# Antibacterial and anti-inflammatory effects of *Platycodon* grandiflorum extracts

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# 도라지 추출물의 항균작용 및 항염작용

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Abstract This study investigated the impact on S. mutans of C. albicans in order to reveal the antimicrobial activity of Platycodon grandiflorum extracts. In addition, it examined NO generation inhibition rate in accordance with the extract concentration from Raw 264.7 cell in order to find out anti-inflammatory activation. As for the study materials, domestically made 100% Platycodon grandiflorum powder was utilized. As for the experimental strain, S. mutans KCTC 3065 and C. albicalns KCTC 7965 were utilized. Consequently, this study obtained the following results. Growth inhibition rate of S. mutans became significantly higher with higher concentration of Platycodon grandiflorum extracts. Growth inhibition rate of C. albicans became significantly higher with higher concentration of Platycodon grandiflorum extracts. NO generation inhibition rate was found to be 29.2% and 26.1% respectively when adding *Platycodon grandiflorum* extract with the concentration of 10 and 20 ug/ml in Raw 264.7 cells. These results mean that Platycodon grandiflorum could be leveraged as antimicrobial and anti-inflammatory substance.

Key Words: Antibacterial Effect, Anti-inflammatory Effect, Platycodon grandiflorum

요 약 본 연구에서는 도라지 추출물의 항균활성을 밝히고자 S. mutans와 C. albicans에 대한 영향을 조사하였다. 또한 항염활성을 알아보고자 Raw 264.7 세포에서 추출물 농도에 따른 NO 생성 저해율을 조사하였다. 연구 재료는 국내산 100% 도라지분말을 구입하여 사용하였으며, 실험균주로는 S. mutans KCTC 3065 와 C. albicans KCTC 7965를 사용하여 다음과 같은 결과를 얻었다. 도라지 추출물의 농도가 높을수록 S mutans의 성장 억제율이 현저히 높아졌 다. 도라지 추출물의 농도가 높을수록 C. albicans의 성장 억제율이 현저히 높아졌다. Raw 264.7세포에서 추출물을 10, 20 μg/ml 의 농도로 첨가하였을 때 각각 29.2, 26.1%의 NO 생성 저해율이 나타났다. 이는 도라지가 항균 및 항염 물질로서 활용 가능함을 의미한다.

주제어: 항균작용, 항염작용, 도라지

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#### 1. Introduction

A variety of bacterial species numbering almost 500 species are residing permanently in the oral cavity. They are forming their unique bacterial flora in each part to seek for a place to reside permanently. Florae are permanently residing together as keeping a balance between them. However, florae might cause an infection when the physical barrier of host and the balance between microorganisms are broken or the immune defense of host is not working properly.

Dental caries and periodontal disease are the most important oral cavity disease; thus, they are generated by the bacteria residing permanently in the oral cavity. As for the causative bacteria for dental caries, *Streptococcus mutans* has been known to be the main causative bacteria. Dental caries is caused by an interaction of bacteria, food and saliva inside the dental plaque. It occurs as lactic acid generated by bacterial fermentation erodes the lime of dental hard tissue[1]. To prevent dental caries, it would be important to utilize antibiotic materials that would counter *S. mutans*, suppress the adhesion of *S. mutans* to tooth surface and also suppress GTase that would generate insoluble glucan.

As for the studies on the anti-caries effect of natural raw materials, Choi et al.[2] have reported that pine leaf and pine tree branch had the antimicrobial activity. In addition, it has been reported that there is the effect of antimicrobial substance for natural raw materials such as green tea, oolong tea, cork tree, machilia, flavonoid, aloe[3][4].

Candida albicans is the atypical fungus who would display the growth of yeast form or mycelial form depending on the growing environment; thus, it is the fungus that would be normally found in the oral cavity, skin, intestinal tract and mucous membrane of vagina of normal people. Not only it shows a strong adhesive ability for the resin surface in the oral cavity but it is also the main microorganism forming the oral biofilm

that would be adhered to the denture surface. Denture may form dental plague since it is also adhered by oral biofilm just like tooth. Oral biofilm of denture and dental plague are the major cause of denture stomatitis. They have more serious impact on weak elderly people than young and healthy people. *C. albicans* has been known to be the major causative bacteria of denture stomatitis [5].

C. albicans would not usually cause a problem to healthy people. However, it would display pathogenicity when the immune function of host was reduced due to systemic factors. For example, elderly people or those patients with AIDS or cancer have a high incidence rate of candidiasis. When people with weakened immunity or those taking antibiotics for a long time use it, opportunistic infections might be caused. It also cause profundus candidiasis and sometimes it may become a deadly infectious disease[6].

Hofling *et al.*[7] observed the constraining effect as to *Candida* species from the 6 natural extracts and Murzyn et al.[8] verified the antifungal effect by extracting propolis by ethanol and applying it to those patients who had been infected with oral cavity candidiasis.

Platycodon grandiflorum is a perennial herbaceous that belongs to campanulaceae; thus, its roots have been utilized primarily for traditional medicinal herb and food. In addition, Platycodon grandiflorum has been utilized as a folk remedy for cold, etc. since thousands of years ago around East Asia. It has been also broadly utilized as medicinal herb of oriental The medicine in South Korea. the main pharmacological component of Platvcodon grandiflorum is saponin of the triterpenoid class. The saponin ingredient contained in Platycodon grandiflorum has been known to have several pharmacological effects for calmness, antipyretic, analgesic, antitussive, expectorant, hypoglycemic, anti-cancer activity, etc. In particular, it has been reported to have an excellent antimicrobial effect

against bacteria causing bronchial tube. As for Platycodon grandiflorum at the time, it is being broadly distributed in the market in the form of processed food such as powder, liquid, juice and tablet[9].

It is clear that *Platycodon grandiflorum* is an useful resource having various development potential that the important physiological substances. Platycodon grandiflorum has been utilized as a remedy in everyday life and it has also been utilized even until recently as tea and processed food and so on. However, there has been almost none of the studies related to oral cavity bacteria. Therefore, this study aimed to verify the anti-inflammatory effect of Platycodon grandiflorum extracts by utilizing the antimicrobial effect and Raw 264.7 cell that would affect S. mutans and C. albicans of Platycodon grandiflorum extracts. In addition, this study aimed to the potential as antimicrobial anti-inflammatory natural substance.

### 2. Materials and method

#### 2.1 Preparation for extracts

As for the study materials, domestically made 100 percent *Platycodon grandiflorum* powder was purchased and utilized. The mixture of the powder of 60g and ethanol of 600ml was preserved at room temperature for 24 hours. And then, it was passed through the vacuum filter after it had been applied by ultrasonic wave for 1 hour. The filtered extracts were concentrated using the decompressing concentrator and then utilized after being refrigerated and stored. .

#### 2.2 Experimental strain

As for the experimental strain, S. mutans KCTC 3065 and C. albicans KCTC 7965 that had been obtained from the Korea Research Institute of Bioscience and Biotechnology were utilized. S. mutans was utilized by incubating it at 37°C for 12 hours from

the liquid medium of Brain Heart Infusion (BHI, Difco, USA). C. albicans was utilized after being incubated at 37°C for 12 hours from the liquid medium of Trypic Soy Broth (TSB, Difco, USA).

#### 2.3 Absorbance measurement

1 percent of S. mutans and C. albicans were added to the liquid medium that had been added by Platycodon grandiflorum extracts with 0, 0.1, 0.5, 1 and 2% respectively. Absorbance was measured while incubating at 37°C with the intervals of 6, 9 and 12 hours. Absorbance was measured by 600 nm at the spectrophotometer and the mean value was obtained by repeating all the experimental groups three times. Growth inhibition rate by the extracts was calculated by utilizing the following equation.

Growth Inhibition Rate (%) = (Absorbance of Control Group - Absorbance of Experimental Group) × 100 / Absorbance of Control Group

# 2.4 Cell culture

Raw 264.7 cell (KCLB, Seoul, Korea) was cultured from DMEM medium that contained 100 unit/mL of penicillin/streptomycin and 10% of FBS. It was cultured at 37°C from the 5% CO<sub>2</sub> incubator and it has been sub-cultured every 3 days. Raw 264.7 cell was replaced with the serum free medium after being dispensed to 12 well plate and cultured for 24 hours. After then, it was cultured for 24 hours after processing both of 100 ng/mL of LPS and Platycodon grandiflorum extracts (0, 0.1, 0.5, 1 and 2%) together.

#### 2.5 Nitric Oxide assay

The generated amount of NO was measured using Griess reagent as the form of NO2 that would exist among the cell culture medium. Absorbance was measured at 540 nm using the microplate reader after mixing 50 µl of the cell culture supernatant and 50 µl of Griess reagent (1% sulfanilamide in 5% phosphoric acid, 1% a-naphthylamide in H2O) and inserting this mixture in the 96 well plate and then letting it to react for 15 minutes.

# 3. Results and Discussion

# 3.1 Growth inhibition rate of S. mutans

Platycodon grandiflorum extracts were added to the liquid medium and cultured with the strain for the concentrations of 0. 0.1, 0.5, 1 and 2%. Growth inhibition rate was measured by comparing with the group after measuring absorbance accordance with the time. As a result, all of the experimental groups were found to have a significant change. It was possible to observe that growth inhibition rate of S. mutans increased substantially with higher concentration. In the case of adding the extracts of 0.1 percent after 12 hours, growth inhibition rate of 55.3±0.26% was found as compared with the control group. In the case of adding 0.5 percent, growth inhibition rate was found to be 77.65±0.13%, whereas it was found to be 82.23±0.35% in the case of adding 1 percent and 85.38±0.57% in the case of adding 2 percent <Table 1>.

<Table 1> Growth inhibition rate of S. mutans

			Mean±SD	
Concent-r	Inhibition rate (%)			
ation - (%)	6hr	9hr	12hr	
0	0.00	0.00	0.00	
0.1	24.92±1.75	23.9±3.04	55.3±0.26	
0.5	22.7±2.03	45.81±3.03	77.65±0.13	
1	38.57±4.11	43.5±2.45	82.23±0.35	
2	47.62±3.6	54.96±3.17	85.38±0.57	

# 3.2 Growth inhibition rate of C. albicans Platycodon grandiflorum extracts were added to the liquid medium and cultured with the strain for the

concentrations of 0. 0.1, 0.5, 1 and 2%. Growth inhibition rate was measured by comparing with the control group after measuring absorbance in accordance with the time. As a result, all of the experimental groups were found to have a significant change. It was possible to observe that growth inhibition rate of C. albicans increased substantially with higher concentration. In the case of adding the extracts of 0.1 percent after 12 hours, growth inhibition rate of 5.96±1.02% was found as compared with the control group. In the case of adding 0.5 percent, growth inhibition rate was found to be 15.41±1.06%, whereas it was found to be 77.11±0.16% in the case of adding 1 percent and 98.06±0.03% in the case of adding 2 percent <Table 2>.

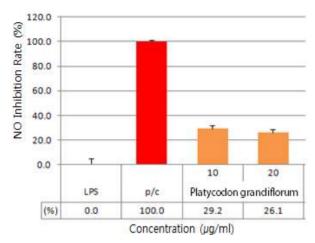
<Table 2> Growth inhibition rate of C. albicans

Mean+SD

			Mean±SD	
Concent-r ation -	Inhibition rate (%)			
	6hr	9hr	12hr	
0	0.00	0.00	0.00	
0.1	7.17±0.65	5.87±0.74	5.96±1.02	
0.5	13.8±0.78	37.52±1.23	15.41±1.06	
1	74.19±0.93	85.62±0.27	77.11±0.16	
2	91.22±0.47	95.81±0.06	98.06±0.03	

## 3.3 NO assay

Of several factors occurred during the generation of inflammation, NO would be easy for experimental evaluation. For the evaluation of anti-inflammatory effectiveness for *Platycodon grandiflorum* extracts, the degree of generation of NO was evaluated using RAW264.7 macrophage. As for NO assay, the extract concentration was set to the extent that would not display cytotoxicity through MTT result. It was confirmed to have NO generation inhibition rate of 29.2 and 26.1% when adding Platycodon grandiflorum extract with the concentration of 10 and 20 µg/ml [Fig. 1].



[Fig. 1] NO generation inhibition rate

Platvcodon grandiflorum is alkaline food that is rich in fiber, calcium and iron. The main pharmacological ingredient is saponin of the triterpenoid class. The total of 17 saponin ingredients contained in Platycodon grandiflorum have been found so far[10]. Moreover, it contains platycodigenin I and platycodigenic acid A, B and C, which are the outstanding saponin of the triterpenoid class that could substitute ginseng in additino to betulin, spinasterol, carbohydrate and fiber. Among the previous studies Platvcodon grandiflorum, there have been some studies related to the anti-inflammatory operation of grandiflorum and the activation of immune system[11].

This study investigated the impact on *S. mutans* and C. albicans in order to reveal the antimicrobial activity of Platycodon grandiflorum extracts. In addition, it examined NO generation inhibition rate in accordance with the extract concentration from Raw 264.7 cell in order to find out anti-inflammatory activation.

Growth inhibition rate was measured as culturing Platycodon grandiflorum extract along with S. mutans after adding it to the liquid medium. As a result, there appeared a significant change in all the experimental groups. It could be verified that growth inhibition rate of S. mutans became significantly higher with higher concentration. Growth inhibition rate was found to be significantly higher with approximately 85 percent in

the case of adding 2 percent as compared with the control group <Table 1>.

S. mutans is the causative bacteria for dental caries; thus, it generates insoluble glucan by polymerizing sugar as producing glucosyltransferase (GTase) after being adhered to the obtained coating film of tooth surface. The generated glucan ultimately causes dental caries by inducing the other bacteria being multiplied on the generated glucan to integrate with tooth easily. As for the studies on the prevention of dental caries, those studies on the development of GTase synthesis inhibitors, the development of antimicrobial agent, etc. are currently under progress. Choi et al.[12] reported that 70 percent ethanol extract of pine leaf would be very effective for the inhibition effect of GTase activation. In the case of antimicrobial substances in plants, most have been known to be phenolic compound of the terpenoid class[13]. They can be integrated with sugar and protein; therefore, they have been known to operate as a defense compound against organisms[14]. Kim et al.[15] reported that phenolic compounds had the antibacterial performance in addition to the function as natural antioxidant as for the antimicrobial effect of persimmon vinegar as to phagocytic caries. They reported that the antimicrobial effect would increase with higher content of phenol substances contained in the vinegar. Such reports support the results of this study.

As for the virulence factors of *C. albicans*, they have been known to include adhesion capability to host epithelial cells, ability of secreting hydrolases and mode conversion capability from yeast form to mycelial form. In particular, proteinase generated by *C. albicans* makes it easy for the adhesion of strain and tissue infection by damaging the membrane of hostcell. In addition, it has been reported that C. albicans yeast model, caustic hyphae and mycelia are closely linked to get involved in the formation of biofilm[16].

Growth inhibition rate was measured as culturing Platycodon grandiflorum extract along with C. albicans after adding it to the liquid medium. As a result, there appeared a significant change in all the experimental groups. It could be verified that growth inhibition rate of *C. albicans* became significantly higher with higher concentration. Growth inhibition rate was found to be significantly higher with approximately 98 percent in the case of adding 2 percent as compared with the control group <Table 2>.

It is believed that such antimicrobial effect was caused by the high degree of polyphenol content in addition to the high content of saponin in Platycodon grandiflorum. It was reported that Platycodon grandiflorum extracts had a strong antimicrobial activity in Staphylococcus aureus. Vibrio parahaemolyticus and Bacillus cereus. Also it was reported that bronchial bacteria would display the antibacterial activity of more than 80 percent. In recent years, there have been many studies on specific ingredients contained in the plants physiologically active substance were contained. In particular, the efficacy of antioxidant, inflammatory and anti-cancer as to phenolic compound or flavonoid has been revealed, through these studies.

As for the studies on the anti-inflammatory effects of *Platycodon grandiflorum* anti-inflammatory, Kim *et al.*[17] reported that methanol extracts and butanol extracts had the anti-inflammatory effect. Choung et al. [18] reported that the saponin ingredient of *Platycodon grandiflorum* displayed a significant effect in concentration-dependent manner in macrophage phagocytic activity, anti-cancer action and antiinflammatory action.

Inflammation is one of defense responses occurred when the body tissue is damaged. An appropriate inflammatory response is essential to protect the living body; however, an excessive and inadequate inflammatory response may become a cuase of necrosis for cell and tissue and chronic disease. Macrophages and monocytes in the inflammatory response would be activated by recognizing various pathogen-associated

molecular patterns including lipopolysaccharide (LPS). They generate NO by producing pro-inflammatory cytokine such as tumor necrotic factor-a (TNF-a), interleukin-6 (IL-6) and IL-1β and synthesizing nitric oxide svnthase (iNOS) inducible cyclooxygenase-2 (COX-2). When an inflammatory reaction occurs, the amount of expression of iNOS in the relevant cells would increase; thereby, producing a large quantity of NO. The excessively produced NO causes tissue damage, genetic mutation, nerve damage, etc. and also accelerates inflammatory reaction such as edema by increasing the vascular permeability.

Of several factors occurred during the generation of inflammation, NO would be easy for experimental evaluation. For the evaluation of anti-inflammatory effectiveness for *Platycodon grandiflorum* extracts, generation inhibition rate of NO was verified using RAW264.7 macrophage. It was confirmed to have NO generation inhibition rate of 29.2 and 26.1 percent when adding *Platycodon grandiflorum* extract with the concentration of 10 and 20 µg/ml [Fig. 1].

As for the inflammatory medications inside the oral cavity, they could be broadly divided into antibiotics, antibacterial medications and anti-inflammatory medications. Antibiotics have such adverse effects as resistant expression of bacteria. irritability. gastrointestinal disorders, etc. Those antibacterial medications that would be represented by NSAID medications such as anti-inflammatory medication, phenolic compound, quaternary ammonium compound, bisbiguanide, etc. would display the dental plaque inhibition and antimicrobial effects. However, they have such problems as surface discoloration of tooth, inducement of peeling gingivitis, hypersensitivity of mucosa, etc. As for the method to overcome such adverse effects, many studies on herbal agents have been actively conducted.

Chang et al.[19] reported the constraining effect of NO production of *Platycodon grandiflorum*. Ko et al.[20] reported that *Platycodon grandiflorum* would

inhibit the secretion of inflammatory cytokine such as TNF-a, IL-6 and IL-8. Such results are also consistent with the results of this study. Moreover, they imply that *Platycodon grandiflorum* could be applied as a substance for the prevention or treatment of inflammatory diseases induced from the pathogens.

### 4. Conclusions

This study investigated the impact on S. mutans of C. albicans in order to reveal the antimicrobial activity of Platycodon grandiflorum extracts. In addition, it examined NO generation inhibition rate in accordance with the extract concentration from Raw 264.7 cell in order to find out anti-inflammatory activation. Consequently, this study obtained the following results.

- 1. Growth inhibition rate of S. mutans became significantly higher with higher concentration of Platycodon grandiflorum extracts. Growth inhibition rate was found to be approximately 85 percent as compared with the control group in the case of adding the extract of 2 percent.
- 2. Growth inhibition rate of *C. albicans* became significantly higher with higher concentration of Platycodon grandiflorum extracts. Growth inhibition rate was found to be approximately 98 percent as compared with the control group in the case of adding the extract of 2 percent.
- 3. For the evaluation of anti-inflammatory effectiveness for *Platycodon grandiflorum* extracts, NO generation inhibition rate was verified. NO generation inhibition rate was found to be 29.2 percent and 26.1 respectively when adding grandiflorum extract with the concentration of 10 and 20 ug/ml.

These results mean that Platycodon grandiflorum could be leveraged as antimicrobial and antiinflammatory substance. However, it would be imperative to conduct follow-up studies on the

expression of iNOS and COX-2 in relation with the inflammtory response of Platycodon grandiflorum extracts. Moreover, it would also be imperative to conduct follow-up studies on the generation of pro-inflammatory cytokines such as IL-6, IL-1β, TNF-a. etc.

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