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Camellia sinensis = green tea
Psoralea corylifolia= ?
Galla Rhois = ?
Salvia miltiorrhiza= danshen, red sage
Platcodon grandiflorum = galloon flower

Effects of Herbal Extracts on Dental Plaque Formation and Human Gingival Fibroblasts

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Abstract. The purpose of this study was to investigate the effects of *Galla Rhois, Psoralea corylifolia, Camellia sinensis, Salvia miltiorrhiza* and *Platcodon grandiflorum* on the inhibition of artificial dental plaque formation and the cytotoxicity of the herbal extracts on human gingival fibroblasts. The analysis was carried out with the measurment of dental plaque weight and the cell survival rate of human gingival fibroblasts. It showed that *Galla Rhois, Psoralea corylifolia, Camellia sinensis* and *Salvia miltiorrhiza* inhibited the artificial dental plaque formation and did not reduce the cell viability of human gingival fibroblasts.

Introduction

Dental plaque is a film of microorganisms on the tooth surface, plays an important part in the development of caries and periodontal disease[1].

Streptococcus mutans can colonize the tooth surface and initiate plaque formation by their ability to synthesize water insoluble glucan, using glucosyltransferase[2-4]. To prevent dental caries, inhibition of glucosyltransferase activity by specific enzyme inhibitors[5,6], inhibition of initial cell adhesion of *S. mutans* by polyclonal and monoclonal antibodies[7], and inhibition of cell growth of *S. mutans* by antibacterial agents have been studied.

Especially antimicrobial agents against *S. mutans* can play an important role in the prevention of dental diseases, particularly those affect plaque formation[8-10].

Natural agents like herbs or herb extracts have been used for thousands of years in folk medicine for prevention and treatment of disease. Using natural agents like herbal extracts can be safe, and it also can reduce the pathogenic bacteria effectively[11]. In cariogenic bacteria, it was reported that Ceanothic acid and ceanothetric acid from the native American plant *Ceanothus ameicanus* showed growth-inhibitory effects against *S. mutans*[12].

Galla Rhois, Psoralea corylifolia, Camellia sinensis, Salvia miltiorrhiza and *Platcodon grandiflorum* which are traditional Chinese medicines, as a natural agent have been studied for antimicrobial activity[13-17], but there are few studies both all in cariogenic oral pathogen and cytotoxicity of the agents. Thus, inhibition study of dental plaque formation of the herbal extracts are necessary to find effective antimicrobial activity against *S. mutans*, and safety of normal cell in human mouth must be considered at the same time.

This study reports whether *Galla Rhois, Psoralea corylifolia, Camellia sinensis, Salvia miltiorrhiza* and *Platcodon grandiflorum* inhibit the plaque formation caused by cariogenic oral pathogen, *S. mutans* and show the cytotoxicity of the herbal extracts on human gingival fibroblasts.

Experimental Methods

Preparation of specimens: *Galla Rhois, Psoralea corylifolia, Camellia sinensis, Salvia miltiorrhiza* and *Platcodon grandiflorum* in this experiment were cultivated in Gwangju area, Chonnam Province in Korea.

The specimens were stored and processed at the Department of Food and Technology of the College of Agriculture, Chonnam National University, Korea by standard methods.

After being air-dried under a fume hood at room temperature, the aerial parts of the plants were crushed using a super mixer (model SM2000, Retch, Germany). The dried material (100g each) was extracted in 100% Distilled Water (D/W), 5 V/W, 80°C for 4 hours and filtered through Whatman No. 2 filter paper. These D/W extracts were concentrated using vacuum evaporator (TYPE N-2N, Eyela, Tokyo, Japan) attached to a cooling aspirator. The concentrated D/W extracts were then lyophilized (FDU-540, Eyela, Tokyo, Japan) to obtain the dried samples.

Measurement of plaque weight: Effect of herbal extracts from *Galla Rhois, Psoralea corylifolia, Camellia sinensis, Salvia miltiorrhiza,* and *Platcodon grandiflorum* were screened at a wide range of concentrations by diluting them to the final concentrations of 0.1%, 0.4% and 0.8% in 40 ml of M17SY growth medium supplemented with 5.0% sucrose, 0.5% yeast extract, and 0.1M 3-(N-Morpholino)propanesulfonic acid (MOPS) in a beaker. And final concentration of control is 0.0%. A 0.016 inch stainless steel wire (ORMCO, Glendora, CA, USA) was suspended in the beaker and inoculated with 100ul of 18 hours cultured *Streptoccus mutans Ingbritt.*

The beakers were then incubated under slow agitations at 37^{o}C for 24 hours. At the end of incubation period, the dry weights of plaque accumulation on the wire were determined. At the end of the incubation time, the accumulated plaque on the wire was placed in 10% neutral formaldehyde for 30 minutes followed by gentle washing in running tab water. The plaque on the wire was then dried on a Petri dish in a Wheaton Dry-Seal desiccator with a porcelain plate under clear plastic cartridges containning blue silica gel at 37°C for 24 hours.

The weight of the wire with dried plaque was assessed using an analytical balance (Mettler Toledo AB series Basic Level Analytical Balance, Model AB54). The wire was weighed after removing the dried plaque by immersing it in 6% sodium hydroxide overnight and then dried in a desiccater. The dried weight was determined by the difference of the two measurements.

Cytotoxicity test: Herbal extracts were dissolved in Dimethylsulfoxide (DMSO) (Sigma, USA) and then those were diluted in media without serum for final concentrations of 0.0004%, 0.0002%, 0.0001%, and 0.0000% as a control.

Human gingival fibroblasts had been taken from the patients who visited Department of Periodontology, Dental hospital of Chonnam National University, Korea. The patients had a sound gingiva with no inflammation and no medication history during 3 month.

Human gingival fibroblasts were incubated in alpha minimum essential medium (Gibco BRL, UK) with gentamycin (10 mg/ml, JBI, Korea) and fetal bovine serum (Gibco BRL, UK) and incubator was used at 37^{o}C, 5% CO_2 condition. Then medium was changed. After 4 days, fibroblasts were dealed with trysin-EDTA solution (Gibco BRL, UK).

Final fibroblasts were selected among 3th-8th times incubated cells. MTT 〔3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide〕 (Sigma, USA) reduction assay was used for survival rate of human gingival fibroblasts.

Statistical analysis: Kruskal - Wallis test and Mann-Whitney test as a post hoc were carried out with SPSS 12.0 (SPSS Inc.,USA) for statistical analysis.

Results and Discussion

The inhibiting activity of herb extracts on artificial dental plaque formation is shown in Table 1. *Galla Rhois* showed the most effective plaque inhibition, and *Psoralea corylifolia*, *Cammellia*

sinensis, Salvia miltiorrhiza showed more effective plaque inhibition than control. However *Platycodon grandiflorum* showed equal or worse inhibition effect than control.

Table 1. The weight of artificial dental plaque in the culture of *S. mutans Ingbritt*

Unit : [mg]

Concentration(%)	Herbal extract				
	*Galla Rhois**	*Psoralea corylifolia**	*Camellia sinensis**	*Salvia miltiorrhiza**	*Platycodon grandiflorum**
0.1	26.75±9.05[b]	121.28±42.30[b]	119.73±17.21[a]	119.82±36.24[a]	151.15±39.57[a]
0.4	1.47±1.06[c]	3.01±1.52[c]	55.87±9.26[b]	86.57±16.66[b]	129.23±16.20[a]
0.8	1.31±1.16[c]	2.15±1.58[c]	3.43±1.39[c]	74.95±24.38[b]	202.07±40.53[b]
control	136.52±18.40[a]	136.52±18.40[a]	136.52±18.40[a]	136.52±18.40[a]	136.52±18.40[a]

Data are expressed as mean±SD (N=9)

*: $p<0.05$, by Kruskal - Wallis test

[a, b, c]: Values with same superscript letter were not statistically significant at each extracts ($p=0.05$)

The MTT reduction assay results on human gingival fibroblasts is shown in Table 2. If there is a cytotoxic effect of the herbal extracts on human gingival fibroblasts, the herbal extracts could not use as a antimicrobial agent for plaque control in human. In this study, regarding cytotoxicity of the herbal extracts, cell viabilities of human gingival fibroblasts were not reduced by *Galla Rhois, Psoralea corylifolia, Camellia sinensis, Salvia miltiorrhiza,* and *Platcodon grandiflorum,* but increased by *Galla Rhois* and *Salvia miltiorrhiza.*

Table 2. MTT reduction assay results on human gingival fibroblasts

Unit : [%]

Concentration(%)	Herbal extract				
	*Galla Rhois**	*Psoralea corylifolia*	*Camellia sinensis*	*Salvia miltiorrhiza**	*Platycodon grandiflorum*
0.0001	97.23±1.66[a]	98.17±1.46	101.98±1.01	100.66±0.26[a]	98.81±0.79
0.0002	108.28±0.88[b]	102.61±1.10	99.5±1.19	102.36±0.27[ab]	101.05±0.77
0.0004	107.15±1.31[b]	103.60±1.34	104.32±0.71	104.29±0.49[b]	101.64±0.66
Control	100.00±1.15[a]	100.00±1.81	100.00±0.66	100.00±0.59[a]	100.00±1.46

Data are expressed as mean±SD (N=8)

*: $p<0.05$, by Kruskal - Wallis test

[a, b, c]: Values with same superscript letter were not statistically significant at each extracts ($p=0.05$)

We evaluated *in vitro* the antimicrobial activities and cytotoxicity of the herbal extracts. *Galla Rhois, Psoralea corylifolia, Camellia sinensis, Salvia miltiorrhiza* showed effective antimicrobial activities in order, especially at 0.4% and 0.8%. If the inhibition effect is similar, the lower concentration can be recommended. Thus, we think that 0.4% of *Galla Rhois, Psoralea corylifolia* and *Salvia miltiorrhiza,* and 0.8% of *Camellia sinensis* are useful concentrations for inhibition of plaque formation by *S. mutans Ingbritt*. Unfortunatly, *Platycodon grandiflorum* could not inhibit the plaque formatin, even though it did not show the cytotoxic effect on human gingival fibroblasts.

It was reported that *Galla Rhois* –derived tannins (methyl gallate and gallic acid) has significant growth inhibitory activity on some intestinal bacteria[13]. We think that one or both of *Galla Rhois* –derived tannins (methyl gallate and gallic acid) may be the active compound of antibacterial effect on *S. mutans.* Thus, further studies are necessary to verify the compounds with antimicrobial effect from those herbal extracts and to evaluate the antimicrobial effects of those herbal extracts against oral pathogen using *in vivo* models.

In this study, we studied the inhibtion effect of plaque formation due to *S. mutans* only, but it could be considered the antimicrobial effect of the herbal extracts to other oral pathogens like *Porphyromonas gingivalis*, *Actinomyces viscosus* etc. which are cause of periodontal disease.

Conclusions

Herbal extracts in this study, excepts *Platcodon grandiflorum* inhibited the artificial dental plaque formation, and it is suggested that 0.4% of *Galla Rhois*, *Psoralea corylifolia* and 0.8% of *Camellia sinensis* are useful concentrations for inhibition of dental plaque formation by *S. mutans Ingbritt*. All of the herbal extracts in this study did not reduce the cell vialbility of human gingival fibroblsts. Therefore, *Galla Rhois*, *Psoralea corylifolia*, *Camellia sinensis*, and *Salvia miltiorrhiza* may be developed as a antibacterial agent against dental caries and periodontal disease.

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