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Antibacterial effect of the *Lonicera japonica* extract on oral microorganisms

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Abstract
In this study, we aim to verify the antibacterial effect of *Lonicera japonica* extract in an attempt to reduce side effects. To examine the antibacterial effect of *Lonicera japonica* extract, paper disc method was used for the measurement. To examine the antibacterial effect of *Lonicera japonica* extract, paper disc method was used for the measurement. It showed antibacterial activity against *S.mutans* with 14mm inhibition at 250mg/disc concentration. For adhesivity, it showed an inhibitory effect of 71.4% at 200mg/ml concentration, 28% at 100mg/ml, 20% at 50mg/ml, and 11.4% at 25mg/ml. We could see that it was dependent on the concentration of *Lonicera japonica*, and confirmed definite inhibitory effect at 200mg/ml concentration. When we measured the pH changes according to inhibition of acid production, we confirmed that *Lonicera japonica* extract inhibited acid production. According to the results of the present study, enhancing the advantage of natural substance *Lonicera japonica* can serve an excellent antibacterial agent for dental caries.

Keywords: *Lonicera japonica*, antibacterial activity, s*treptococcus mutans*

Introduction

Dental caries and periodontal diseases are two major types of dental diseases that commonly occur in modern society [1]. They occur when people are aware of preventive measures but do not execute them properly due to busy lifestyle and little dental health knowledge, or are not aware of such measures and thereby cannot execute them due to little dental health knowledge [2].

In 2011 National Health Survey, incidence of dental caries on permanent teeth showed consistent decline from 38.3% in 2007 to 32.1%, but it is still reported to be higher than 23.7% of the U.S [3].

Dental caries is a disease caused by bacteria on teeth surfaces; when sugar and starch break down and produce acid by bacteria inhabiting the mouth, this acid damages enamel of teeth and creates cavity. Therefore, prevention is very important for dental caries.

Streptococcus mutans called *S.Mutans, Streptococcus sorbinus, Lactobacillus,* and *Actinomyces* are major bacteria that cause dental caries. Among them, *S.Mutans,* a major invasive bacterial species, adheres to dental surfaces in the mouth to proliferate and produces acid which causes dental caries. *S.Mutans* secretes glucosyltransferase (GTase) which is the enzyme that induces dental caries, and GTase degrades sucrose and produces insoluble and adhesive glucan [4]. Through this, it increases the adhesion of bacteria to teeth surfaces and facilitates acid production, resulting in dental caries.

Many supplementary products are commercially available to cope with this. These include mouth wash, tongue cleaner, mouth wash that contains Listerine, and dental floss. Among these various supplementary products, for antibacterial substances used in mouth wash, chlorhexidine, triclosan, and cetylpyridinium chloride (CPC) are widely used [5]. However, long-term utilization of such products has caused many problems such as emergence of bacteria that are resistant to the antibacterial substances and its influence on the normal oral flora [6]. Thus, as the need for improvement to deal with these problems is growing, research on utilizing natural ingredients is gaining attention.

Therefore, in this study, we aim to verify the antibacterial effect of Lonicera japonica extract in an attempt to reduce side effects.

Materials and Methods

Extract preparation and fractionation

Herbal medicines used in this study were purchased from Hwalim Natural Drug Co. Ltd (Busan, Korea). We selectively removed foreign substances contained in the collected petals, and after washing with water and freeze-drying, we pulverized the petals to make them into a powder. We added 80% ethanol solution with a mass of 20 times the mass of the powder, performed shaking extraction for 24 hours at room temperature, and then filtered using Whatman No. 2 filter paper. Following this filtration, we added 80% ethanol solution to the residue in the same quantity as before, and repeated 12 hours of shaking extraction and filtration at room temperature 3 times. After collecting together all the extracted filtrate obtained from the above process, we filtered it again using Whatman No. 2 filter paper. We then used a decompression concentrator at 50°C to evaporate off all the ethanol, and concentrate the extract to a watery state. This concentrate was frozen at a low temperature, below -50°C, and freeze-dried, then processed to a powder state before the extract was used to test its antiseptic effect.

Bacterial strains

To measure antibacterial activity and GTase inhibitory function, the cavity-inducing strain *Streptococcus mutans* KCTC 3065(brain heart infusion; BHI agar, 37°C) were obtained from Korean Agricultural culture collection (KACC) and Korean Collection for Type Cultures (KCTC) to use in this study.

Measurement of antibacterial activity against oral pathogens

To examine the antibacterial effect of Lonicera japonica extract, paper disc method was used for the measurement. Lonicera japonica extract was used at a concentration of 15mg/disc. Each strain was cultured at 37°C (optimal culture condition) for 24 hours and the antibacterial activity was measured from the diameter of the clear zone (mm).

Measurement of the inhibition on the adhesivity of dental pathogens

GTase

Streptococcus mutans KCTC 3065 which is a strain that produces GTase was incubated in BHI(brain heart infusion) medium at 37°C for 24 hours, and inoculated at 2%(v/v) in 450ml of BHI medium and incubated under the same condition. To supernatant obtained from centrifugation at 6,000 rpm for 20 min at room temperature, 300ml of ice-cold ethanol was added for protein precipitation. After storing at 4°C overnight, it was centrifuged at 8,000 rpm at 4°C for 30 min, and the precipitate was suspended with 1ml of 0.05M potassium phosphate buffer (pH 6.8). This was considered as crude GTase, and kept frozen at -20°C.

GTase adhesivity experiment

In a test tube, 0.8ml of substrate solution (12.5g sucrose and 0.25g NaN_3 contained in 1L of 0.0625M potassium phosphate buffer (pH 6.5)), 0.025ml of crude GTase, and 0.175ml of Lonicera japonica extract at 250mg/ml concentration were added so the total volume would be adjusted to 1ml, and 0.175ml distilled water was used for the control sample. The test tube was inclined 30°angle from the ground. The reaction was carried out for 16 hours at 37°C. After reaction, supernatant was discarded, and 3ml of distilled water was added and the sample was sonicated for 5 sec to disperse the glucan that has been formed. After dispersion, absorbance was measured at 550nm using a spectrophotometer to calculate the level of inhibition on adhesivity by Lonicera japonica extract.

Insoluble adhesive glucan synthesis inhibition (%) = A-B/A*100

Here, A is the absorbance of adhesive glucans formed in the control sample, and B is the absorbance of insoluble adhesive glucans formed in the presence of Lonicera japonica extract.

Measurement of inhibitory effect on acid production by *S.mutans*

The effect of Lonicera japonica extract on acid production by *S.mutans* was analyzed. To 2ml of bacterial suspension in each test tube (OD_{600}=1.2), 0.5ml of fraction with different concentrations (2, 4mg/ml) and, for control, 1ml of 2% glucose solution were added and mixed. While they were incubated at the optimal temperature, pH was measured every 30 min for 240 min to verify the level of inhibition on acid production.

Result

Measurement of antibacterial activity

Measurement of antibacterial activity of Lonicera japonica extract showed that it has antibacterial activity against all pathogens. It showed inhibition of approximately 14mm at 250mg/disc concentration, showing antibacterial activity.

Table 1. Antibacterial activity of Lonicera japonica extract from the diameter of the clear zone (mm).

	Lonicera japonica extract
S.mutans	14
Sample concentration :250mg/ml paper disc: 8mm clear zone unit: mm	

Inhibitory effect of Lonicera japonica extract on GTase adhesion

Inhibition of adhesivity against *S.mutans* was highest with 71.4% at 200mg/ml concentration, followed by 28% at 100mg/ml, 20% at 50mg/ml, and 11.4% at 25mg/ml. This shows that Lonicera japonica extract has an inhibitory effect on the adhesion of the fractions.

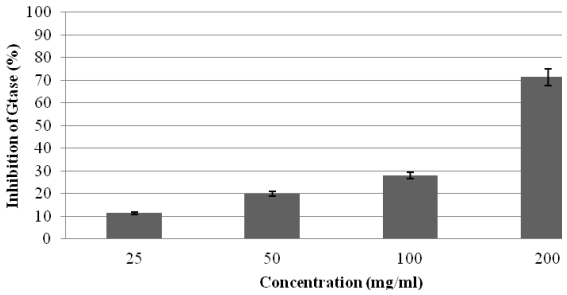

Figure 1. Inhibition of adhesivity against *S.mutans*

Inhibitory effect of Lonicera japonica extract on acid production

To examine whether Lonicera japonica extract inhibits acid production by *S.mutans*, we measured the changes of pH during incubation and displayed it as a graph below. At 0 hr, pH of the control sample in which glucose was added to *S.mutans* was 6.15. When 2mg/ml and 4mg/ml of Lonicera japonica extract were added, pH was 6.75 and 6.67, respectively, showing a slight difference. After 5 hr, pH of the control sample dropped to 5.84 whereas pH of the samples with 2mg/ml and 4mg/ml of Lonicera japonica extract were 6.57 and 6.16, respectively, confirming the inhibition of acid production compared to the control sample.

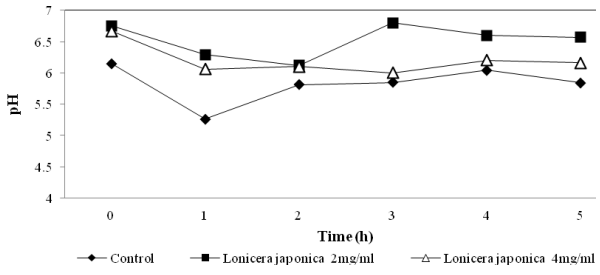

Figure 2. Inhibitory effect on acid production by changes of pH.

Comparison and Discussion

Dental caries occurs when dental enamel gets demineralized by organic acid such as lactic acid which is a carbohydrate metabolite produced by bacteria on oral biofilm, and the *S.mutans* group are known as the major cause among such bacteria [7].

Chemical materials used to inhibit oral bacterial pathogens are mainly used for antibacterial purposes, and in most cases antibiotics are used. Although strong chemical substances can be added to mouth wash, this can possibly eliminate oral normal flora along with pathogens [8]. Therefore, as an attempt to inhibit only pathogens while healthily maintaining oral normal flora, utilizing natural extracts instead of synthetic chemical substances is recently being highlighted [9]. Research on using natural extracts for preventing dental diseases that have little side effects and can be safely used over a long period of time is increasing.

Lonicera japonica is a flower of a honeysuckle (Lonicera japonica Thumb) that has been harvested and dried, and its known major medicinal functions include antibacterial function [10] and anti-inflammatory function [11]. In this paper, in order to reduce side effects, we used Lonicera japonica extract which is one of the natural substances to verify its antibacterial effect on oral bacterial pathogens, inhibition on the adhesion of oral bacterial pathogens, and inhibitory effects on acid production.

It showed antibacterial activity against *S.mutans* with 14mm inhibition at 250mg/disc concentration. For adhesivity, it showed an inhibitory effect of 71.4% at 200mg/ml concentration, 28% at 100mg/ml, 20% at 50mg/ml, and 11.4% at 25mg/ml. We could see that it was dependent on the concentration of Lonicera japonica, and confirmed definite inhibitory effect at 200mg/ml concentration. When we measured the pH changes according to inhibition of acid production, we confirmed that Lonicera japonica extract inhibited acid production.

As an alternative to overcome the shortcomings of antibiotics, Lonicera japonica extract, which inhibits the growth of oral bacterial pathogens and can be widely used to prevent dental diseases, showed superior antibacterial activity against the bacterial pathogens that causes dental caries. Taken these results together, enhancing the advantage of natural substance Lonicera japonica can serve an excellent antibacterial agent for dental caries.

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