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# Antimicrobial Activity of Coptischinensis Extract against Dental Caries

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## Abstract

**Background/Objectives:** Many microorganisms reside in the oral cavity, and they are referred to as oral-resident bacteria. Oral microorganisms are an important cause of oral diseases, and as such, many studies have been conducted to control them. This study aimed to investigate the antimicrobial activity against *S. intermedius* and *S. gordoii* among the oral-resident bacteria using *Coptischinensis*, a natural extract.

**Method/Statistical Analysis:** To investigate the antimicrobial activity of *Coptischinensis*, the clear-zone confirmation method using filter paper and colony-forming unit (CFU) was used to check the bacterial proliferation. As the concentration of *Coptischinensis* increased, the size of the clear zone also increased. As a result of the CFU, bacterial proliferation was not observed in the experimental group as compared to the control group.

**Findings:** The results of this study proved the antimicrobial activity of *Coptischinensis* against the oral-resident bacteria *S. intermedius* and *S. gordoii*. The clear zone results showed that *S. intermedius* and *S. gordoii* were larger in size than the control group.CFU showed a large amount of colony in the control group but not exist colony in the experimental group.The result showed its potential as an antimicrobial agent applicable to oral-resident bacteria.

**Improvements/Applications:** *S. intermedius* and *S. gordoii* bacteria found in the oral cavity showed antimicrobial effect on the extracts. *Coptischinensis* was confirmed to be a preventative and therapeutic agent of tooth decay. In conclusion, the possibility that it can be applied to various intraoral antibacterial products in the future.

***Keywords:*** *Antibacterial activity, Coptischinensis, Dental caries, Dental pathogens, Microorganism.*

## Introduction

In the 21st century, with the Westernization of people's lifestyles, oral diseases have become diverse and have emerged as a serious social problem. According to a recent WHO report on dental diseases, 60% of adults worldwide are suffering from dental caries and periodontal diseases[1].The most important factor causing oral diseases, as represented by dental caries and periodontal diseases, has been known to be dental plaque, composed of oral-resident bacteria that form a cluster on the tooth surface, or on and beneath the gingiva[2]. It has been reported that more than 500 types of bacteria reside in the human oral cavity, and that $10^8$-$10^9$ CFU/mg of bacteria are present in dental plaque[3]. Dental caries is an oral and maxillofacial disease that

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has plagued humanity since the ancient times, and its harmful effects have become a serious problem both economically and socially in both developing and developed countries[4]. Dental caries occurs when the bacteria attached to the tooth surface form dental plaque, and then the bacteria of the dental plaque generate acids by fermenting the carbohydrates obtained from food[5]. Among the many bacteria present in dental plaque, oral streptococci is considered a causative bacteria of dental caries[6]. In particular, the *Streptococcus gordonii* (*S. gordonii*) in the oral cavity is known to play an important role in the adhesion of bacteria in the oral cavity by specifically binding to a specific glycoprotein of saliva[7]. *Streptococcus intemedius*(*S. intemedius*) is an oral-resident bacterium clinically characterized by forming abscesses in the oral cavity[8]. Moreover, it has been reported to form dental calculus like streptococci, and to cause endocarditis in vitro[9].

Medicinal plant remedies have been attracting attention of late in South Korea for the prevention of oral infectious diseases and for the ongoing inhibition of oral pathogens. In particular, medicinal plants are expected to be able to overcome the problems of safety or tolerance, which the conventional natural therapeutic agents used from the olden times also had[10]. Among the various medicinal plants, *Coptidisrhizoma* is a perennial vegetation belonging to *Coptis japonica* Makino (Japan), *Coptischinensis Franch* (China), or other wisteria (Ranunculaceae), and is a rootstock with almost no roots. *Coptidisrhizoma* is slightly odorous, with a very bitter taste, and has a persistently yellow color. It has strong antimicrobial activity against various pathogens as well as excellent anti-inflammation, antioxidant, hemostatic, blood pressure lowering, and anticancer effects[11]. The studies on the antimicrobial activity of the natural extract of *Coptischinensis* against oral streptococci have been limited. Thus, this study was conducted to investigate the antimicrobial activity against dental caries of *Coptischinensis* extract, and to identify its potential for the prevention and treatment of oral diseases.

Method

*Coptischinensis* was purchased from Foodsynergy Co., Ltd. (Seoul, South Korea). After adding 80% ethanol to 100 g crushed Coptischinensis, extraction was done at 65°C for 12 hours. The extract was filtered using filter paper, and the Coptischinensis extract was concentrated using a rotary vacuum evaporator (N-1300E.V.S EYELA Co., Japan). The concentrated Coptischinensis extract was again lyophilized using a freeze dryer (FD5508, Ilshin Lab, Yangju-kun, Kyunggi-do, South Korea). It was diluted in distilled water to form the 5, 10, 20, and 40 mg/mL concentrations, and as a control group, PBS (phosphate-buffered saline, Gibco$^{TM}$) was applied as a medium. *S. intermedius* (ATCC 9895) and *S. gordonii* (ATCC, 10558) were purchased from Korean Culture Center of Microorganisms (KCCM). Each microorganism was activated by brain heart infusion (BHI; Sigma-Aldrich, St. Louis, MO, USA) and was diluted at a $2x10^6$ ratio

100 µL ($2x10^6$) of *S. intermedius* and *S. gordonii*, respectively, were applied on a solid medium; 100 µL of each experimental group was dropped onto a paper disc, and it was carefully placed on the solid medium inoculated with the bacteria. After keeping it at 37°Cfor 24 hours in each environment, the diameter of the clear zone was measured on a paper disc. The average value and standard deviation were obtained after three repeated experiments, to measure the diameter of the clear zone, where the growth was inhibited. Only the average value was recorded, however, because the degree of deviation was insignificant.

The media for each bacterium and bacteria ($1X10^5$) were mixed at a ratio of 9 (medium):1 (bacteria). Mixed extracts were prepared at the 5, 10, 20, and 40 mg/mL concentrations, and 100 µL was inoculated into the solid medium. After keeping the extracts in a 37°C bacterial incubator for 24 hours, the number of CFUs present in the solid medium was checked.

## Result

1. **Clear zone results:** As a result of the application of the *Coptischinensis* experimental group to *S. intermedius*, a slight bacterial concentration decrease of 1-2 mm around the filter paper was observed at the 5 mg concentration, but there was no definite zone. At the 10 mg concentration, a 10mm clear zone was observed, and at the 20 mg concentration, a 20 mm clear zone [Figure 1].

   For the results of the application of the *Coptischinensis* experimental group to *S. gordonii*, a slight death of about 1 mm appeared around the 5 mg filter paper, and a 10mm clear zone was shown in the 10 mg group while a 15 mm clear zone was shown in the 20 mg group [Figure 2].

2. **CFU Results:** To examine the ability of the

*Coptischinensis* extract to inhibit bacterial proliferation, the group with only bacteria and the groups with 5, 10, and 20 mg of the extract with bacteria were incubated for 24 hours. As a result, it was confirmed that many bacteria survived in the control group with only bacteria, not mixed with the extract, and *S.intermedius*and *S.gordonii* were both killed in all the experimental groups (5, 10, and 20mg groups) overall [Figure 3].

As a result of the application of *Coptischinensis* extract to the opportunistic, infectious oral-resident bacteria, a definite clear zone appeared on the paper disc of *S. intermedius* when 10mg extract was applied, and became larger when 20mg extract was applied. Even in *S. gordoii*, a 10mm clear zone appeared in the 10mg extract, showing an antimicrobial activity almost similar to the one against *S. intermedius*, but a smaller clear zone was shown in the 20mg extract compared to the one in *S. intermedius*. In the comparison of CFUs, it was confirmed that bacteria were killed in all the 5, 10, and 20mg extracts [Figure 4].

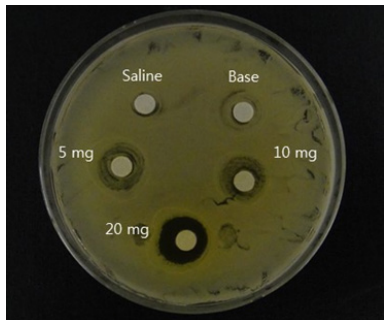


**Figure 1. Results of the clear zone of Coptischinensis extract against S. intermedius. (Saline: 0mm, base: 0 mm, 5mg: 1mm, 10mg: 10mm, 20mg: 20mm)**

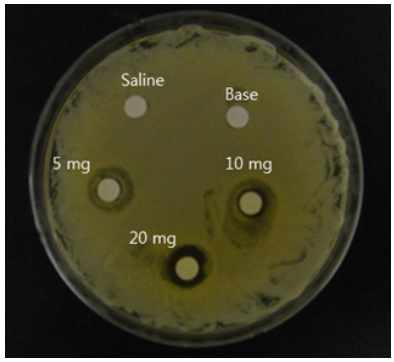


**Figure 2. Results of the clear zone of Coptischinensis extract against S.gordonii. (Saline: 0mm, base: 0mm, 5mg: 1mm, 10mg: 10mm, 20mg: 15mm)**

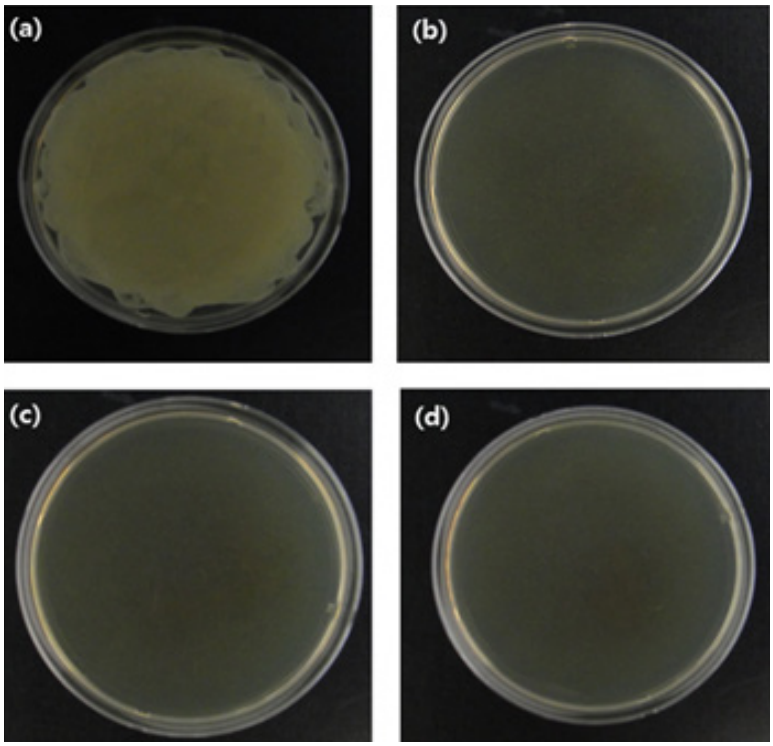


**Figure 3. CFU results of Coptischinensis extract against S.intermedius: (a) control; (b) 5 mg/mL; (b) 10 mg/mL; (c) 20 mg/mL; and (d) 40 mg/mL**

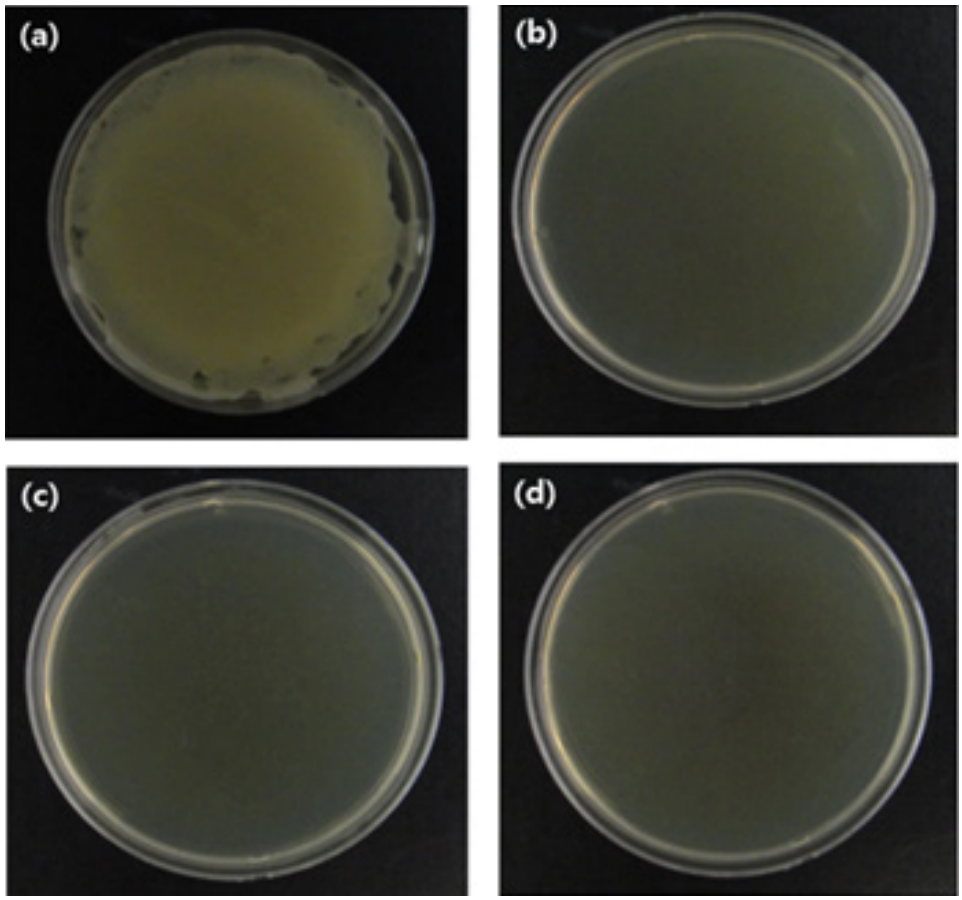


**Figure 4. CFU results of Coptischinensis extract against S.gordonii: (a) control; (b) 5 mg/mL; (b) 10 mg/mL; (c) 20 mg/mL; and (d) 40 mg/mL**

## Discussion

Studies with the aim of finding new antimicrobial substances in natural resources like plants have been actively conducted of late. As such, this study was conducted to evaluate the antimicrobial activity against two kinds of oral-resident bacteria, using a substance isolated from *Coptischinensis* ethanol extract.

The environment in the oral cavity consists of various microorganisms linked together to form an ecosystem, in which biofilm is formed in various orders. The biofilm is usually present in the oral cavity, and if a trigger factor that causes opportunistic infection is involved, an oral disease may occur [12-13]. In this study, *S. intermedius* and *S. gordoii*, which are normally residing in the oral cavity, were included in the experiment. *S. intermedius* in the root canal of the natural tooth is known to be present in the oral cavity, pharynx, and gastric juice, and is known to be capable of causing brain tumor and liver abscess through opportunistic infection[14].On the other hand, *S. gordoii* is not only associated with pulpitis but can also cause systemic diseases like infective endocarditis and infectious arthritis [15-17].

Looking at the previous studies on the antimicrobial activity of *Coptischinensis* extract against oral bacteria, Yoo et al. reported a 10-22 mg killing range on average as a result of the application of Coptischinensis to oral disease bacteria, including *S. sobrinus*, *A. viscocus*, and *S. mutans*[18].

Cha et al. reported that *Coptischinensis* showed a strong antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* in the study on the antimicrobial activity using various native cosmetic plants [19]. In addition, various studies have shown the results of the excellent antimicrobial activity of *Coptischinensis*, which were similar to the results of this study[18]. The excellent antimicrobial activity of *Coptischinensis* is attributed to a component called "berberin," which is known to inhibit the metabolism of carbohydrates and the synthesis of the glycoproteins in the bacteria[19]. Additionally, this component was found

to form a complex with the DNA of the bacterium, thereby affecting the DNA replication, inhibiting the growth and propagation of the bacterium, and exhibiting the antimicrobial activity[20].

When evaluating the antimicrobial activity of *Coptischinensis* extract, the influence of the solvent used to make *Coptischinensis* extract should also be considered. In the case of *Coptischinensis*, it is known that its antimicrobial activity can be confirmed in all the extracts obtained from solvents like water, ethanol, and methanol, and its effect has been proven[21]. Even native plants with excellent antimicrobial activity may have stronger antimicrobial activity in a wide range of bacteria, when mixed with other substances rather than when used alone. Thus, it is necessary to verify the antimicrobial activity in the future using this type of stable complex.

## Conclusion

This study analyzed the antimicrobial activity of *Coptischinensis* ethanol extract as a natural plant by applying it to oral-resident bacteria. As a result of the analysis of the clear zone of *Coptischinensis* extract against two kinds of oral-resident bacteria, *S. intermedius* and *S. gordoii*, which may cause opportunistic infections, excellent antimicrobial activity was shown, and 20 and 15mm clear zones were observed at a high concentration of 20 mg, proving an excellent antibacterial activity. These results suggest that *Coptischinensis* extract can be used to efficiently control and manage oral-resident bacteria. Further studies using a combination of various extracts with similar antimicrobial activities and analyses of the component causing antimicrobial activity other than berberine of *Coptischinensis* need to be conducted.

**Ethical Clearance:** Not required

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**Conflict of Interest:** Nil

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