

Screening of Antimicrobial Activity among the Therapeutic Herbal Extracts on Dental Pathogens

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The periodontal diseases and dental caries are major infectious diseases in oral cavity. Many of the preventive and therapeutic dental products contain the antimicrobial and anti-inflammatory components. But some of these antimicrobial have weak points such as coloration, burning sensation and insolubility in water. We have screened the therapeutic herbal extracts of the Plant Extract Bank for the antimicrobial activity on the major dental pathogens by growth inhibition assay. For the *Porphyromonas gingivalis*, 8 herbal extracts had an antimicrobial activity, 11 herbal extracts for the *Prevotella intermedia*, 43 herbal extracts for the *Haemophilus actinomycetemcomitans* and 61 herbal extracts for the *Streptococcus mutans*. Among these extracts, 6 herbal extracts had an antimicrobial activity for more than 3 species of dental pathogens. These extracts are *Araliae Cordatae Radix*, *Crassirhizomae Rhizoma*, *Mori Radicis Cortex*, *Psoraleae Semen*, *Pini Ramulus* and *Siegesbeckiae Herba*. All of effective extracts were CA group, ethanol extracts. Among these 6 herbal extracts, only *Crassirhizomae* is known to have a antibacterial effects. Therefore these herbal extracts have a possibility to be a candidate for a major antibacterial components in dental products.

Keywords: Herbal extract, Dental pathogen, Antimicrobial activity

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Introduction

The periodontal diseases and dental caries are major infectious diseases occurred in oral cavity. Many pathogenic bacteria were found to be related with these diseases. For dental caries, *Streptococcus mutans* (Fitzgerald *et al.*, 1960, and Michalek *et al.*, 1977), *Lactobacillus* spp. (De Stoppelaar *et al.*, 1969, and Krasse, 1954), and *Actinomyces* spp. (Jordon *et al.*, 1972) are known to be pathogenic bacteria. The caries on the all of tooth surfaces including early caries lesion and root surface is related with *Streptococcus mutans*, and *Lactobacillus* spp. are related with the progression of dental caries by lowering the environmental pH (Gibbons *et al.*, 1968).

The periodontal disease is the chronic infectious disease, which may cause the inflammation of periodontal tissue and lead to the tooth loss (Trans and Ludney, 1996). The known pathogenic bacteria are *Haemophilus actinomycetemcomitans*, *Bacteroides forsythus*, *Campylobacter rectus*, *Eikellena corrodens*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia* and spirochetes (Darveau *et al.*, 1997, Genco *et al.*, 1985, Mandell, 1984, Slot and Genco, 1984, Zambon *et al.*, 1985 and Michalek *et al.*, 1977).

The oriental medicine was used as a primary cure for many diseases in many countries. Recently, the major effective components of the ancient medicine has been found and used as a lead compound of new drug development (Lawrence, 1999).

The strategy for the oral disease is changing to the prevention from the treatment and reconstruction. The population using the preventive remedies such as mouthwash, dental floss and fluoridation are increased. In the mouthwash, many of the products available at this time are containing fluoride, essential oil or antiseptics. Because many antiseptics in mouthwash are synthetic materials, there would be many side-effects such as coloring of oral tissue and burning

sensation of tongue. Also mouthwash containing these antiseptics should contain the alcohol for the insolubility of synthetic antiseptics to the water.

To overcome these weak points, many attempts are focused to find the new antimicrobial compound from natural product (Ooshima *et al.*, 1998, Sarkar *et al.*, 2000, Tsuchiya, 1999 and Yanagida *et al.*, 2000). We have screened the therapeutic herbal extracts made by the Plant Extract Bank (Daejeon, Korea) to find a candidate for natural antimicrobial activity for major dental pathogens.

Materials and Methods

Therapeutic Herbal extracts

All of the therapeutic herbal extracts were purchased from the Plant Extract Bank (Daejeon, Korea). The "CA" groups were prepared by extraction with 95% ethanol. And the "CW" groups were extracted by traditional boiling with water. Total 800 extracts (400 extracts for CA and 400 extracts for CW) were used for screening.

Bacteria

Three periodontal pathogens (*Porphyromonas gingivalis*, ATCC 33277, *Haemophilus actinomycetemcomitans* ATCC

33384 and *Prevotella intermedia* ATCC 25611) and one cariogenic bacteria (*Streptococcus mutans*, ATCC 27351) were purchased from the Korean Collection for Type Cultures (KCTC, Daejeon, Korea). The *P. gingivalis* and *P. intermedia* were cultured with anaerobic Brain Heart Infusion broth containing 1.0 µg/ml Vit K₁ and 5 µg/ml Hemin at anaerobic condition. *H. actinomycetemcomitans* and *S. mutans* were cultured with anaerobic BHI broth.

Screening for antimicrobial activity

Each of bacteria was cultured and diluted with sterile media at 0.1 absorbance at 450 nm. In the 96-well plate, 200 µl of bacterial solution was added to the each well. Each of the therapeutic herbal extracts was added to the each well at final concentration of 2 mg/ml. After 24 hrs, the bacterial growth was checked by the absorbance at 450 nm., The ampicillin was added at 100 microgram/ml concentration as a positive control. The sterile media was used as negative control. For standardization, the absorbance was divided with the absorbance of bacterial culture without any addition.

Results and Discussions

We have screened 800 kinds of therapeutic herbal extracts

Table 1. Therapeutic herbs that have an antibacterial activity against major pathogens in dental caries and periodontal diseases.

Species Name	P.g	P.i	H.a	S.m	Species Name	P.g	P.i	H.a	S.m
<i>Chrysanthemi Flos</i>	+				<i>Alpiniae Semen</i>			++	
<i>Glycyrrhizae Radix</i>			+		<i>Broussonetiae Fructus</i>				+++
<i>Angelicae koreanae Radix</i>			+++	+++	<i>Peucedani Radix</i>				++
<i>Cinnamomi Ramulus</i>			+		<i>Cnidii Rhizoma</i>			++	
<i>Pogostemonis Herba</i>			++		<i>Biotae Orientalis Folium</i>				+
<i>Sophorae Flos</i>			+		<i>Biotae Orientalis Folium</i>				+
<i>Arisaematis Rhizoma</i>			+++		<i>Tiglii Semen</i>				+++
<i>Angelicae Sinensis Radix</i>			+++		<i>Lygodii Spora</i>				++
<i>Persicae Semen</i>			+++	++	<i>Nepetae Spica</i>				+
<i>Araliae Cordatae Radix</i>	+	+		+	<i>Benincasae Semen</i>				++
<i>Aristolochiae Fructus</i>				++	<i>Malvae Semen</i>				++
<i>Cannabis Semen</i>			+++		<i>Thujae Semen</i>				++
<i>Equiseti Herba</i>			++		<i>Psoraleae Semen</i>	++		++	++
<i>Saussureae Radix</i>			+++		<i>Amomi Semen</i>				++
<i>Sterculiae Scaphigeriae Semen</i>			+++	+++	<i>Pini Ramulus</i>	+	+	+	+
<i>Peucedani Japonici Radix</i>			++	++	<i>Schizandrae Fructus</i>			+	
<i>Amomi Cardamomi Fructus</i>				+	<i>Plantaginis Semen</i>				+++
<i>Cynanchi Radix</i>				++	<i>Ligustici Rhizoma</i>				++
<i>Zedoariae Rhizoma</i>			++		<i>Gastrodiae Rhizoma</i>			++	
<i>Quisqualis Fructus</i>				+++	<i>Harphagophytum</i>				+
<i>Torilidis Fructus</i>				+++	<i>Zanthoxylis Pericarpium</i>				+
<i>Ligustici sinensis Rhizoma et Radix</i>				+++	<i>Rubiae Radix</i>				+
<i>Angelicae tenuissimae Radix</i>				++	<i>Coptidis Rhizoma</i>			+	+

P. g : *Porphyromonas gingivalis*

P. i : *Prevotella intermedia*

H. a : *Haemophilus actinomycetemcomitans*

S. m : *Streptococcus mutans*

Table 1 (Continued)

Species Name	P.g	P.i	H.a	S.m	Species Name	P.g	P.i	H.a	S.m
<i>Angelicae tenuissimae Radix</i>				++	<i>Coptidis Rhizoma</i>			+	+
<i>sophorae Radix</i>	+			+	<i>Imulae Radix</i>				+++
<i>Crassirhizomae Rhizoma</i>	+	+		+	<i>Cuscutae Semen</i>				++
<i>Allii tuberosi Semen</i>				+++	<i>Tetrapanacis Medulla</i>				++
<i>Gossypii Semen</i>			+++	++	<i>Corydalis Tuber</i>				+
<i>Ledebouriellae Radix</i>				++	<i>Cartami Semen</i>				+++
<i>Amomi Semen</i>				++	<i>Cartami Semen</i>				+++
<i>Zizyphi Spinosi Semen crudus</i>			+++	+	<i>Cartami Semen</i>			++	+++
<i>Mori Radicis Cortex</i>	++	++		+	<i>Phellodendri Cortex</i>			+	+
<i>Dendrobii Herba</i>				+	<i>Phellodendri Cortex</i>				+
<i>Imulae Flos</i>			+		<i>Glycine Semen nigra</i>			+	+++
<i>Anethi Fructus</i>				+++	<i>Siegesbeckiae Herba</i>		++	+	++
<i>Magnoliae Flos</i>			+++		<i>Rehmaniae Radix Preparata</i>			+	
<i>Schizandrae Fructus</i>			++		<i>Rehmaniae Radix Preparata</i>			+	
<i>Hydnocarpus Semen</i>		++		++	<i>Portulacae Herba</i>			+	
<i>Ledebouriellae Radix</i>				++	<i>Psoraleae Semen</i>				+
<i>Zizyphi Spinosi Semen</i>				+++	<i>Plantaginis Semen</i>		+	++	
<i>Perillae Semen</i>			++		<i>Lycopi Herba</i>			+	
<i>Lini Semen</i>				++	<i>Cuscutae Semen</i>		+		+
<i>Acanthopanax Cortex</i>			++		<i>Illicii Veri Fructus</i>			+	
<i>Pruni Nakaii Semen</i>				+++	<i>Rhei Rhizoma</i>			+	
<i>Curcumae longae Rhizoma</i>			+		<i>Coptidis Rhizoma</i>				+
<i>Polygalae Radix</i>				++	<i>Trigonellae Semen</i>		+	++	
<i>Cinnamomi Cortex Spissus</i>			+		<i>Polygoni Cuspidati Radix</i>		+		
<i>Myristicae Semen</i>	+++	++			<i>Scutellariae Radix</i>			+	

for the antimicrobial activity on major dental pathogens. For the *Porphyromonas gingivalis*, 8 herbal extracts had an antimicrobial activity, 11 herbal extracts for the *Prevotella intermedia*, 43 herbal extracts for the *Haemophilus actinomycetemcomitans* and 61 herbal extracts for the *Streptococcus mutans* (Table 1). Among these extracts, 6 herbal extracts had an antimicrobial activity for more than 3 species of dental pathogens. These extracts are *Araliae Cordatae Radix*, *Crassirhizomae Rhizoma*, *Mori Radicis Cortex*, *Psoraleae Semen*, *Pini Ramulus* and *Siegesbeckiae Herba*. All of effective extracts were CA group, ethanol extracts.

Among these 6 herbal extracts, only *Crassirhizomae* is known to have a antibacterial effects. This species is a pteridophyte and its rhizome has been used clinically in the Korea, Japan and China as a vermicide (Namba *et al.*, 1993). In addition, phloroglucinol derivatives and kaempferol acetyl rhamnosides have been isolated from this plant (Min *et al.*, 2001). The phloroglucinol derivatives possessed antibacterial (Mathekga *et al.*, 2000), anti-tumor promoting (Kapadia *et al.*, 1996), nitric oxide inhibitory (Ishii *et al.*, 2001) and anti-reverse transcriptase activities (Nakane *et al.*, 1991).

Araliae cordatae (*A. cordata*) has been used in treatment of arthritis and low back pain. It has been reported that *A. cordatae* inhibited COX-2 dependent PGE2 generation (Dang *et al.*, 2005) and showed effectiveness regarding analgesia, hypothermia, duration of pentobarbital-induced

anesthesia (Okuyama *et al.*, 1991). *Mori* has been used in oriental medicine in remedies for immune disorders such as asthma (Kim *et al.*, 2000). *Psoraleae Semen* has an effects on adhesion and migration of melanocytes and may play a role through this effect in the treatment of vitiligo (Zhang *et al.*, 2005). *Siegesbeckiae* has anti-inflammatory activities (Park *et al.*, 2007), immunosuppressive activities (Sun *et al.*, 2006) and inhibitory effect on IgE production (Kim *et al.*, 2001).

In our experiment, *Mori Radicis Cortex*, *Psoraleae Semen*, and *Siegesbeckiae Herba* have better antibacterial effects on major dental pathogens than others. Therefore these herbal extracts have a possibility to be a candidate for a major antibacterial components in dental products.

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