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Evaluation of the Promotion for Antibacterial Activity on *Cibotium barometz J. Smith* with Low-Temperature Plasma from Electric Currents and Magnetic Fields

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Many studies have been conducted in pursuit of health and safety. This study aims to suggest a preventive and therapeutic plan to address dental caries by confirming the antibacterial effect of *Cibotium barometz J. Smith* (*Cibotium barometz J. S*), a natural material, on *Streptococcus mutans* (*S. mutans*), a type of bacteria that can cause dental carries, and to maximize the efficiency of antibacterial activity by applying low-temperature plasma from electric currents and magnetic fields. Low-temperature plasma can be applied to living tissue without thermal damage, and the composition of plasma is diverse, so the application field is increasing. *Cibotium barometz J. S* extract was treated with *S. mutans* diluted to 1×10^5 CFUs/mL from low concentration (0.01 mg/mL) to high concentration (45 mg/mL) for 6 and 24 hours. CFUs (colony forming units) were counted for the corresponding time, and lowtemperature plasma was used in combination to maximize antibacterial effect. The higher the concentration of *Cibotium barometz J. S* extract, the higher the death rate was, and antibacterial activity increased when combined with low-temperature plasma, compared to using *Cibotium barometz J. S* extract alone. The application of low-temperature plasma improves the penetration of *Cibotium barometz J. S* extract safely and effectively to suppress the occurrence of dental caries, and has good antibacterial effect. Therefore, low-temperature plasma is considered excellent in promoting oral health.

Keywords : low-temperature plasma, magnetic fields, *Streptococcus mutans*, *Cibotium barometz J. Smith*, antibacterial effect

1. Introduction

The increase in life expectancy has led to keener interest in quality of life. Among them, interest in oral heath, which is closely related to diet, is rapidly increasing [1]. If dental treatment is not received at an early stage, the economic burden increases, preventing proper treatment, and can further worsen oral health problems [2]. Therefore, it is necessary to effectively manage oral health, and remove the causes of oral diseases [3].

Dental caries is the most representative oral disease. It is a multifactorial disease caused by the interaction of bacteria, food, and saliva in plaque, and is a progressively infectious disease accompanied by odontoclasis [4]. Among various oral bacteria, *mutans streptococci* such as *Streptococcus mutans* (*S. mutans*) are the main cause of dental caries, as a result of the demineralization of hemorrhoids by acid accumulated in the dental plaque [5]. Brushing the teeth is recommended as an effective way to prevent dental caries, but such recommendation has a limitation because the bristles may find it difficult to access certain areas due to anatomical structure, such as deep fossae or fissures of the teeth [6]. Accordingly, various studies to explore other methods have been conducted.

Medicines based on natural ingredients have been used for a long time, and continuous research has been conducted to properly use ingredients in treating specific diseases [7]. The name *Cibotium barometz J. Smith* (*Cibotium barometz J. S*) was given because the shape of the medicine resembles that of a dog's spine, and has golden hairs [8]. *Cibotium barometz J. S*, a traditional Asian herbal medicine, is a perennial pteridophyte that

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has been reported to have several biological activities, including nerve regeneration and anti-inflammatory effects on bones. Accordingly, activity studies such as antioxidant [9] and anti-inflammatory effects [10], hemostasis after tooth extraction [11], regeneration and recovery of nerve cells [12], and inhibition of osteogenesis [13] have been conducted. In addition, interest rose in various natural ingredients that can prevent dental caries and disease progression.

With the extension of life expectancy in the 21st century, many studies are being conducted throughout society, especially in the health and medical fields [14]. Low-temperature plasma from electric currents and magnetic fields is the fourth state of matter, and consists of electrons, ions, charged particles, and electric and magnetic fields. Both electric and magnetic fields can be generated through the movement of charged particles, such as electrons and ions [15]. Since the low-temperature plasma source can be applied to living tissue without thermal damage, it can be applied to treatment, and has various fields of application [16]. With these characteristics of low-temperature plasma, interest in bio-application has emerged [17], and most of plasma research is being applied to biomedical fields, such as sterilization, tooth whitening, hemostasis, wound healing, cell activity, and cancer treatment [18-20]. However, there are few reports on studies that use low-temperature plasma to promote the delivery of substances that have antibacterial effects on oral diseases.

Therefore, this study aims to confirm the antibacterial effect on *S. mutans*, a cariogenic streptococcus, by co-treating *Cibotium barometz J. S* extract, which are natural, material-based substances. This was performed to evaluate whether anticariogenic properties was caused by maximizing the antibacterial effect by increasing the penetration of the drug through exposure to low-temperature plasma.

2. Materials and Methods

2.1. Plasma device

Figure 1 shows a handy type of device that can generate low-temperature plasma (magnetic fields) below 40 °C. It is equipped with a high voltage circuit that can generate a peak-to-peak voltage of 5 kV inside the device. High-purity (99.999 %) argon gas is introduced through the hose at the back of the equipment at a flow rate of 2 SLM (standard liter per minute), and a DC voltage of 12 V is applied to the plasma device through the DC power supply connected to the equipment. Its output has a high voltage of peak 5 kV, and is applied to the electrode of the plasma-generating source.

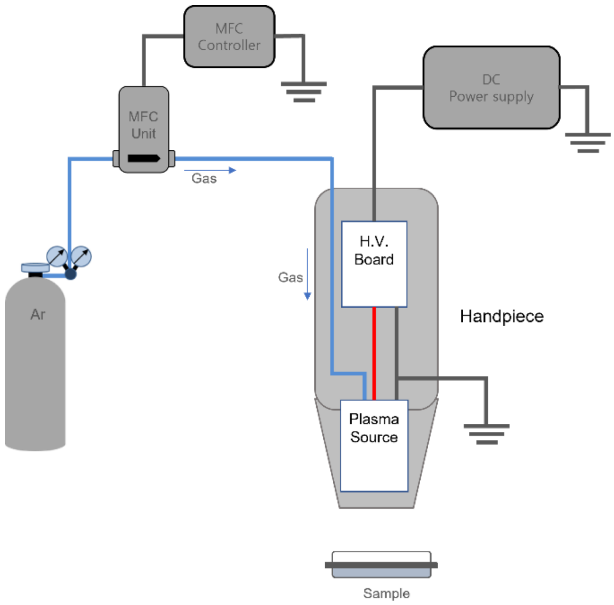

Fig. 1. (Color online) Schematic diagram of the plasma device used to increase the penetration efficacy of *Cibotium barometz J. S* extract.

The governing equations for non-isothermal gas flow are given in the following fluid equations:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho v) = 0 \tag{1}$$

$$\frac{\partial}{\partial t}(\rho \mathbf{v}) + \nabla \cdot (\rho \mathbf{v}\mathbf{v}) = -\nabla p - \nabla \cdot (\boldsymbol{\tau}) + \mathbf{f} \tag{2}$$

$$\boldsymbol{\tau} = -\mu \left[\left(\nabla \mathbf{v} + (\nabla \mathbf{v})^{\mathrm{T}} \right) - \frac{2}{3} (\nabla \cdot \mathbf{v}) \mathbf{I} \right] \tag{3}$$

Here, r, **v**, p, and μ refer to the density, average velocity, pressure, and molecular viscosity, respectively. ***I*** is the unit matrix, and t is the time. The second term on the right-hand side of the equation (3) describes the volume dilation effect, where **f** is the net force per unit volume. In addition to these equations, energy conservation is included in a conventional form. The governing equations for the charged particles and radicals were obtained from the Boltzmann equation.

The governing equations for the considered discharge system were derived from the moments of the Boltzmann equation, called the plasma fluid model [21]. The zeroth moment equation is called the continuity equation, which is as follows:

$$\frac{\partial n_e}{\partial t} + \nabla \cdot J_e = R_e \tag{4}$$

$$\frac{\partial n_p}{\partial t} + \nabla \cdot J_p = R_p \tag{5}$$

$$\frac{\partial n_q}{\partial t} + \nabla \cdot J_q = R_q \tag{6}$$

where n_e, n_p, and n_q are the electron density, positive ion density, and negative ion density, and J_e, J_p, and J_q are the electron flux, positive ion flux, and negative ion flux, respectively. R_e, R_p, and R_q are denoted for the electron production or loss terms, positive ions, and negative ions, respectively, which are related to volumetric reactions such as ionization, recombination, and excitation. The subscripts *e*, *p*, and *q* stand for electron, positive ion, and negative ion, respectively. The fluxes are derived by the first moment of the Boltzmann equation. In particular, in an atmospheric pressure discharge, the drift-diffusion approximation is adopted to simplify J_e, J_p, and J_q as follows due to the atmospheric pressure condition:

$$J_e = -D_e \nabla n_e + \mu_e n_e \nabla \phi \tag{7}$$

$$J_p = -D_p \nabla n_p - \mu_p n_p \nabla \phi \tag{8}$$

$$J_q = -D_q \nabla n_q + \mu_q n_q \nabla \phi \tag{9}$$

where, μ and D are the mobility and diffusion coefficients, respectively. Finally, the electron magnetic energy balance was obtained by:

$$\frac{\partial (n_e \varepsilon)}{\partial t} + \nabla \cdot \left[\frac{5}{3} n_e \varepsilon \mathbf{v}_e - \frac{5}{3} n_e D_e \nabla \varepsilon \right] = -e J_e \cdot E - n_e N k_L(\varepsilon) \tag{10}$$

where ε, v_e, N, E, and k_L refer to the electron magnetic energy, electron velocity, neutral density, electric field, and collision rate for loss of electron magnetic energy, respectively. The production rates in Eqs. (4) to (6) and the collision reaction rate of electrons in Eq. (10) can be obtained by references for atmospheric pressure plasma discharges [21]. Finally, the electric field is calculated from the negative gradient of the electric potential ϕ, which is calculated from the Poisson's equation, where ε_0 is the permittivity of free space, and *e* is the electron charge:

$$\nabla^2 \phi = \frac{e}{\varepsilon_0}\left(n_p - n_e - n_q\right) \tag{11}$$

Equation (11) applies a handy type device that can generate a low-temperature plasma (magnetic field) with the electric field calculated by the Poisson equation.

2.2. Bacterial strain and culture

S. mutans (KCTC 3065/ATCC 25175) was used after the subculture in brain-heart infusion (BHI; Sigma-Aldrich, St. Louis, MO, USA) broth. *S. mutans* was incubated in BHI broth at 37 °C for 24 hours, and was diluted to a concentration of 1×10^5 colony forming units per milliliter (CFUs/mL) by measuring absorbance at 660 nm to match the culture conditions of the same bacteria.

2.3. Preparation of plant material

Cibotium barometz J. S is from Vietnam, and was purchased from Miryung Bio Pharm. Co., Ltd. (Dong-daemun-gu, Seoul, South Korea), a company that manufactures, sells, and distributes imported oriental medicines. After adding 70 % ethanol to 100 g of crushed *Cibotium barometz J. S*, the extraction was done in a heating mantle at 60 °C for 12 hours. The extract was concentrated and lyophilized by using a rotary vacuum evaporator (N-1300E.V.S. EYELA Co., Tokyo Rikakikai Co., Ltd., Tokyo, Japan) after filtration via filter paper (Advantec No. 2, Tokyo, Japan). The concentrated *Cibotium barometz J. S* was lyophilized by using a freeze dryer (Ilshin Lab Co., Yangju-si, South Korea) to obtain the powder.

2.4. Antibacterial activity

In order to evaluate the antibacterial effect of *Cibotium barometz J. S* extract on *S. mutans*, the activity was confirmed by treating the extract from low concentration to high concentration (0.01 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.3 mg/mL, 0.5 mg/mL, 1 mg/mL, 3 mg/mL, 5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL, 35 mg/mL, 40 mg/mL, and 45 mg/mL). After diluting *Cibotium barometz J. S* extract by concentration in BHI liquid medium, 1×10^5 CFUs/mL of *S. mutans* was mixed and cultured at 37 °C for 24 hours. Then, 1 mL of culture medium was uniformly spread on BHI solid medium and incubated at 37 °C. Changes in CFUs at 6 hours and 24 hours were measured.

In the case of the low-temperature plasma combination, *Cibotium barometz J. S* extract was treated at concentrations of 1 mg/mL, 3 mg/mL, 5 mg/mL, 10 mg/mL, and 20 mg/mL, while low temperature plasma was irradiated for 1, 3, 5, and 10 minutes. After culturing at 37 °C for 6 hours, 1 mL of culture medium was uniformly spread on BHI solid medium. CFUs were counted to evaluate antibacterial activity.

2.5. Statistical analysis

Significant analysis was carried out using SPSS (Ver. 26.0 SPSS Inc., Chicago, IL, USA). The difference in each concentration and the antibacterial effect of the combination treatment with low temperature plasma was

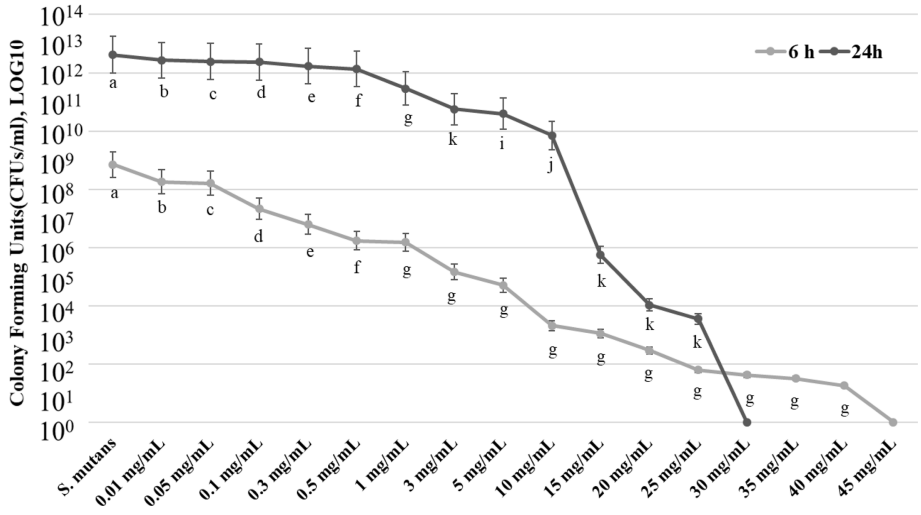

Fig. 2. Changes in the cell death of *S. mutans* from low to high concentrations of *Cibotium barometz J. S* extract and the antibacterial effect according to 6 hours and 24 hours.

evaluated by one-way analysis of variance (ANOVA), followed by the Tukey test at the 0.05 level.

3. Results

3.1. Growth inhibitory effect of the extract

As shown in Fig. 2, the bacterial proliferation of *S. mutans* clearly decreased, depending on the concentration of *Cibotium barometz J. S* extract and time ($p<0.05$). Statistical analysis showed no significant difference from 1 mg/mL at 6 hours, and from 15 mg/mL to 45 mg/mL at 24 hours ($p>0.05$). At the low concentration of 0.01 mg/mL, the bacterial growth inhibition was 72 % at 6 hours, and 34 % at 24 hours. The concentration showing 99 % antibacterial response was 0.3 mg/mL at 6 hours, and 5 mg/mL at 24 hours. Complete death of *S. mutans* according to the concentration of the extract by time was achieved at 45 mg/mL at 6 hours, and 30 mg/mL at 24 hours.

3.2. Antibacterial effect of plasma exposure

The antibacterial effect according to the low-temperature plasma irradiation time when *Cibotium barometz J. S* extract was treated by concentration was analyzed (Fig. 3). As the irradiation time increased, more pronounced antibacterial activity was observed ($p<0.05$). CFUs of *S. mutans* treated with 1 mg/mL *Cibotium barometz J. S* extract were $1.50 \pm 0.2 \times 10^6$ CFUs without low-temper-

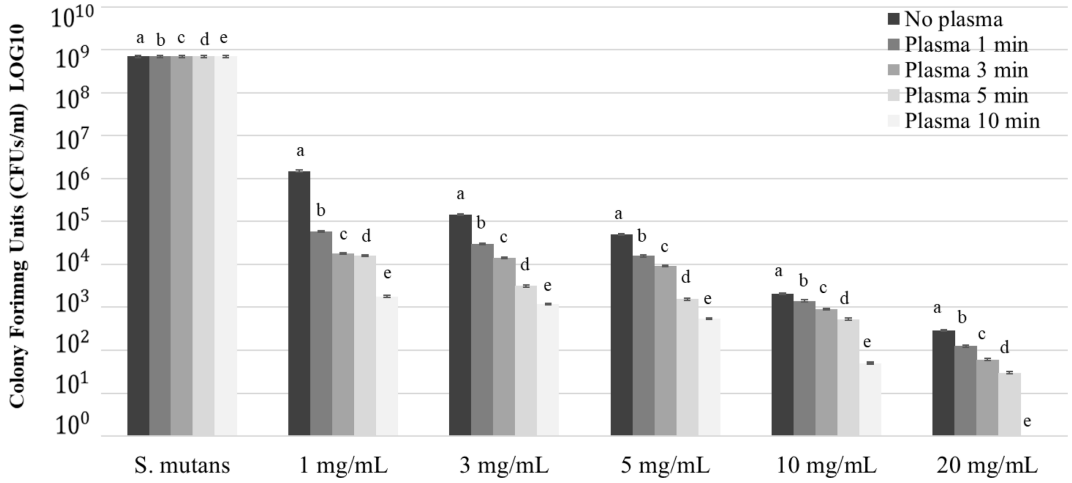

Fig. 3. The *S. mutans* survival rate by combination treatment with *Cibotium barometz J. S* extract and low-temperature plasma from electric currents and magnetic fields.

Table 1. The mean ± SD number of CFUs and p-value of the antibacterial activity by combination treatment with *Cibotium barometz J. S* extract and low-temperature plasma from electric currents and magnetic fields.

Group	*S. mutans*	*Cibotium barometz J. S* 1 mg/mL	*Cibotium barometz J. S* 3 mg/mL	*Cibotium barometz J. S* 5 mg/mL	*Cibotium barometz J. S* 10 mg/mL	*Cibotium barometz J. S* 20 mg/mL	ANOVA p-value
No plasma	$7.00 \pm 0.5\ 10^{8a}$	$1.50 \pm 0.2\ 10^{6b}$	$1.46 \pm 0.3\ 10^{5c}$	$5.06 \pm 0.3\ 10^{4d}$	$2.06 \pm 0.1\ 10^{3e}$	$2.92 \pm 0.1\ 10^{2f}$	0.000*
Plasma 1 min	$7.00 \pm 0.5\ 10^{8a}$	$5.83 \pm 0.1\ 10^{4b}$	$3.00 \pm 0.1\ 10^{4c}$	$1.58 \pm 0.2\ 10^{4d}$	$1.41 \pm 0.2\ 10^{3e}$	$1.25 \pm 0.2\ 10^{2f}$	0.000*
Plasma 3 min	$7.00 \pm 0.5\ 10^{8a}$	$1.80 \pm 0.1\ 10^{4b}$	$1.42 \pm 0.2\ 10^{4c}$	$9.30 \pm 0.2\ 10^{3d}$	$9.00 \pm 0.1\ 10^{2e}$	$6.10 \pm 0.1\ 10^{1f}$	0.000*
Plasma 5 min	$7.00 \pm 0.5\ 10^{8a}$	$1.60 \pm 0.2\ 10^{4b}$	$3.13 \pm 0.2\ 10^{3c}$	$1.54 \pm 0.1\ 10^{3d}$	$5.32 \pm 0.1\ 10^{2e}$	$3.00 \pm 0.1\ 10^{1f}$	0.000*
Plasma 10 min	$7.00 \pm 0.5\ 10^{8a}$	$1.80 \pm 0.1\ 10^{3b}$	$1.20 \pm 0.1\ 10^{3c}$	$5.40 \pm 0.1\ 10^{2d}$	$5.00 \pm 0.2\ 10^{1e}$	0.00 ± 0.0^{f}	0.000*

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

ature plasma treatment, $5.83 \pm 0.1 \times 10^4$ CFUs with treating plasma for 1 minute, $1.80 \pm 0.1 \times 10^4$ for 3 minutes, $1.60 \pm 0.2 \times 10^4$ CFUs for 5 minutes, and $1.80 \pm 0.1 \times 10^3$ CFUs for 10 minutes, showing a gradually decreasing trend. When *Cibotium barometz J. S* extract was treated with 20 mg/ml and low-temperature plasma was irradiated for 10 minutes, the CFUs was 0.0±0.0, indicating complete bacterial death (Table 1). In addition, when exposed to low-temperature plasma for 1, 3, 5, and 10 minutes, respectively, an increase in the concentration of *Cibotium barometz J. S* extract (1 mg/mL, 3 mg/mL, 5 mg/mL, 10 mg/mL, and 20 mg/mL) showed a remarkable antibacterial effect ($p<0.05$).

4. Discussion

Dental caries is the most serious disease that threatens people's oral health throughout their lifespan. Efforts to prevent dental caries have been carried out for a long time. As a result, prevalence has decreased sharply, but it is stuck at a certain level, and remains a significant problem [22]. Therefore, in order to prevent and treat dental caries, studies on antibacterial activity through killing and inhibiting growth of *S. mutans* are being actively conducted.

Following the global trend of pursuing well-being and well-dying, the public's awareness of using natural materials has been renewed, and interest in natural ingredients has also increased. Currently, in terms of health and safety, there is a tendency to prefer the development of new drug products derived from natural materials rather than those based on synthetic chemicals [23]. Peppermint and tea tree essential oils are known to have excellent antibacterial properties by inhibiting the activity of *S. mutans* [24]. In addition, *Forsythiae fructus* extract [25], *Nelumbo nucifera* leaf extract [26], *Coptischinensis* extract [27], and *Glycyrrhiza uralensis* extract [28] have been reported as effective natural agents against cariogenic streptococcus. *Cibotium barometz J. S* extract applied in this study showed an antibacterial effect on *S. mutans*. When the extract was treated for 6 hours, it showed 99 % antibacterial activity at 0.3 mg/mL, but 100 % complete killing of bacteria was shown at 45 mg/mL. Whereas when treated for 24 hours, it showed 99 % antibacterial activity at 5 mg/mL, but 100 % complete killing was shown at 30 mg/mL. This means that if the treatment time is short, it shows a rapid antibacterial effect, but complete killing of the bacteria is possible at a high concentration. If the treatment time is long, it does not show a rapid antibacterial effect at a low concentration, but complete killing is shown rapidly at a certain concentration. According to the results of this study, *Cibotium barometz J. S* extract was confirmed to contribute to controlling dental caries, and conditions of appropriate concentration and time for inhibition of *S. mutans* activity during low-temperature plasma irradiation could also be established.

Low-temperature plasma is generated at a temperature like that of the human body. Studies on surgical trauma treatment, burn treatment, and skin disease control and treatment that use various components from plasma have been reported [29]. Plasma has high tissue penetration, and is used as a new medical technology in the field of skin regeneration, as important basic research results are being revealed [30]. Compared to laser or radiation therapy, it has less tissue damage, shorter treatment time, and fewer side effects such as pain from treatment, so it is expected to be used in various fields in the future. Medical device equipment that uses plasma is also recognized as an important business item [31]. Research for the treatment and prevention of oral diseases by using low-temperature plasma has been widely conducted. When low temperature plasma is applied to bacteria, it has a safe antibacterial effect because it destroys harmful

microorganisms by collapsing the bacterial wall through free radicals, and reduces pathogens without changing the tooth surface [32]. As a result of measuring the antibacterial activity against oral bacteria by using plasma, 99 % of the bacterial species that cause oral diseases were killed. In addition, it showed high antibacterial activity of more than 3-log reduction even on the resin block that reproduces the deep and narrow tooth surface and the root canal that is difficult to access [33].

Changes according to the concentration of *Cibotium barometz J. S* extract (1, 3, 5, and 10 mg/mL) and the irradiation amount of the low-temperature plasma (1, 3, 5, and 10 minutes) were confirmed when low-temperature plasma is used in combination. According to the results of this study, the antibacterial effect on *S. mutans* was more pronounced as the plasma irradiation time increased. As the concentration and plasma irradiation time of the extract increased, the death rate of bacteria increased and showed complete death, verifying that the use of low-temperature plasma clearly contributed to the enhancement of the killing effect of dental caries pathogens. CFUs decreased by 2-log when 1 mg/mL *Cibotium barometz J. S* extract and low temperature plasma were treated in combination for 1, 3, and 5 minutes, respectively, and decreased by 3-log when treated in combination for 10 minutes. CFUs decreased by 1-log when 3 mg/mL of extract was treated with low-temperature plasma for 1 and 3 minutes, respectively, and decreased by 2-log when combined for 5 and 10 minutes, respectively. When 5 mg/mL of extract was irradiated with low-temperature plasma for 1 minute, there was no log change, but the death of bacteria was significant. The CFUs decreased by 1-log when treated in combination for 3 and 5 minutes, respectively, and by 2-log when treated in combination for 10 minutes. The trend at 5 mg/mL was also observed at 10 mg/mL and 20 mg/mL, and the log change became smaller as the concentration of extract increased. With these results, the extract alone shows an excellent antibacterial effect, and the death of bacteria is further increased during plasma treatment, though the width of the change is not large. *Cibotium barometz J. S* extract 20 mg/ml with low-temperature plasma treatment for 10 minutes led to the complete killing of bacteria that cause dental caries.

Based on the above-mentioned results, low-temperature plasma promotes penetration of natural medicines to increase delivery efficiency, and has excellent antibacterial effects, so it is expected to be established as a safe oral medical device. In addition, low-temperature plasma can be useful for patients who are anxious or afraid of treatments because it does not generate vibrations during use, and causes little discomfort to the patient [34]. This will not only help patients overcome their fear of dental treatment, but also improve oral health and gain access to the narrow and deep tooth structure, which is the most common site for dental caries. Therefore, with the combined treatment of natural extracts and low-temperature plasma to maximize the efficiency of penetration and delivery, it will be possible to avoid dental caries disease and maintain a healthy oral environment. The limitation of this study is that it is limited to only the main pathogens, so the evaluation will be performed by expanding the related bacterial species that can cause dental caries. In addition, animal experiments and clinical trials will be conducted through future studies to evaluate the effectiveness of enhancing the effective penetration of natural substances into low-temperature plasma.

5. Conclusions

Application of low-temperature plasma is a new and practical technology used in the treatment and prevention of dental caries. Thus, it will be possible to establish and invigorate the research base for plasma medical devices.

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