ISSN 2093-5986(Print)
ISSN 2288-0666(Online)

The Korean Journal of Health Service Management
Vol.13 No.2 pp.135-142 June 2019
https://doi.org/10.12811/kshsm.2019.13.2.135

도라지 추출물의 구강미생물에 대한 항균효과

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Anti-microbial Activity of Platycodon Grandiflorum Extracts Against Oral Microbes

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<Abstract>

Objectives: The objective of this study was to assess the antimicrobial effect of Platycodon grandiflorum extracts against oral microorganisms. **Methods:** The anti-microbial activity and minimal inhibitory concentration were measured the agar dilution method. **Results:** Platycodon grandiflorum extracts grew in the free agar plates all of the oral microorganisms. In the bark-free Platycodon grandiflorum extracts all the oral microorganisms grew in the free agar plates. Growth was inhibited at a concentration of 0.5 mg/ml. Oral microorganisms showed an absence of growth at a concentration of 1 mg/ml. **Conclusions:** It was confirmed that the extracts of Platycodon grandiflorum having a higher saponin content than the bark - free Platycodon grandiflorum extract showed excellent antimicrobial effect.

Key Words : Antibacterial Effect, Oral Microbes, Platycodon Grandiflorum Extracts

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• Received : Feb 24, 2019 • Revised : Apr 17, 2019 • Accepted : Jun 27, 2019

Ⅰ. Introduction

According to World Health Organization (WHO) report on dental disease, about 60% to 90% of the world's children have caries experience, and about 20% of adults have undergone extraction due to periodontal disease [1]. In the past five years, dental caries outpatient clinics in Korea have increased by 4.7 million (39.5%) from about 11.88 million in 2011 to about 16.58 million in 2015 and the total medical expenses have increased by about 630.5 billion won (91.5%) from about 688.9 billion won in 2011 to about 1.13 trillion won by 2015 [2]. It is estimated that since July 2013, medical staff has increased greatly due to the introduction of scaling treatment to the health insurance [3].

There are about 700 kinds of microorganisms in the oral cavity [4]. Oral nutrients are continuously supplied for oral microbial growth, resulting in the incidence ofvarious oral diseases such as dental caries and periodontitis [5]. Streptococcus mutans is a major causative organism of dental caries [6,7], and plays a role as a cohesive factor for other types of bacteria [8], leading to endogenous mixed infections.

In clean teeth Streptococcus spp. [8] And actinomyces viscosus bacteria proliferate, forming dental plaque [8], coagulating with other bacteria, Actinomyces naeslundii and Actinomyces odontolyticus [9] and displaying symptoms accompanied by edema. The main cause of oral diseases such as dental caries and periodontitis is the proliferation of dental flora. In order to suppress the dental flora, physical methods using toothpaste and oral aids, and chemical methods such as oral cleansers and antibiotics are employed [10]. However, in reality, there is are limitations the removal of the bacterial membrane through tooth brushing or the use of oral auxiliary products, Furthermore, the prolonged use of chemical agents such as antibiotics could result in side effects such as toxicity and tolerance [11] Hence, there is an increased interest in the development of natural products that can be safely used for the prevention and treatment of oral diseases [12].

Platycodon grandiflorum is a calcium-rich, alkaline compound that has long been consumed as food or as a health food and is known to be good effective in bronchial, asthma, and pneumonia [13]. In particular, saponin and inulin are considered to strengthen immune system and relieve inflammation. These ingredients are generally contained in the roots of rosewood, and saponin content is approximately 2 to 3% more in the rosewood [14].

Studies have reported the antimicrobial effects of natural extracts against oral microorganisms A previous study reported the antibacterial effect of Platycodon grandiflorum powder on oral microorganisms. In this study, we investigated the antimicrobial effect of the Platycodon grandiflorum extracts on oral microorganisms.

Ⅱ. Methods

1. Preparation for extracts

Platycodon grandiflorum extracts, the raw material in this experiment, was cultivated in

Sangju, Gyeongbuk province for 3 years. In order to prepare the extract, 180 g of dried shell-free Platycodon grandiflorum was separated, and 1,000 ml of primary distilled water was added to the boiling water. The mixture was heated until the amount of water halved, and using a filter paper (Adantec No.2, Japan) subjected to filtration under reduced pressure.

2. Experimental strains

Experiments were carried out using Streptococcus mutans (KCOM 1147), Streptococcus anginosus (KCOM 1348), Streptococcus sobrinus (KCOM 1185), Streptococcus oralis (KCOM 1505), Streptococcus mitis (KCOM 1050), Actinomyces naeslundii (KCOM 1517), Actinomyces odontolyticus (KCOM 1473), Actinomyces viscosus (KCOM 1472), Actinomyces oris (KCOM 1471) and Streptococcus salivarius (PO 4296). Escherichia coli (ATCC 25922) was used as a contamination indicator. These strains were purchased from the Korean Center for Oral Microbiology, Gwangju, Korea, and the Korean Center for Pathology Resources and ATCC (American Type Culture Collection for Chonbuk National University).

3. The MIC of Platycodon grandiflorum extracts

To determine the minimum inhibitory concentration (MIC) of the extract, the solid medium dilution method was used. The plate medium was prepared so as to have the concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml, and 1 mg/ml, and the test strain was cultured in a BHI liquid medium, LB liquid medium for 18 hours at 36 °C in a shaking incubator, and then subjected to 1% inoculation in a new liquid medium. The cultured strain was diluted to 1 × 104 CFU/ml, and 5 μl of each strain was inoculated on a plate culture medium containing the Platycodon grandiflorum extract for 24 to 48 hours at 36 °C. In order to evaluate the antimicrobial activity of Platycodon grandiflorum extract, the concentration without growth of each strain was regarded as the minimum growth inhibitory concentration.

4. The MIC of bark-free Platycodon grandiflorum extracts on the growth

To determine the viability of the oral microorganisms according to the concentration of extracts, plate culture media were prepared so that the concentration of the extract solution was 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml. The test strain was cultured in a BHI liquid medium, LB liquid medium for 18 hours at 36 ° C in a shaking incubator and the number of colonies were compared by culturing on a plate culture medium after diluting the cultured medium to 1 × 104 CFU/ml. The viable cell count was repeated three times under the same conditions.

Ⅲ. Results

1. The MIC of Platycodon grandiflorum extracts

The minimum growth inhibitory concentration of the Platycodon grandiflorum extract on the oral microorganisms was measured as shown in Table 1 for the barked Platycodon grandiflorum extracts. The MIC values of S. sobrinus, S. salivarius, A. naeslundii, A. odontolyticus and A. viscosus including E. coli were 0.25 mg/ml, S. mutans, S. anginosus, S. oralis, The MIC value of S. mitis and A. oris was 0.5 mg/ml. Table 2 shows the results for bark-free Platycodon grandiflorum extracts. MIC values of S. sobrinus, A. naeslundii, A. odontolyticus, and A. viscosus were 0.25 mg/ml, including S. mutans, S. anginosus, S. oralis, S. mitis, The MIC value of S. salivarius, and A. oris was measured at 0.5 mg/ml.

<Table 1> MIC of Platycodon grandiflorum extracts on the growth of oral bacteria

Strains	Concentration (mg/ml)						MIC (mg/ml)
	0	0.0625	0.125	0.25	0.5	1	
E. coli	+	+	+	+	−	−	0.25
S. mutans	+	+	+	+	+	−	0.5
S. anginosus	+	+	+	+	+	−	0.5
S. sobrinus	+	+	+	+	−	−	0.25
S. oralis	+	+	+	+	+	−	0.5
S. mitis	+	+	+	+	+	−	0.5
S. salivarius	+	+	+	+	−	−	0.25
A. naeslundii	+	+	+	+	−	−	0.25
A. odontolyticus	+	+	+	+	−	−	0.25
A. viscosus	+	+	+	+	−	−	0.25
A. oris	+	+	+	+	+	−	0.5

<Table 2> The MIC of bark-free Platycodon grandiflorum extracts on the growth of oral bacteria

Strains	Concentration (mg/ml)						MIC (mg/ml)
	0	0.0625	0.125	0.25	0.5	1	
E. coli	+	+	+	+	+	−	0.5
S. mutans	+	+	+	+	+	−	0.5
S. anginosus	+	+	+	+	+	−	0.5
S. sobrinus	+	+	+	+	−	−	0.25
S. oralis	+	+	+	+	+	−	0.5
S. mitis	+	+	+	+	+	−	0.5
S. salivarius	+	+	+	+	+	−	0.5
A. naeslundii	+	+	+	+	−	−	0.25
A. odontolyticus	+	+	+	+	−	−	0.25
A. viscosus	+	+	+	+	−	−	0.25
A. oris	+	+	+	+	+	−	0.5

2. The MIC of bark-free Platycodon grandiflorum extracts on the growth

1) Platycodon grandiflorum extracts

Colonies were observed in the medium, 0.25 mg/ml, and 0.5 mg/ml medium, but the number of colonies was decreased as the concentration increased. Colony formation did not occur in the 1 mg/ml medium. In the case of S. sobrinus and S. salivarius, colonies were formed in the medium, 0.25 mg/ml, but the number gradually decreased, and the colonies failed to grow in the medium of 0.5 mg/ml and 1 mg/ml. Colonies were formed in A. naeslundii, A. odontolyticus, and A. viscosus at a concentration of 0.25 mg/ml, but the number of colonies was decreased with the increasing concentration. At a concentration of 0.5 mg/ml, no colonies grew in the medium. Colony formation was not observed in A. oris in the medium, and at concentrations of 0.25 mg/ml, and 0.5 mg/ml, and in 1 mg/ml medium. E. coli, colony formation was seen in the medium of 0.25 mg/ml and 0.25 mg/ml in the medium, but the colony was killed in the medium of 0.5 mg/ml and 1 mg/ml as shown in Table 3.

2) Bark-free Platycodon grandiflorum extracts

Colonies were formed in the medium of 0.25 mg/ml and 0.5 mg/ml, but As the concentration increased, the number of colonies decreased and Colony formation was not observed in 1 mg/ml medium. Colonies were observed in S. sobrinus at a concentration of 0.25 mg/ml in the normal medium, but the number decreased gradually, and colonies failed to grow at the concentration of 0.5 mg/ml and 1 mg/ml. Colonies were formed in A. naeslundii, A. odontolyticus, and A. viscosus at a concentration of 0.25 mg/ml, but the number of colonies was decreased with the increasing concentration. At the concentrations of 0.5mg/ml, no colonies appeared in the medium. Colony formation was not observed in A. oris in the medium, at concentrations of 0.25 mg/ml, 0.5 mg/ml, and in the 1 mg/ml medium. The colony counts of E. coli were 0.25 mg/ml and 0.5 mg/ml, but the number of colonies was decreased and no colony was formed in the 1 mg/ml medium as shown in Table 4.

<Table 3> Inhibitory effect of Platycodon grandiflorum extracts on the growth of oral bacteria Unit: 105 CFU/ml

strains	Free	0.25 mg/ml	0.5 mg/ml	1 mg/ml
E. coli	33.5	24.7	-	-
S. mutans	43.5	32.8	30.3	-
S. anginosus	32.5	26.6	20.4	-
S. sobrinus	35.3	21.4	-	-
S. oralis	20.4	14.7	9.8	-
S. mitis	32.6	24.8	8.2	-
S. salivarius	29.9	9.1	-	-
A. naeslundii	62.3	30.3	-	-
A. odontolyticus	22.7	17.2	-	-
A. viscosus	37.4	28.1	-	-
A. oris	29.7	25.1	13	-

<Table 4> Inhibitory effect of bark-free Platycodon grandiflorum extracts on the growth of oral bacteria

Unit: 105 CFU/ml

strains	Free	0.25 mg/ml	0.5 mg/ml	1 mg/ml
E. coli	34.5	22.9	19	-
S. mutans	54.9	48.5	38	-
S. anginosus	40.5	33	24	-
S. sobrinus	22	16.2	-	-
S. oralis	19	15.8	5.9	-
S. mitis	26.1	14.8	8.5	-
S. salivarius	29.9	20.1	2.8	-
A. naeslundii	62.3	44.4	-	-
A. odontolyticus	11.1	9.7	-	-
A. viscosus	37.9	23.7	-	-
A. oris	30.5	26.9	18	-

Ⅳ. Discussion

Dental caries and periodontal disease are typical oral diseases, which are increasing every year, and the formation of dental plaque is a common cause. The oral cavity is directly exposed to the external environment and microorganisms at all times, resulting not only in the incidence of oral diseases but also systemic diseases by providing an environment in which microorganisms can thrive. In recent years, antibiotic abuse and the development of tolerance have become serious issues, and there is a growing interest in the use and necessity of natural extracts. Kim et al. [15] have reported the antibacterial effects of the Platycodon grandiflorum powder on Streptococcus mutans and Candida albicans. However, the antibacterial role of the extract on oral microorganisms is yet to be established. Hence, this study investigated the antimicrobial activity of the Platycodon grandiflorum extract against oral microorganisms, which is previously reported to be useful in bronchial asthma.

In this study, the antimicrobial activity was evaluated using E. coli and dental caries strains S. mutans, S. anginosus, S. oralis, S. mitis, S. sobrinus, S. salivarius and gingivitis strains A. odontolyticus, A. naeslundii, A. viscosus, and A. oris. The minimum growth inhibitory concentration of Platycodon grandiflorum extracts on oral microorganisms was 0.25 mg/ml, including S. sobrinus, S. salivarius, A. naeslundii, A. odontolyticus and A. viscosus.

The measurement of the number of viable cells according to the concentration of the Platycodon grandiflorum extract, demonstrated that all 10 strains including the E. coli, which is a contaminant indicator bacterium, formed colonies in the medium without the Platycodon grandiflorum extract and when the concentration of Platycodon grandiflorum extract was 0.25mg/ml in the culture medium. Colonies were formed in all of the 11 strains, but the colony colonies decreased in the culture medium without the Platycodon grandiflorum extract.

In the medium inoculated with 0.5 mg/ml, colony formation was not observed in S. sobrinus, S. salivarius, A. naeslundii, A. odontolyticus, and A. viscosus, including E. coli,

Colonies were formed in S. mutans, S. anginosus, S. oralis, S. mitis and A. oris but the number of colonies was decreased in the medium with 0.25 mg/ml concentration. In the medium supplemented with 1 mg/ml, no growth of bacteria was observed across all strains. In the case of extracts without Platycodon grandiflorum, all 11 strains formed colonies in the normal medium. In the culture medium supplemented with 0.25 mg/ml, the 11 strains grew colonies but were decreased compared to the colony formation in the normal medium. Colony formation was not detected in S. sobrinus, A. naeslundii, A. odontolyticus, and A. viscosus in the medium inoculated with 0.5 mg/ml of the Platycodon grandiflorum extract. In the culture medium with a 1 mg/ml concentration, bacterial growth was absent.

The saponin in the Platycodon grandiflorum educes inflammation, and it is known to contain about approximately 2 to 3% more than in the Platycodon grandiflorum shell. The result of this study shows that the platycodon grandiflorum extract has a higher saponin content than the Platycodon grandiflorum extract. The results confirmed that the antimicrobial activity. In addition, the results of the measurement of the number of viable cells according to the concentration were in agreement with those of the minimum growth inhibitory concentration, and the antimicrobial effect of A. naeslundii, A. odontolyticus, and A. viscosus, which cause gingivitis. Based on these results, further studies on oral microorganisms using various extracts are necessary, with the antimicrobial activities being investigated concurrently on strains responsible for periodontitis, as well as and gingivitis.

Ⅴ. Conclusion

This study investigated the antimicrobial effect of Platycodon grandiflorum on 10 strains of oral microorganisms.

1. Antimicrobial effects were more evident on A. naeslundii, A. odontolyticus, and A. viscosus, which cause gingivitis, han on strains causing dental caries.
2. It was confirmed that the extracts of Platycodon grandiflorum having a higher saponin content than the bark-free Platycodon grandiflorum extract showed excellent antimicrobial effect.
3. The number of viable cell counts according to the concentration of Platycodon grandiflorum extracts showed that the colonies were formed in the medium of all 11 strains in case of the barked Platycodon grandiflorum.

These results suggest that these natural extracts may have antimicrobial effects against oral diseases and gum diseases.

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<국문초록>

Objectives: 도라지 추출액을 이용하여 구강미생물에 대한 Platycodon grandiflorum 추출물의 항균 효과를 실험하고자 한다. **Methods:** 항균 활성 및 최소 저해 농도는 한천 희석법을 사용하여 측정하였다. 추출물 (Platycodon grandiflorum 추출물과 껍질이 없는 Platycodon grandiflorum 추출물)을 준비하고 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml의 농도로 한천 플레이트를 만들었다. **Results:** 치아우식균보다 치은염을 유발시키는 A. naeslundii, A. odontolyticus, A. viscosus 세 균주에서 더 우수한 항균효과를 나타내었으며, 껍질이 없는 도라지 추출물 보다 사포닌 함량이 더 많은 껍질이 있는 도라지 추출물에서 우수한 항균효과를 나타낸 것을 확인하였다. **Conclusions:** 도라지 추출물 결과에서 보듯이 천연 추출물이 구강질환을 유발시키는 치아우식균 및 잇몸질환균에 대해 항균효과가 있을 수 있음을 시사한다.