colony forming units (CFU/mL).

### Sample extraction

*Chamaecyparisobtusa* used in this study was grown in Gyeongsan, Gyeongsangbuk, South Korea, was purchased from Cheongmyeong Co., Ltd. After adding 70% ethanol to 100 g of crushed *Chamaecyparisobtusa*, extraction was done in a heated mantle at 60°C for 12 hours. The extract was filtered by using filter paper (Advantec No. 2, Toyo, Japan), and the *Chamaecyparisobtusa* extract was concentrated and lyophilized by using a rotary vacuum evaporator (N-1300E.V.S. EYELA Co.,Japan). The concentrated *Chamaecyparisobtusa* was

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lyophilized using a freeze dryer (Ilshin Lab Co., South Korea) to obtain the *Chamaecyparisobtusa* powder. The final samples were stored at -20°C until use.

**Disc diffusion method**

A paper disc was placed on top of BHI agar medium, and 100 μL of each experimental group (1.25, 2.5, 5, 10, 20, 30 and 40 mg/mL) was dropped onto an 8 mm filter paper disk (Advantec Toyo Kaisha, Ltd.). After keeping it at 37°C for 24 hours, the diameter of the clear zone was measured.

**Antibacterial test**

Mixed extracts were prepared at the 1.25 mg/mL, 2.5 mg/mL, 5 mg/ml, 10 mg/mL, 20 mg/mL, 30 mg/mL and 40 mg/mL concentrations, and *S. mutans*-containing solution of 100 μL was inoculated into the medium. The microplate was incubated anaerobically for 24 hours at 37°C, and then the mixture in each well was uniformly smeared in an BHI agar medium. After incubation for 24 hours, the number of CFU present in the agar plates was checked.

**Statistical analysis**

The software (IBM SPSS Statistics 24.0, SPSS Inc., Chicago, IL, USA) was used to evaluate significant differences in the antibacterial activity with one-way ANOVA. In post hoc analysis used the Duncan test at a 0.05 significance level

## Results

This study investigated the antibacterial effect of *Chamaecyparisobtusa* extract on *S. mutans*, the causative agent of dental caries. *Chamaecyparisobtusa* ethanol extract was isolated and concentrated to measure its antibacterial effect and properties against *S. mutans* using the paper disk method. The results are as follows (Figure 1). When *Chamaecyparisobtusa* extract was applied to *S. mutans* at concentrations of 20, 30, and 40 mg/mL, it inhibited the growth of the bacteria by 14, 15.5, and 17 mm, respectively. When *Chamaecyparisobtusa* extract was applied at concentrations of 20, 30, and 40 mg/mL, bacterial growth inhibition zones were observed in proportion to the increase in the concentration of the extract. The strongest antibacterial effect was observed in the 40mg/mL group, which showed a 17mm bacterial growth inhibition zone.

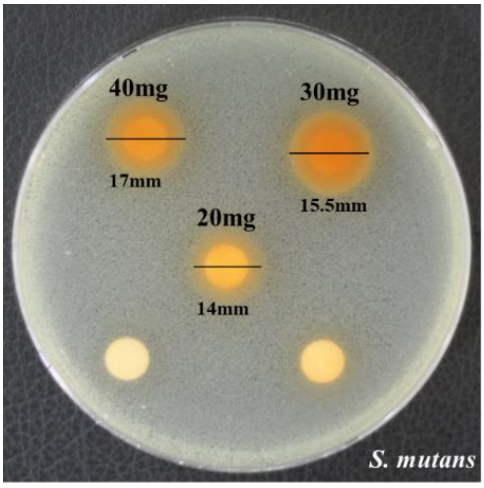

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**Figure 1. Antibacterial activity of the ethanol extract of *Chamaecyparisobtusa* on the growth of *S.mutans*via paper disk method.**

To observe the antibacterial activity of *Chamaecyparisobtusa*extract against *S. mutans*, *Chamaecyparisobtusa* extract was added to *S. mutans* at the concentrations of 1.25, 2.5, 5, 10, 20, 30, and 40 mg/mLon a BHI agar medium, respectively. After 24 hours, the number of CFU of the *S. mutans* and the growth inhibition rate compared to the control were measured. The results are as follows (Figure 2).

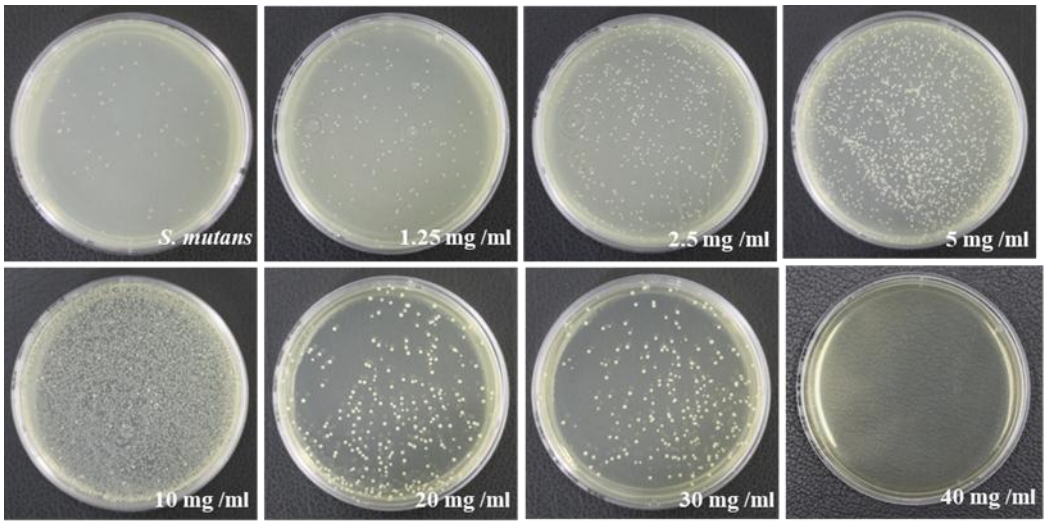


**Figure 2. Inhibitory effect of *Chamaecyparisobtusa* on biofilm formation *by S. mutans*, as observed by the number of CFU.**

In the control, which was not treated with *Chamaecyparisobtusa* extract, a high number of *S. mutans* bacteria was detected: $3.7\pm0.2 \times 10^{11}$mg/mL. In the medium to which 1.25 mg/ml of *Chamaecyparisobtusa* extract was added, the number of *S. mutans* CFU was $1.8\pm0.2 \times 10^{8}$ CFU; in the medium to which 2.5 mg/mLof the extract was added, $2.8\pm0.1 \times 10^{7}$ CFU; in the medium to which 5 mg/mLof the extract was added, $1.5\pm0.1 \times 10^{5}$CFU; in the medium to which 10 mg/mLof the extract was added, $5.8\pm0.2 \times 10^{3}$ CFU/ml; in the medium to which 20 mg/mLof the extract was added, $2.1\pm0.1 \times 10^{2}$CFU; in the medium to which 30 mg/mLof the extract was added, $1.5\pm0.1 \times 10^{2}$CFU; and in the medium to which 40 mg/mLof the extract was added, 0.0 CFU/ml. As shown in Figure 2, compared with the control, even in the medium to which only 1.25 mg/mLof the extract was added, the *S. mutans* colony formation was significantly reduced; and in the medium to which 40 mg/mLof the extract was added, no *S. mutans* colony formation was observed at all (Figure 3).

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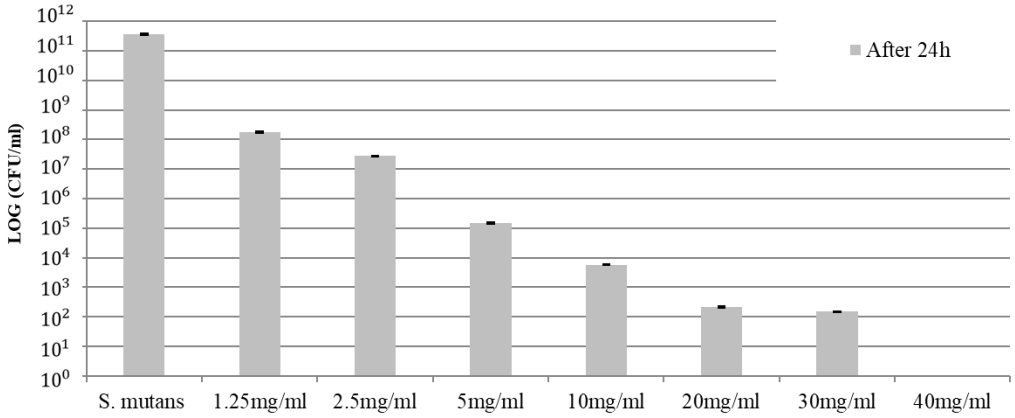


**Figure 3. Inhibitory effect of *Chamaecyparisobtusa* on the number of CFUsecreted by *S. mutans*.**

The statistical analyses showed that the antibacterial effect of *Chamaecyparisobtusa*extract on *S. mutans* significantly differed according to the concentration of the extract that was applied (Table 1). The CFU of the control was $3.7\pm0.2 \times 10^{11a}$, and the number of CFUs decreased significantly as the concentration of the *Chamaecyparisobtusa*extract increased ($p < 0.05$). However, there was no statistically significant difference in the number of CFU when 20-40 mg/mLof the extract was applied ($p > 0.05$). At the 40mg/mLconcentration of the extract, no CFU appeared, so 40 mg/mLcan be considered the optimal concentration in terms of antibacterial power.

**Table 1.** Antibacterial activity of ethanol extract of *Chamaecyparisobtusa* via number of CFU.

| Group | *S. mutans* | 1.25 mg/mL | 2.5 mg/mL | 5 mg/mL | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| 24h | $3.7\pm0.2 \times 10^{11a}$ | $1.8\pm0.2 \times 10^{8b}$ | $2.8\pm0.1 \times 10^{7c}$ | $1.5\pm0.1 \times 10^{5d}$ | $5.8\pm0.2 \times 10^{3e}$ | $2.1\pm0.1 \times 10^{2f}$ | $1.5\pm0.1 \times 10^{2f}$ | $0.0^{f}$ | 0.000 |

***The significant difference among groups in one-way ANOVA. Different letters (a, b, c, d, e and f) by the presented significant result of the post hoc Duncan test ($p<0.05$).**

## Discussions

*S. mutans*, a resident oral flora, causes dental caries, which leads to tooth loss. Therefore, to prevent oral disease, it is important to manage disease-causing bacteria among resident oral flora [25]. However, the use of antibiotics may discolor the teeth or dry the mouth. Therefore, studies have been conducted to find out if plant extracts with antibacterial effects can be used as antibacterial agents without the side effects of antibiotics [26]. In particular, studies have been conducted on the prevention or suppression of oral diseases through bacteriostasis or sterilization of oral bacteria using natural extracts [27]. Many of these studies used the *Chamaecyparisobtusa* leaf extract, as its biological effects are known. In particular, its antibacterial and anti-inflammatory activities have been reported [25]. As *Chamaecyparisobtusa* essential oil has been found to have high effects on physiological activities, such as anti-inflammatory and antioxidant effects as well as antibacterial and insect repellent effects that alleviate atopy and prevent mold and mites, research and other efforts to find more functional substances are continuing [28].

According to a paper on the environmental change and inhibition of adhesion of *S. mutans* to the natural extract, gold, as an antibacterial activity medium, when *S. mutans*was administered to the culture medium with a concentration of 50 mg/mL, the polysaccharide formation was reduced, which showed antibacterial power [29]. However, the *Chamaecyparisobtusa* extract that was used in this study showed a bacteria killing effect at the lower concentration of 40 mg/mL, which confirmed its stronger antibacterial activity compared to that of gold.

According to a previous study, the measurement of the clear zone of *Chamaecyparisobtusa* extract against Gram-positive and Gram-negative bacteria that cause food poisoning showed antibacterial effects on all the bacteria [30]. Meanwhile, the results of a study that showed the antibacterial activity of curcuma longa, ginger, and finger root against *S. mutans* were similar to the results of this study [31].

Therefore, the results of this study confirmed the antibacterial activity of *Chamaecyparisobtusa* extract against cariogenic bacteria. Particularly, *Chamaecyparisobtusa* extract has antibacterial activity against *S. mutans*, which is a typical bacterium that induces dental caries. It can be said that the antibacterial activity of an active ingredient can be exhibited by its penetration into cells or its inhibition of the synthesis of the bacteria with cells [32]. Based on the above results, *Chamaecyparisobtusa* extract is expected to be used as an antibacterial agent against *S. mutans*.

## Conclusion

This study evaluated the antibacterial activity of *Chamaecyparisobtusa* extract, which has been used as medicine for a long time, against *S. mutans*, a cariogenic bacterium. The antibacterial power of *Chamaecyparisobtusa* extract was found to be higher at higher concentrations. Its optimal concentration in terms of its antibacterial activity against *S. mutans*was 40 mg/mL, at which concentration the bacteria were killed completely. As *Chamaecyparisobtusa* extract exhibited an antibacterial effect against *S. mutans*, the causative agent of dental caries, we suggest its applicability as a natural antibacterial agent against oral cavities.

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