

ANTIMICROBIAL ACTIVITY OF ARTEMISIA SPECIES AGAINST CLINICALLY ISOLATED STREPTOCOCCUS MUTANS

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Abstract

Streptococcus mutans plays a major role in the formation of dental plaque and it is considered one of the important pathogens in the development of dental caries. Established dental plaque can be more resistant to antimicrobial agents and offers nutrient rich and relatively stable cariogenic conditions for bacteria. Thus bacteria growing in dental plaque have strong resistance to antimicrobial agents and they are not removed easily by the flow of saliva. Many researchers have been performed using natural materials, especially herbal extracts to prevent dental plaque. However, the strains of mutans streptococci used in the researches were not from Koreans. Therefore, it would be necessary to evaluate the antimicrobial activity of herbal extracts against clinical isolates of *Streptococcus mutans* isolated from saliva of Koreans living in Jeollabuk-do.

For this study four clinical isolates were isolated from saliva samples of seventeen Korean people to investigate whether essential oils of *Artemisia* spp. have antimicrobial activity against them including four reference strains of *Streptococcus mutans*. Minimum inhibitory concentrations, Minimum bactericidal concentrations and time kill studies were performed and the results showed that *Artemisia lavandulaefolia* DC, *Artemisia scoparia*, and *Artemisia capillaries* have antimicrobial activity against the *Streptococcus mutans* clinical isolates and reference strains

Key words : Artemisia, Antimicrobial agent, *Streptococcus mutans*

I. Introduction

Streptococcus mutans (*S. mutans*) is a Gram-positive, facultatively anaerobic bacteria which plays a major role in the formation of dental plaque and it is considered one of the important pathogens in the development of dental caries¹⁻³⁾. Initial adherence of *S. mutans* to the tooth surface occurs in a sucrose-independent way by Antigen I/II (Ag I/II)⁴⁾ and glucans synthesized by glucosyltransferases of *S. mutans* in a sucrose dependent way, which mediate attachment of bacteria to the tooth

surface and form dental plaque⁵⁻⁷⁾, a complex microbial biofilm which is a well organized, cooperating community of microorganisms^{8,9)}. Once *S. mutans* attaches to the surface of the tooth, it produces acid, lactic acid and it demineralizes tooth enamel inducing dental decay¹⁰⁾. Glucan-dependent adherence and accumulation by mutans streptococci is critical for the plaque formation^{11,12)} and a well established dental plaque, biofilm, can be more resistant to antimicrobial agents and offers nutrient rich and relatively stable cariogenic conditions for bacteria. Thus bacteria growing in dental plaque have

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strong resistance to antimicrobial agents and they are not removed easily by the flow of saliva so tooth-brushing, flossing and using a mouthwash containing antimicrobial agents would be good methods to prevent dental plaque.

The effects of antimicrobial agents such as Chlorhexidine¹³⁾, Alkylamine salts and amides¹⁴⁾, quaternary ammonium salts¹⁵⁾, phosphorylated polyethylene glycol¹⁶⁻¹⁸⁾ and herbal extracts have long been studied to reduce dental plaque. However, the strains of mutans streptococci used in the researches were not from Koreans. Therefore, it would be necessary to evaluate the antimicrobial activity of herbal extracts against clinical isolates of mutans streptococci, especially *S. mutans*, isolated from saliva of Koreans.

Artemisia is used for antioxidant, therapeutic applications and also have been suggested that it is an effective antimicrobial agent^{19,20)}, but there are only few studies on its antimicrobial activity against *S. mutans* and the strains used in the researches were not clinical isolates isolated from Koreans but reference strains.²¹⁾

Three species of the genus *Artemisia* were used in this study: *Artemisia lavandulaefolia* DC (*A. lavandulaefolia*), *Artemisia scoparia* (*A. scoparia*) and *Artemisia capillaries* (*A. capillaries*). *A. scoparia* is used for mosquito repellent and has hepatoprotective effects against carbon tetrachloride^{22,23)}. *A. capillaries* is used for herbal medicine and inhibits the activity of herpes simplex virus^{24,25)}.

The purpose of the present study was to isolate *S. mutans* clinical isolates (Ch, P, L1 and L2) from Korean patients with more than one dental caries and investigate antimicrobial activity of *A. lavandulaefolia* DC, *A. scoparia* and *A. capillaries* against the *S. mutans* clinical isolates including reference strains, KCTC 3298, KCTC 3605 (ATCC 25175), KCTC 3306 and GS5 with Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and time-kill studies.

II. Material and Methods

1. Bacterial strains

1-1. *Streptococcus* strains

Streptococcus mutans GS5 was kindly provided by F. Macrina, Virginia Commonwealth University, Richmond, VA, USA. The other reference strains were

KCTC 3298, KCTC 3605 (ATCC 25175), KCTC 3306. The four clinically isolated strains were Ch, P, L1 and L2.

1-2. Isolation of *S. mutans*

Saliva samples were collected from 17 patients (aged 6-7 years) who had more than 1 dental caries in 50 ml conical tubes and stored at 4°C before analysis. The samples were serially diluted 10-fold twice and 100 µl of each dilution was transferred onto a selective medium of *S. mutans*, medium plates (90 g Mitis salivarius agar and 100 g sucrose in 1L distilled water, autoclave and cooling to 50°C, then 0.2 g sulfisoxazole, 300 units bacitracin and 10 mg valinomycin) and grown at 37°C in an anaerobic chamber for 16 hrs. *S. mutans* exhibit a distinct colonial morphology on mitis salivarius agar²⁶⁾. Mitis salivarius agar was originally devised to isolate fecal streptococci but it has mostly used for the isolation of oral streptococci, including *S. mutans*, because of its selective and differential properties.

The samples were cultured selectively on MS-MUTV medium and brain heart infusion (BHI) medium plates. After several rounds of selection a colony from each plate was inoculated into BHI broth and grown at 37°C in an anaerobic chamber for 16 hrs and qualified at PCR screening using PCR primers for *S. mutans* AgI/II gene (also known as protein B, P1, Pac, and SpaP).

1-3. PCR screening

The PCR reaction mixture (50 µL) contained Taq polymerase, dNTPs mixture (dATP, dCTP, dGTP and dTTP), primers (Ag I/II F: 5'-ACATGCATGCGGACAAAAGGTTTTGCCGATG-3' and Ag I/II MR: 5'-GTATACCAGAGCTAGCGAACCGGGATC-3') and distilled water. The amplification reaction was 5 minute denaturation at 95°C, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 1 minute with a final extension at 72°C for 5 minutes.

2. Extraction of *Artemisia* spp.

Essential oils of *Artemisia* spp. (*A. lavandulaefolia* DC, *A. scoparia*, and *A. capillaries*) were supplied by Cha et al²⁷⁾ and Cha et al²⁸⁾. In brief, essential oils were prepared by distillation of air-dried *Artemisia* with a modified Clevenger-type apparatus.

3. Antibiotic

Ampicillin was used to compare the sensitivity with the essential oils.

4. MICs and MBCs

MICs and MBCs are the tests to monitor the activity of antimicrobial agents²⁹⁾. MIC is defined as the lowest concentration of antibiotic sufficient to inhibit bacterial growth when tested *in vitro* or as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MBC is defined as the lowest concentration of antibiotic required to kill an organism.

MICs of the essential oils of *A. lavandulaefolia* DC, *A. scoparia* and *A. capillaries* were defined by broth dilution method. Two fold dilutions were made for the test. The initial concentration of *A. lavandulaefolia* DC and *A. scoparia* was 25.6 ug/mL and 102.4 ug/mL for *A. capillaries*, respectively. About 1×10^8 CFU/ml of each strain was inoculated into 96 well containing BHI broth and the essential oils and grown at 37°C in an anaerobic chamber. After 16 hrs, the presence or absence of turbidity was evaluated. Optical Density (OD) of each well was measured at 550 nm using spectrophotometer (Optizen 2120uv, Mecasys, Korea). The lowest concentration that did not show growth was determined as the minimum inhibitory concentration. MICs were tested in quadruplicate and results are presented as the means of them.

MBCs were the lowest concentration of the essential oils that prevented the growth of the *S. mutans* strains following broth dilution procedures. The cells in the wells

of $0.5 \times \text{MIC}$, $1 \times \text{MIC}$, $2 \times \text{MIC}$, $4 \times \text{MIC}$, and $8 \times \text{MIC}$ were transferred onto the BHI plates. The dishes were incubated for 16 hrs at 37°C in the anaerobe chamber. The minimum concentration showing no growth on the plates was determined as the MBC of the essential oil. MBCs were tested in quadruplicate and results are the means of them.

5. Time-kill studies

Time-kill studies are generally used in investigations of antimicrobial activity of antimicrobial agents^{30,31)}. *A. lavandulaefolia* DC and ampicillin were chosen for time-kill studies. Viability counts of *A. lavandulaefolia* DC and ampicillin-containing *S. mutans* strains in BHI broth were performed at 0.5 hr, 1 hr, 2.5 hrs and 4 hrs by plating onto BHI plate. The plates were incubated at 37°C in the anaerobic chamber for 16 hrs. Time-kill activities were measured in duplicate and *A. lavandulaefolia* DC and ampicillin concentration chosen was $1 \times \text{MIC}$ for each strain. Results are the means of two studies.

III. Results

1. PCR screening to select clinical isolates

Clinical isolates were from saliva samples collected from Korean patients who had more than one dental caries. The isolates were selected based on the colony morphology on MS-MUTV agar plate and screened by PCR (Fig. 1) using PCR primers of *S. mutans* AgI/II gene. Four clinical isolates (Ch, L1, L2, and P) were selected from seventeen samples.

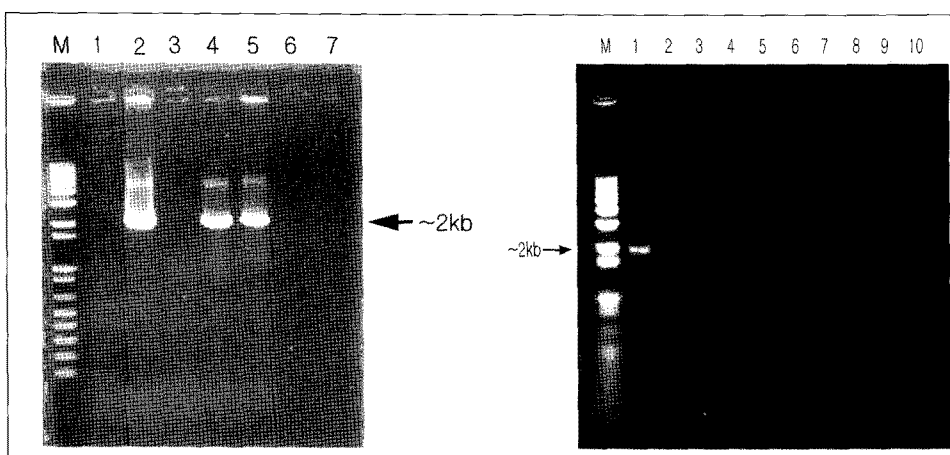


Fig. 1. PCR screening to select clinical isolates of *S. mutans*. The colonies from 17 patients were selected and qualified at PCR screening using primers of AgI/II gene, Ag I/II F and Ag I/II MR, and 4 of them were identified as *S. mutans* (M: marker).

2. Susceptibility

The selected clinical isolates were tested for antimicrobial activity of *A. lavandulaefolia* DC, *A. scoparia* and *A. capillaris* with four reference strains. MICs and MBCs of the essential oils of the *Artemisia* spp. are shown in Table 1. According to Table 1, *A. lavandulaefolia* DC, *A. scoparia* and *A. capillaris* have antimicrobial activity against all *S. mutans* strains.

A. lavandulaefolia DC MICs were the same as 0.8 ug/mL for reference strains (Fig. 2) and ranging between 0.4 and 0.8 ug/mL for clinical isolates (Fig. 3). *A.*

scoparia MICs were 0.8 to 1.6 ug/mL for reference strains (Fig. 4) and 0.4 to 0.8 ug/mL for clinical isolates (Fig. 5). *A. capillaris* MICs were 0.2 to 25.6 ug/mL for reference strains (Fig. 6) and 1.6 to 3.2 ug/mL for clinical isolates (Fig. 7).

A. lavandulaefolia DC MBCs were 1.6 to 6.4 ug/mL for reference strains and 0.8 to 3.2 ug/mL for clinical isolates. *A. scoparia* MBCs were 1.6 to 12.8 ug/mL for reference strains and 0.8 to 3.2 ug/mL for clinical isolates. *A. capillaris* MBCs for KCTC 3306, KCTC 3605, KCTC 3298, and GS5 were not possible to read and were 6.4 to 12.8 ug/mL for clinical isolates.

Table 1. MICs and MBCs of *A. lavandulaefolia* DC, *A. scoparia*, *A. capillaris*, and ampicillin for *S. mutans* four reference strains (KTCC 3306, KTCC 3605, KTCC 3298 and GS5) and four clinical isolates (P, L1, L2, and Ch) (ug/mL)

Strains/agents	<i>Artemisia lavandulaefolia</i> DC		<i>Artemisia scoparia</i>		<i>Artemisia capillaris</i>		Ampicillin	
	MICs	MBCs	MICs	MBCs	MICs	MBCs	MICs	MBCs
3306	0.8	1.6	0.8	6.4	12.8	↑	1	1
3605	0.8	6.4	1.6	1.6	12.8	↑	1	1
3298	0.8	6.4	1.6	6.4	0.2	↑	1	1
GS5	0.8	6.4	0.8	12.8	25.6	↑	1	1
P	0.8	3.2	0.4	0.8	3.2	12.8	1	1
L1	0.4	3.2	0.8	3.2	3.2	6.4	1	1
L2	0.4	3.2	0.8	3.2	3.2	6.4	1	1
Ch	0.4	0.8	0.4	0.8	1.6	6.4	1	1

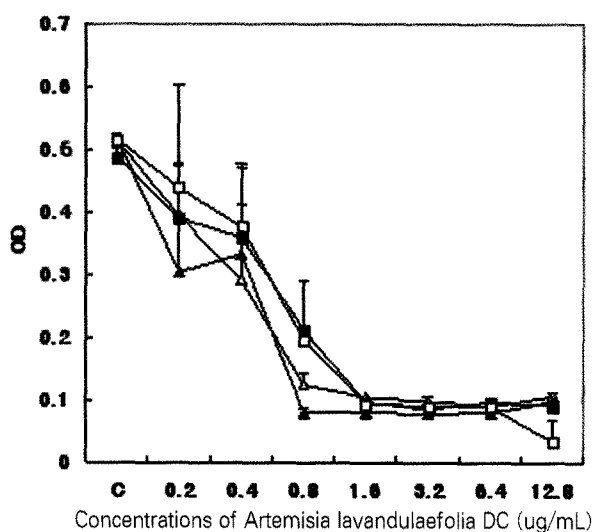


Fig. 2. *A. lavandulaefolia* DC MICs against *S. mutans* reference strains. KTCC 3605 (Δ), KTCC 3298 (\blacksquare), KTCC 3306 (\blacktriangle) and GS 5 (\square); Optical Density was measured at 550 nm. The results are mean values SD from four tests. C: control

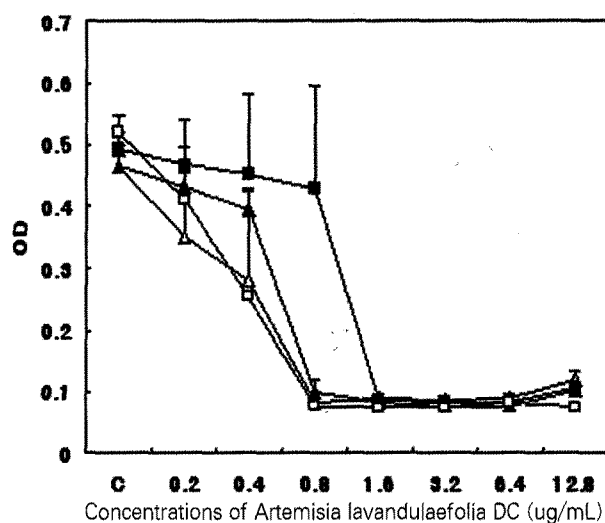


Fig. 3. *A. lavandulaefolia* DC MICs against *S. mutans* clinical isolates; Ch (Δ), P (\blacksquare), L1 (\blacktriangle) and L2 (\square). Optical Density was measured at 550nm. The results are mean values SD from four tests. C: control.

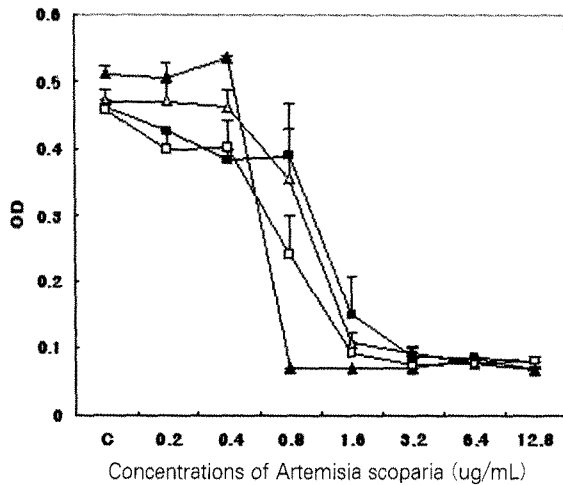


Fig. 4. *A. scoparia* MICs against *S. mutans* reference strains; KTCC 3605 (Δ), KTCC 3298 (\blacksquare), KTCC 3306 (\blacktriangle) and GS 5 (\square). Optical Density was measured at 550 nm. The results are mean values SD from four tests. C: control.

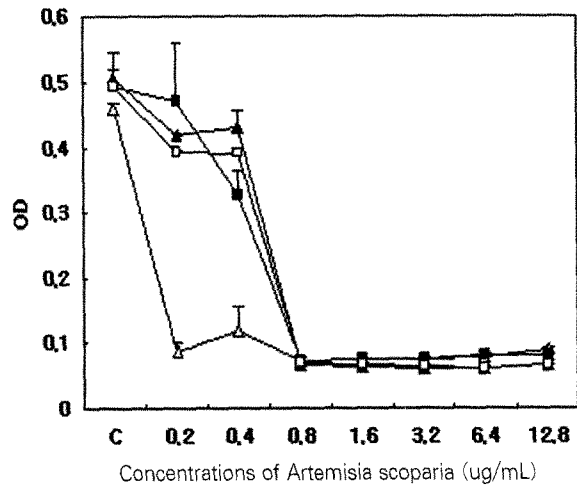


Fig. 5. *A. scoparia* MICs against *S. mutans* clinical isolates; Ch (Δ), P (\blacksquare), L1 (\blacktriangle) and L2 (\square). Optical Density was measured at 550 nm. The results are mean values SD from four tests. C: control.

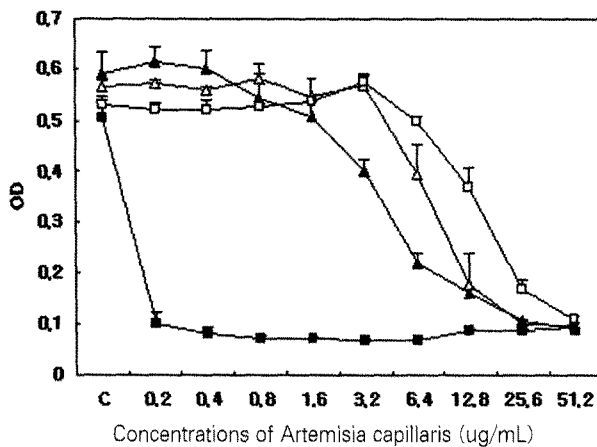


Fig. 6. *A. capillaris* MICs against *S. mutans* reference strains; KTCC 3605 (Δ), KTCC 3298 (\blacksquare), KTCC 3306 (\blacktriangle) and GS 5 (\square). Optical Density was measured at 550 nm. The results are mean values SD from four tests. C: control.

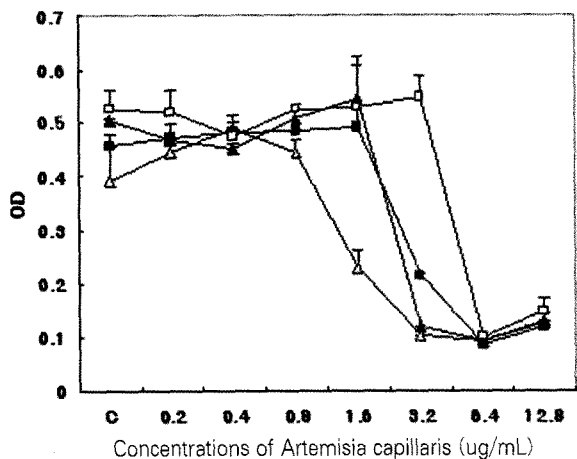


Fig. 7. *A. capillaris* MICs against *S. mutans* clinical isolates; Ch (Δ), P (\blacksquare), L1 (\blacktriangle) and L2 (\square). Optical Density was measured at 550 nm. The results are mean values SD from four tests. C: control.

3. Time-kill studies

According to the Time-kill studies, *A. lavandulaefolia* DC killed 100% of KCTC 3298 and almost 100% of GS5 at 4 hrs (data not shown) and between 99% and 99.90% of other strains at 0.5 hr and killed > 99.99% of KCTC 3298 and GS5 at 0.5 hr (data not shown). All the strains were killed between 99.90% and 99.99% at 2.5 hrs (Fig. 8). Thus *A. lavandulaefolia* DC was bacteri-

dal for all the *S. mutans* strains at $1 \times \text{MIC}$ after 0.5 hr. Ampicillin was used as a standard antibiotic to compare the sensitivity of the essential oil of *A. lavandulaefolia* DC (Fig. 9).

The results of MICs, MBCs of the essential oils of *A. lavandulaefolia* DC, *A. scoparia* and *A. capillaris* and the time-kill studies of *A. lavandulaefolia* DC showed that they had antimicrobial activity against all the *S. mutans* strains tested in this study.

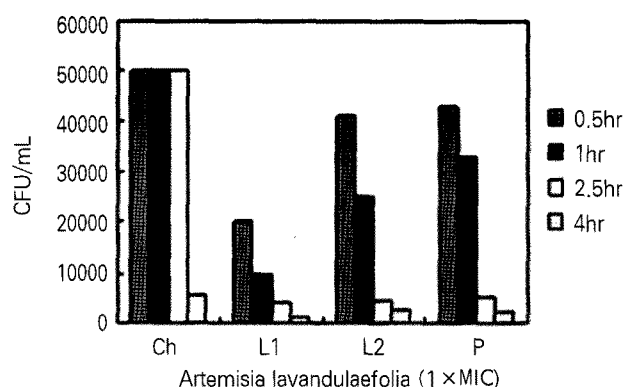


Fig. 8. The results of time-kill studies of *A. lavandulaefolia* DC against clinical isolates. The No. is CFU/mL and the concentration of *A. lavandulaefolia* DC was $1 \times \text{MIC}$. Viability counts of *A. lavandulaefolia* DC-containing *S. mutans* suspensions were performed at 0.5 hr, 1 hr, 2.5 hrs and 4 hrs by plating 100 μL from each suspension. The initial concentration of *S. mutans* was 1×10^8 CFU/mL.

IV. Discussion

S. mutans was isolated from human carious lesions in 1924, confirmed by a number of investigators and also found that *S. mutans* is related with the development of dental caries^{32,33}. Clinical observations in humans and animals indicate that plaque formation is an essential requirement for both dental caries and periodontal disease. *S. mutans* comprises the primary component of dental plaque, metabolizes exogenous dietary carbohydrates including sucrose and makes lactic acid⁹. The accumulation of lactic acid in the plaque biofilm results in a localized drop in pH and subsequent demineralization of tooth enamel. Therefore, there have been researches on elimination of dental plaque with antimicrobial agents³⁴.

There are a number of studies to evaluate the antimicrobial activity of herbal extracts such as Eucalyptol and Thymol³⁵, *Streblus asper*³⁶, garlic and tea tree oil³⁷, green tea³⁸ and *Uncaria tomentosa* against *S. mutans*³⁹. The purpose of this study was to isolate *S. mutans* from saliva samples of Koreans and investigate whether the essential oils of *A. lavandulaefolia* DC, *A. scoparia*, and *A. capillaries* have antimicrobial activity against them including reference. According to a study used the extract of *Artemisia* spp., Mugwort, on *S. mutans*²¹, the MIC was 0.5 mg/mL against a reference strain, KCTC 5316 and this is much higher than the MICs of the essential oils of *A. lavandulaefolia* DC, *A. scoparia*, and *A.*

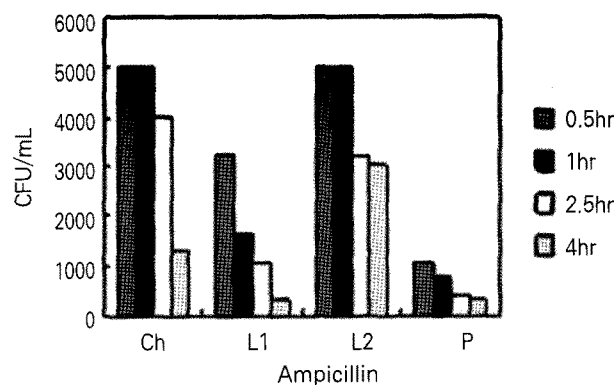


Fig. 9. The results of time-kill studies of ampicillin against clinical isolates. The No. is CFU/mL and the concentration of ampicillin was $1 \times \text{MIC}$ (1 $\mu\text{g/mL}$). Viability counts of ampicillin-containing *S. mutans* suspensions were performed at 0.5 hr, 1 hr, 2.5 hrs and 4 hrs by plating 100 μL from each suspension. The initial concentration of *S. mutans* was 1×10^8 CFU/mL.

capillaries used in this study against reference strains: 0.8 $\mu\text{g/mL}$, 0.8 to 1.6 $\mu\text{g/mL}$ and 0.2 to 25.6 $\mu\text{g/mL}$, respectively and also higher than the MICs against clinical isolates: 0.4 to 0.8 $\mu\text{g/mL}$, 0.4 to 0.8 $\mu\text{g/mL}$ and 1.6 to 3.2 $\mu\text{g/mL}$, respectively (Table 1). It may suggest that *A. lavandulaefolia* DC, *A. scoparia*, and *A. capillaries* have more antimicrobial activity than Mugwort against *S. mutans*. The MICs of *A. capillaries* were higher (1.6 to 3.2 $\mu\text{g/mL}$ against clinical isolates and 0.2 to 25.6 $\mu\text{g/mL}$ against reference strains) than the MICs of *A. lavandulaefolia* DC and *A. scoparia* (0.4 to 0.8 $\mu\text{g/mL}$ and 0.4 to 0.8 $\mu\text{g/mL}$ against clinical isolates and 0.8 $\mu\text{g/mL}$ and 0.8 to 1.6 $\mu\text{g/mL}$ against reference strains, respectively). The MBCs of the essential oils of *A. capillaries* (6.4 to 12.8 $\mu\text{g/mL}$) were slightly higher than the MBCs of *A. lavandulaefolia* DC and *A. scoparia* (0.8 to 3.2 $\mu\text{g/mL}$) for clinical isolates. These results suggest that *A. lavandulaefolia* DC and *A. scoparia* have more antimicrobial activity than *A. capillaries* against *S. mutans*.

The essential oil of *A. lavandulaefolia* DC could kill *S. mutans* clinical isolates within 0.5 hr (90% - 99%) at $1 \times \text{MICs}$ (0.8 $\mu\text{g/mL}$ for P and 0.4 $\mu\text{g/mL}$ for Ch, L1 and L2, respectively). The time-kill studies also indicate that the essential oil of *A. lavandulaefolia* DC is bactericidal against the *S. mutans* clinical isolates.

In conclusion, according to the results of MICs, MBCs and time-kill studies, the essential oils of *A. lavandulaefolia* DC, *A. scoparia*, and *A. capillaries* have antimicro-

bial activity against the *S. mutans* clinical isolates including reference strains and this suggests that they could be used to control dental plaque formation and subsequent dental caries formation.

References

1. Walter RG, Shklair IL : *Streptococcus mutans* in caries-free and caries-active naval recruits. *J Dent Res*, 61:1229-1232, 1982.
2. Hamada S, Koga T, Ooshima T : Virulence factors of *Streptococcus mutans* and dental caries prevention. *J Dent Res*, 63:407-411, 1984.
3. Loesche WJ : Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev*, 50:353-380, 1986.
4. Russell MW, Lehner T : characterization of antigens extracted from cells and culture fluids of *Streptococcus mutans* serotype c. *Arch Oral Biol*, 23:7-15, 1978.
5. Ooshima T, Matsumura M, Hoshino T, et al. : Contributions of three glycosyltransferases to sucrose-dependent adherence of *Streptococcus mutans*. *J Dent Res*, 80:1672-1677, 2001
6. 김수경, 김재곤, 백병주 등 : *Streptococcus mutans* GS-5 Glucosyltransferase의 클로닝과 발현. *대한소아치과학회지*, 35:73-82, 2008
7. Miller CH, Kleinman JL : Effect of microbial interactions on *in vitro* plaque formation by *Streptococcus mutans*. *J Dent Res*, 53:427-434, 1974.
8. Marsh PD, Bradshaw DJ : Dental plaque as a biofilm. *J Industrial Microbiology*, 14:91-94, 1995.
9. Costerton JW, Lewandowski Z, DeBeer D : Biofilms, the customized microniche. *J Bacteriology*, 176:2137-2142, 1994.
10. Hamada S, Slade HD : Biology, immunology, and cariogenicity of *Streptococcus mutans*. *Microbiol Rev*, 44:331-384, 1980.
11. Schilling KM, Bowen WH : Glucans synthesized *in situ* in experimental salivary pellicle function as specific binding sites for *Streptococcus mutans*. *Infect Immun*, 60:284-295, 1992.
12. Russell RRB : Glucan-binding proteins of *Streptococcus mutans* Serotype c. *J Gen Microbiol*, 112:197-201, 1979.
13. Hemp SE, Loe H : Long term effect of chlorhexidine on developing gingivitis in the Beagle dog. *J Periodontol Res*, 8:63-70, 1973.
14. Warner VD, Sane JN, Warner AM, et al. : Alkylamine salts and amides: *in vitro* inhibition of *S. mutans* 6715. *J Dent Res*, 56:1599-1602, 1977.
15. Harrap GJ : Assessment of the effect of dentifrices on the growth of dental plaque. *Journal of Clinical Periodontology*, 1:166-174, 1974.
16. Shimotoyodome A, Koudate T, Kobayashi H, et al. : Reduction of *Streptococcus mutans* adherence and dental biofilm formation by surface treatment with phosphorylated polyethylene glycol. *Antimicrob Agents Chemother*, 51:3634-3641, 2007.
17. Estafan D, Gultz J, Kaim JM, et al. : Clinical efficacy of an herbal toothpaste. *J Clin Dent*, 9:31-33, 1988.
18. 이선영, 김재곤, 백병주 등 : 구강내 세균에 대한 Essential oil의 항균효과에 관한 연구. *대한소아치과학회지*, 36:1-11, 2009
19. Zheng WF, Tan RX, Yang L, Liu ZL : Two flavones from *Artemisia giraldii* and their antimicrobial activity. *Planta Med*, 62:160-162, 1996.
20. Mangena T, Muyima NY : Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strains. *Lett Appl Microbiol*, 28:291-296, 1999.
21. Min Jung Jo, Kyung Jin Min : Anti-microbial activities against oral microbes and growth-inhibitory effect on oral tumor cell of extracts of *Perilla* and *Mugwort*. *Kor. J. Env. Hlth*, 33:115-122, 2007.
22. Zhou T, Guo J, Han D, et al. : Chemical components of *Artemisia scoparia* volatile oil and its poison activity to mosquito. *Ying Yong Sheng Tai Xue Bao*, 17:907-910, 2006.
23. Gilani AH, Janbaz KH : Hepatoprotective effects of *Artemisia scoparia* against carbon tetrachloride: an environmental contaminant. *J Pak Med Assoc*, 44:65-68, 1994.
24. Chen CP, Lin CC, Namba T : Screening of Taiwanese crude drugs for antibacterial activity against *Streptococcus mutans*. *J Ethnopharmacol*, 27:285-295, 1989.
25. Cheng HY, Lin LT, Huang HH, et al. : Yin Chen Hao Tang, a Chinese prescription, inhibits both herpes simplex virus type-1 and type-2 infections *in vitro*. *Antiviral Res*, 77:14-19, 2008.
26. Takada K, Hirasawa M : A novel selective medium

- for isolation of *Streptococcus mutans*. J Microbiol Methods, 60:189-193, 2005.
27. Cha JD, Jeong MR, Choi HJ, et al. : Chemical composition and antimicrobial activity of the essential oil of *Artemisia lavandulaefolia*. Planta Med, 71:575-577, 2005.
28. Cha JD, Jeong MR, Jeong SI, et al. : Chemical composition and antimicrobial activity of the essential oils of *Artemisia scoparia* and *A. capillaries*. Planta Med, 71:186-190, 2005.
29. Coudron PE, Stratton CW : Use of time-kill methodology to assess antimicrobial combinations against metronidazole-susceptible and metronidazole-resistant strains of *Helicobacter pylori*. Antimicrob Agents Chemother, 39:2641-2644, 1995.
30. Masuda K, Ohta M, Ito M, et al. : Bactericidal action of tachyplestin I against oral streptococci. Oral Microbiol Immunol, 9:77-80, 1994.
31. Weber DA, Howard-Nordan K, Buckner BA, et al. : Microbiological assessment of an improved stannous fluoride dentifrice. J Clin Dent Spec, 97-104, 1995.
32. Hamada S, Masuda N, Ooshima T, et al. : Epidemiological survey of *Streptococcus mutans* among Japanese children. Identification and serological typing of the isolated strains. Jpn J Microbiol, 20:33-44, 1976.
33. Masuda N, Tsutsumi N, Sobue S, Hamada S : Longitudinal survey of the distribution of various serotypes of *Streptococcus mutans* in infants. J Clin Microbiol, 10:497-502, 1979.
34. Jenkins S, Addy M, Newcombe RG : A comparison of cetylpyridinium chloride, triclosan and chlorhexidine mouthrinse formulations for effects on plaque regrowth. J Clin Periodontol, 21:441-444, 1994.
35. Fine DH, Furgang D, Barnett ML, et al. : Effect of an essential oil-containing antiseptic mouthrinse on plaque and salivary *Streptococcus mutans* levels. J Clin Periodontol, 27:157-161, 2000.
36. Taweekhaisupapong S, Wongkham S, Chareonsuk S, et al. : Selective activity of *Streblus asper* on *Mutans streptococci*. J Ethnopharmacol, 70:73-79, 2000.
37. Groppo FC, Ramacciato JC, Simões RP, et al. : Antimicrobial activity of garlic, tea tree oil, and chlorhexidine against oral microorganisms. Int Dent J, 52:433-437, 2002.
38. Simonetti G, Simonetti N, Villa A : Increased microbicidal activity of green tea (*Camellia sinensis*) in combination with butylated hydroxyanisole. J Chemother, 16:122-127, 2004.
39. Ccahuana-Vasquez RA, Santos SS, Koga-Ito CY, Jorge AO : Antimicrobial activity of *Uncaria tomentosa* against oral human pathogens. Braz Oral Res, 21:46-50, 2007.

국문초록

임상 분리된 *Streptococcus mutans*에 대한 *Artemisia species*의 항균 활성도

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*Streptococcus mutans*는 Mutans streptococci 그룹에 속하는 치아우식 유발균으로 알려져 있다. 국내 자생균 종의 추출물이 이러한 치아우식을 유발하는 *Streptococcus mutans*균에 대해 활성 억제 효과가 있다는 것이 표준균주를 이용한 실험에서 밝혀져 있으며, 본 연구에서는 환자에서 분리된 *S. mutans*를 중심으로 쑥의 항균 효과를 알아보았다.

네 가지 임상 분리균주; Ch, L1, L2, P와 네 가지 표준균주; KCTC (Korean Culture for Type Collection) 3298, KCTC 3605 (ATCC 25175), KCTC 3306, *Streptococcus mutans* GS5를 이용하여 세 가지 종류의 쑥; 참쑥(*Artemisia lavandulaefolia* DC), 비쑥(*Artemisia scoparia*), 사철쑥(*Artemisia capillaries*)의 효능을 검증하였다. 항균 활성은 세 가지 종류의 쑥 추출물의 *Streptococcus mutans*에 대한 최소 억제농도(MIC)와 최소살균농도(MBC), time-kill studies를 통해 알아보았다.

최소억제농도는 표준균주에 대해 각각 참쑥이 0.8 ug/mL, 비쑥이 0.8-1.6 ug/mL, 사철쑥이 0.2-25.6 ug/mL이었으며, 분리된 균주에 대해서는 참쑥이 0.4-0.8 ug/mL, 비쑥이 0.4-0.8 ug/mL, 사철쑥 1.6-6.4 ug/mL이었다.

최소살균농도는 표준균주에 대해서 참쑥이 1.6-6.4 ug/mL, 비쑥이 6.4-12.8 ug/mL 이었다. 분리균주에 대해서는 참쑥이 0.8-3.2 ug/mL, 비쑥이 0.8-3.2 ug/mL, 사철쑥은 6.4-12.8 ug/mL이었다.

Time-kill assay에서 KCTC 3298이 4 hrs에 100% 살균되었으며, GS5도 거의 100% 살균되었고, L1과 L2, P는 0.5 hrs에 99.90% 살균되었다. 0.5 hrs에 KCTC 3298과 GS5는 99.99% 이상 살균되었고, 모든 균주들이 2.5 hrs에 99.99% 살균되었다. 전체적인 실험 결과 세 가지 종류의 쑥은 표준 균주 및 네 가지 분리 균주 모두에서 항균 효과가 있음을 확인하였다.

주요어 : 쑥, 항균효과, *Streptococcus mutans*