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# THE EFFECT OF 5 FRACTIONAL EXTRACTS OF *ACER TEGMENTOSUM MAXIM.* CONCENTRATED IN DECOMPRESSED VACUUM ON DENTAL CARIES-INDUCING BACTERIA

**Hae-Gyung Yoon[1], Gil-Hyun Lee[2] and Kyung-Yae Hyun[3,4]***

[1]Division of Basic Sciences, Dong-Eui University, Busan 47340, Korea.
[2]Depart. of Clinical Laboratory Science, Kyungwoon University, Gumi 39160, Korea.
*[3]Depart. of Clinical Laboratory Science, Dong-Eui University, Busan 47340, Korea.
[4]Institute of Acoustics, Dong-Eui University, Busan 47340, Korea.

***Corresponding Author: Kyung-Yae Hyun**

Depart. of Clinical Laboratory Science, Dong-Eui University, Busan 47340, Korea.

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## ABSTRACT

*Acer tegmentosum Maxim.* (*Acer TM*) was concentrated in decompressed vacuum to obtain 5 factional extracts, and the amount of dental caries-inducing bacteria and periodontitis-inducing bacteria that were inhibited depending on the concentration of *Acer TM* was examined. The concentration of proteins contained in *Acer TM* Fractions 1, 2, 3, 4 and 5 was 200 (mg/mL), 225 (mg/mL), 110 (mg/mL), 150 (mg/mL) and 90 (mg/mL) respectively. The protein concentration of *Acer TM* Fraction 2 was the highest. The antibacterial effect of each *Acer TM* fraction on *S. mutans* was examined, and no antibacterial effect was observed in Fractions 1, 2 and 3. In the extract of Fraction 4, 2mm and 4mm antibacterial zones were observed at 50mg/ml and 100mg/ml respectively. In the extract of Fraction 5, 2mm and 4mm antibacterial zones were observed at 50mg/ml and 100mg/ml respectively. In the case of *P. gingivalis*, no antibacterial effect was observed in Fractions 1, 2 and 3. In Fraction 4, 1mm, 3mm, 6mm and 8mm antibacterial zones were observed at 10mg/ml, 25mg/ml, 50mg/ml and 100mg/ml respectively. In the extract of Fraction 4, an antibacterial effect was observed across the entire concentration range from the low concentration to the high concentration. In the extract of Fraction 5, 4mm and 10mm antibacterial zones were observed at 50mg/ml and 100mg/ml respectively, showing the highest antibacterial effect. Therefore, the extract of *Acer TM* Fractions 4 and 5 seems to be used as a candidate natural plant to treat periodontal diseases that induce dental caries-causing bacteria.

**KEYWORDS:** *Acer tegmentosum Maxim.*, fractional extract, *S. mutans, P. gingivalis,* antibacterial effect.

## INTRODUCTION

*Acer tegmentosum Maxim.* (hereinafter, "*Acer TM*"), one of the maple family Aceraceae, grows in temperate regions in the northern hemisphere and has been reported to be effective in stopping the bleeding, detoxifying alcohol, reducing inflammation and treating liver diseases. Periodontal diseases do not entail observable symptoms before they become serious, and about 80~90% of people aged over 40 years are found to have experience of periodontal diseases. In particular, they are chronic diseases and cause tooth loss. They are normally treated with anti-proteinase (anti-MMP)[1], non-steroidal anti-inflammatory drugs (NSAID), anti-cytokine drugs, COX-2 inhibitors and nitric oxide synthase inhibitors[2], but they need to be administered for a long time and thus have side effects such as gastrointestinal tract, nephrotoxicity and skin rash, and, in the case of patients with asthma, cramps in bronchial tubes.[3,4,5] Studies have been conducted on replacement therapies using oral indigenous bacteria to prevent dental diseases.[6,7,8] Against this backdrop, the possibility of using natural plant extracts as a substitute was examined in this study to address the side effects of these drugs. In this study, among candidate natural plants, *Acer TM* of which anti-inflammatory effect was proved in earlier studies was selected. Periodontal disease bacteria used in this study include *Streptococcus mutans* (hereinafter, "*S. mutans*") and *Porphyromonas gingivalis* (hereinafter, "*P. gingivalis*"). *S. mutans* is known to play an important role in causing dental plaques and dental caries, and bacteria that live in dental plaques are produced in the process of metabolism.[9] Clarke[10] separated it from dental plaques and names it *S. mutans*, and it has been known as the most important bacterium that causes dental caries.[11,12] *S. mutans* excretes glucosyltransferase and synthesizes glucans, an insoluble polysaccharide, from sucrose. These glucans serve as a base substance for dental plaques, and are combined with the glucan receptors of *S. mutans*, being attached to the surface of teeth. *S. mutans* within dental plaques generates a large

amount of lactic acid in the process of carbohydrate metabolism, and demineralizes teeth, causing dental diseases. *P. gingivalis* is a gram-negative anaerobic pathogenic bacterium that forms dental plaques[13,14,15] that infiltrates gingival fibroblasts and survives for a long time and, in particular, is known to be related to rheumatoid arthritis.[16,17,18] It contains peptidyliarginie deiminase. However, *Enterococcus faecalis* in the mouth generates bacteriocins that plays as an antibiotic[19,20,21], which inhibits the growth of *streptococci*, and thus has been studied as a substitute. In this experiment, *Acer TM* was concentrated in decompressed vacuum, and 5 fractional extracts were used to examine the amount of dental caries and periodontitis bacteria inhibited by the extracts depending on their concentration.

## MATERIALS AND METHODS

### Separation of *Acer TM*

*Acer TM* 200g was completely ground, and was concentrated in decompressed vacuum using a C18 column on a PREP-LC System 1L/min (Agela Technologies) to obtain 5 fractional extracts. Water was used as a solvent. Figure 1 shows the results of the separated and purified *Acer TM*. After concentrating *Acer TM* in decompressed vacuum, 300mL of *Acer TM* extracts were obtained. Table 1 shows the start time and end time of the separation and purification process. The start time and end time of Fraction 1) were 7.5 (min) and 19.00 (min) respectively, and those of Fraction 2 were 19.00 (min) and 33.50 (min) respectively. The start time and end time of Fraction 3) were 33.50 (min) and 41.00 (min) respectively, and those of Fraction 4) were 41.00 (min) and 52.60 (min) respectively. The start time and end time of Fraction were 52.60 (min) and 59.00 (min) respectively.

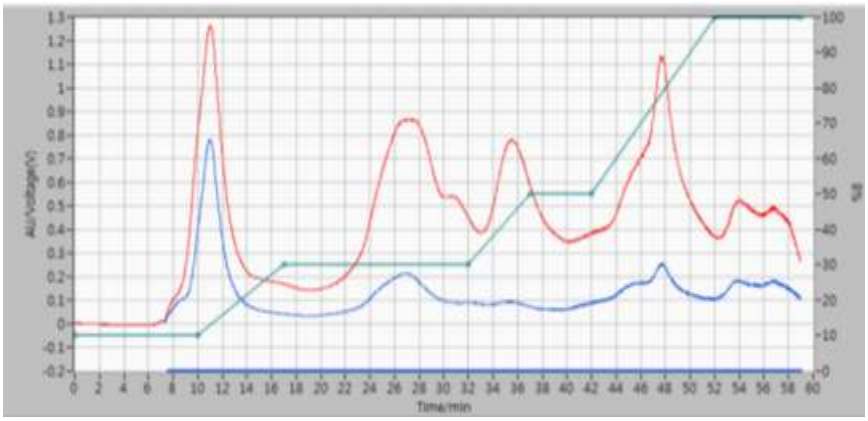

**Figure 1: Separation and purification of *Acer TM* using a C18 column.**

**Table 1: Start time and end time of each separated and purified fraction.**

| Start Time (min) | End Time (min) | Fraction Number |
|---|---|---|
| 7.50 | 19.00 | **1** |
| 19.00 | 33.50 | **2** |
| 33.50 | 41.00 | **3** |
| 41.00 | 52.60 | **4** |
| 52.60 | 59.00 | **5** |

### Preparation of Bacteria and Culture Media

Bacterial strains used in this study include gram-positive *Streptococcus mutans* ATCC (25175$^{TM}$) and *Porphyromonas gingivalis* ATCC (33277$^{TM}$). The method of culture is as shown in Table 2.

**Table 2: Bacterial strains and culture requirement.**

| Strains | Straining properties | Culture requirement | Temperature |
|---|---|---|---|
| *Streptococcus mutans* | Gram positive | Facultative anaerobes | 37℃ |
| *Porphyromonas gingivalis* | Gram negative | Anaerobes | 37℃ |

The culture medium used for *S. mutans* was Brain Heart infusion Agar (ATCC Medium 44) (BHA, BD, New jersey, USA), and that for *P. gingivalis* was Tryptic Soybean–Casein digest Medium (BactoTM Tryptic Soy Broth BD, New jersey, USA). Each of the obtained strains was stored in a frozen and dried state, and was suspended in a 300 ㎕ liquid culture medium. They were cultured in a solid culture medium for 48 hours until colonies were observed with the naked eye. The identified colonies were put into 20% glycerol 500㎕ and were stored in a frozen state at –70℃. To culture the strains, the petri dishes of Brain Heart infusion Agar and Tryptic Soybean–Casein digest Medium agar were streaked. Each medium was divided into 4 sections, and each fractional extract (10, 25, 50, 100mg/mL) was placed at the center of a sterilized disk (Table 3). Until colonies were observed with the naked eye, each strain was cultured according to the conditions for culturing each strain. The method of culturing suggested by van der Veen[22] was revised in this study. Each experiment was repeated twice.

**Table 3: Each concentration's location on an antibacterial disk.**

| Disk Concentration (mg/mL) | |
|---|---|
| 10 | 100 |
| 25 | 50 |

## RESULTS AND CONCLUSIONS

Table 4 shows the concentration of proteins measured in each fraction of *Acer TM*. The concentration of proteins measured in Acer TM Fraction 1, 2, 3, 4 and 5 was 200 (mg/mL), 225 (mg/mL), 110 (mg/mL), 150 (mg/mL) and 90 (mg/mL) respectively. The concentration of proteins that were extracted from *Acer TM* in a decompressed state using water was the highest in Fraction 2.

**Table 4: Protein concentration in each fraction of *Acer tegmentosum Maxim.***

| Sample name | Protein Concentration (mg/mL) |
|---|---|
| Acer TM fraction 1 | 200 |
| Acer TM fraction 2 | 225 |
| Acer TM fraction 3 | 110 |
| Acer TM fraction 4 | 150 |
| Acer TM fraction 5 | 90 |

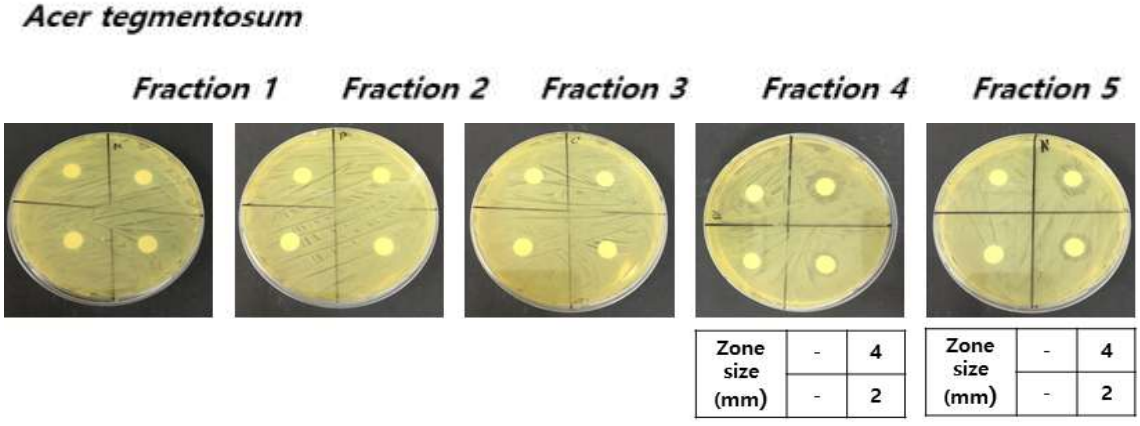


**Figure 2: Each *Acer TM* fraction's antibacterial effect on *S. mutans.***

Figure 2 shows each *Acer TM* fraction's antibacterial effect on *S. mutans* observed on a disk. No antibacterial zone was observed in Fractions 1, 2 and 3, while 2mm and 4mm antibacterial zones in Fraction 4 were observed at 50mg/ml and 100mg/ml respectively. In Fraction 5, 2mm and 4mm antibacterial zones were observed at 50mg/ml and 100mg/ml respectively.

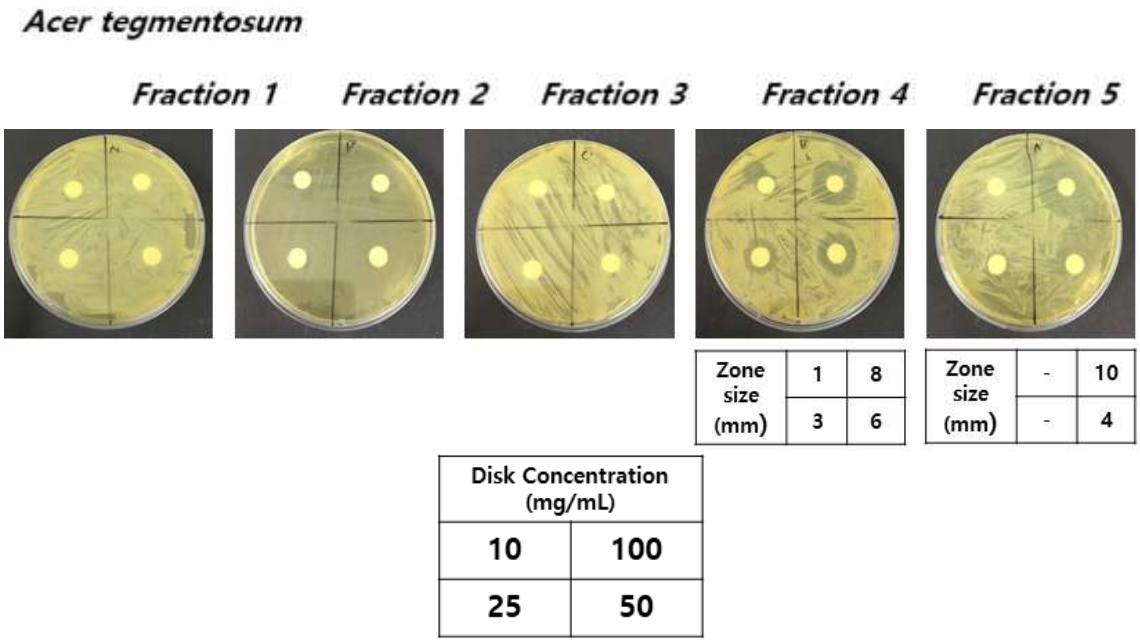


**Figure 3: Each *Acer TM* fraction's antibacterial effect on *P. gingivalis.***

Figure 3 shows each *Acer TM* fraction's antibacterial effect on *P. gingivalis* observed on a disk. No antibacterial zone was observed in Fractions 1, 2 and 3, while 1mm, 3mm, 6mm and 8mm antibacterial zones in Fraction 4 were observed at 10mg/ml, 25mg/ml, 50mg/ml and 100mg/ml respectively. In Fraction 4, the antibacterial effect was observed across the entire concentration range. In Fraction 5, 4mm and 10mm antibacterial zones were observed at 50mg/ml and 100mg/ml respectively, showing a strong antibacterial effect. Various microorganisms interact each other and permanently live in the mouth.[23,24] Pathogenic bacteria are controlled by these indigenous bacteria. In particular, *S. mutans* examined in this study is one of the bacteria that cause dental caries and tooth loss, and *P. gingivalis* also causes periodontal diseases among other dental diseases. The results of this study showed that *Acer TM* extract, especially Fraction 4 and 5, had an antibacterial effect on bacteria that cause periodontal diseases. Therefore, the possibility of *Acer TM* as a candidate natural plant to treat periodontal diseases that induce bacteria that induce dental caries was found, but it will be necessary to conduct an additional study on which substances composed Fractions 4 and 5.

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