

Antimicrobial Activity of Ethanol Extracts of *Xanthium strumarium* L. against Oral Microorganisms

Yeon-Hee Kim¹, Jeong Hwan Kim², Hyung-Joo Jin² and Si Young Lee^{1*}

¹Department of Microbiology and Immunology, College of Dentistry, Research Institute of Oral Science and ²Department of Marine Molecular Biotechnology, College of Life Sciences, Gangneung-Wonju National University, Gangneung, 210-702, Korea.

(Received: 20 December 2013; accepted: 03 February 2014)

Xanthium strumarium L. is an annual or a short-lived perennial that is readily found in the fields. In this study, the antimicrobial activity of ethanol extracts of *X. strumarium* on oral microbial species was examined for possible usage of extracts of *X. strumarium* in dental care products. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by a microdilution method in culture medium. The MICs for oral streptococci were between 125 and 500 µg/ml. The MBCs for streptococci were between 250 and 2,000 µg/ml. *Actinomyces naeslundii*, *Actinomyces odontolyticus* and *Aggregatibacter actinomycetemcomitans* showed similar MICs and MBCs as oral streptococcal strains. The MICs were 62.5 and 15.6 µg/ml for *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, respectively. In the experiment for examining the effect of concentration of extracts and incubation time on the killing of bacteria, the killing of *Streptococcus mutans* was dependent on concentration of extracts and incubation time. The treatment of bacteria with extracts changed the cell surface texture of *S. mutans* and *P. gingivalis*. The surface of bacteria treated with extracts was rougher than untreated bacteria and small bumps appeared on the surface of both bacteria after treatment with extract. Based on the results of this study, extracts of *X. strumarium* would be a useful material for the development of antimicrobial agents against oral pathogens.

Key words: Antibacterial agents; dental plaque; extract; pathogen; *Xanthium*.

Oral microbial species cause several types of oral diseases. For example, mutans group streptococci are known as etiological bacterial species for dental caries¹ and some of Gram-negative anaerobic bacteria have been suggested to cause periodontal diseases². The need for effective antimicrobial agents against these pathogenic oral microorganisms necessitated a search for new antimicrobial agents. For this purpose, a lot of investigation on plants as sources of new antimicrobial agents has been carried out³.

Several kinds of plant products were utilized for prevention and treatment of dental diseases^{4, 5}.

Xanthium strumarium L. is an annual or a short-lived perennial that is readily found in the fields in Korea, northeast Asia and Europe⁶. It has been used in treating fever, skin disease, pain and malignant tumor⁷. Previous study reported that extracts from flowering twigs of *X. strumarium* has antimicrobial properties against certain bacterial species such as *Vibrio cholerae*⁸. In this study, the antimicrobial activity of ethanol extracts of *X. strumarium* on oral microbial species was investigated for possible usage of extracts of *X. strumarium* in dental care products.

* To whom all correspondence should be addressed.
Tel.: +82-33-640-2455; Fax: +82-33-642-6410;
E-mail: siyoung@gwnu.ac.kr

MATERIALS AND METHODS

Preparation of ethanol extract of *X. strumarium*

X. strumarium was collected in October, 2011 and dried completely at room temperature for 3 days in the dark. Leaves and fruits of *X. strumarium* were collected separately and extraction procedures were carried out for leaves and fruits separately. Then powder was made with an electric grinder and stored in sealed containers at -20°C. For each 20 mg sample, 1 ml of 100% ethanol was used to extract the ethanol-soluble fraction at room temperature for 1 day⁹. The ethanol extraction was repeated three times and combined. The ethanol fraction was transferred to a new tube and the extract weight was determined after completely evaporating ethanol under vacuum for 4 h at 30°C. For a stock solution of the ethanol-soluble fraction, 1 ml of ethanol was added for every 100 mg dried extract. After dissolving ethanol fraction, the solution was filtered through a 0.22 micron syringe filter before use.

Microbial Strains

Streptococcus sobrinus 6715 was a laboratory strain stocked in Department of Microbiology and Immunology, College of Dentistry, Gangneung-Wonju National University. *Streptococcus mutans* KN405 and *Streptococcus mitis* KN156 were isolated strains from Korean dental plaque and stocked in the same laboratory. *Actinomyces naeslundii* CCUG 35333, *Actinomyces odontolyticus* ATCC 17929, *Candida albicans* KCTC 7270, *Aggregatibacter actinomycetemcomitans* ATCC 33384, *Fusobacterium nucleatum* ATCC 23726 and *Porphyromonas gingivalis* ATCC 33277 were obtained from Korean Collection for Oral Microbiology (Chosun University, Gwangju, Korea).

Culture Conditions

Oral streptococci were grown in brain heart infusion broth (BHI) (Becton, Dickinson and Company, Sparks, MD, USA) for 18 h at 37°C in aerobic condition supplemented with 5% CO₂ and actinomyces were cultured in BHI for 18 h at 37°C under an aerobic conditions (Bactron Anaerobic Chamber, Sheldon Manufacturing Inc. Cornelius, Oregon, USA) with an atmosphere of 90% N₂, 5% CO₂ and 5% H₂. *A. actinomycetemcomitans* was inoculated in BHI

broth and *F. nucleatum* and *P. gingivalis* were cultured in prereduced trypticase soy broth (Becton, Dickinson and Company) containing 1mg/ml of yeast extract (Becton, Dickinson and Company), 5µg/ml of hemin (Sigma Chemical Co., St. Louis, MO, USA) and 1µg/ml of menadione (Sigma Chemicals Co.). These three species were incubated in an aerobic conditions (Bactron Anaerobic Chamber, Sheldon Manufacturing Inc.). Sabouraud Dextrose medium (Becton, Dickinson and Company) was used for culturing *C. albicans* and candida was incubated for 18 h at 25°C under aerobic condition. The culture media supplemented with bacteriological agar were used for solid agar plates. For plating *F. nucleatum* and *P. gingivalis*, sheep blood (5%) was added additionally to culture medium for these species described above.

Determination of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) by Broth Dilution Method

The concentration of extracts of *X. strumarium* was 100 mg/ml. To determine the MIC of ethanol extracts of *X. strumarium*, a stock solution of extracts was prepared in microbial culture medium for each microbial species (final concentration 2 mg/ml). The MICs were determined by a microdilution method in culture medium which was modified from methods for antimicrobial susceptibility tests of Clinical and Laboratory Standards Institute (CLSI)¹⁰. Using a microbial culture in late log phase or stationary phase, a suspension equivalent to that of the 0.5 McFarland standard (approximately 1 x 10⁸ CFU/ml) in each microbial culture medium was prepared. The bacteria were inoculated into serially diluted extracts of *X. strumarium* solutions in 96-well round bottom microtitration plates for final concentrations of 5 x 10⁵ CFU/ml. The final volume was 100µl in a microtitration plate well. The microdilution trays were incubated in the same conditions described in bacterial culture conditions. After incubation for 18 h for aerobic bacteria and 48 h for anaerobic bacteria, MIC was determined. The microtitration plates were read visually and the minimum concentration of the extracts showing no turbidity was recorded as the MIC. MBC of extracts was determined by spreading a 100µl culture medium from the wells showing no visible growth on agar plates for each bacterial species. The agar plates were incubated for 3 days for aerobic bacteria and

7 days for anaerobic bacteria. The MBC was determined as the minimum concentration of extracts that showed $\leq 99.9\%$ reduction of the original inoculums. The experiments for determining MIC and MBC were repeated at least three times.

Effects of concentrations of extracts and incubation time on killing of bacteria

Among bacterial species tested in this study, *S. mutans* was used in this experiment because this is one of the representative bacteria known as the etiological agent for dental caries. For examining the effects of concentrations of extracts of leaves of *X. strumarium*, serially diluted ethanol extracts were added in wells containing *S. mutans* KN405 and wells were incubated for 3 h at 37°C. In another set of experiment, the effect of incubation time on the killing of bacteria by extracts of *X. strumarium* was investigated by increasing incubation time at 500 µg/ml of final concentration of leaf extracts. Each experimental group was consisted of triplicate wells. After incubation, Live/Dead® BacLight™ Bacterial viability Kits (Molecular Probes, Invitrogen, Eugene, OR, USA) was used for enumeration of viable bacteria¹¹⁻¹³. With this kit of an appropriate mixture of SYTO9 and propidium iodide, bacteria with intact cell membranes were stained as fluorescent green, whereas bacteria with damaged membranes were stained as fluorescent red. Fluorescence was measured with Multilabel reader (VICTOR X, PerkinElmer, Turku, Finland). Excitation was obtained at a wavelength of 485 ± 14 nm. Emission in the green was read at 535 ± 25 nm and in the red at 610 ± 10 nm. A standard curve (Green/red fluorescence ratio vs. % live bacteria) was determined and used for calculating the % live bacteria in the each well¹⁴.

Scanning electron microscopy (SEM)

Variable Pressure Field Emission scanning electron microscopy (VP-FE-SEM) (SUPRA55VP, Zeiss, Germany) was used to examine the morphological changes in the *S. mutans* KN405 and *P. gingivalis* ATCC 33277. *S. mutans* and *P. gingivalis* were selected because these species were known as the etiological bacteria for inducing two major oral diseases, dental caries and periodontal diseases, respectively. The bacteria were treated with 500 µg/ml of leaf extract of *X. strumarium* for 4 h at 37°C. The bacterial

suspensions after treatment were centrifuged and bacterial pellets were fixed in 2.5% glutaraldehyde in PBS (pH 7.4) for 1 h at room temperature. The fixed samples were then washed three times with PBS for 10 min and dehydrated for 30 min in a graded series of ethanol. After critical point drying, the samples were mounted on stub, coated with gold and observed with SEM.

Statistical analysis

The Kruskal-Wallis test was used to compare the effects of the incubation time and the extract concentration on bacterial killing. The level of significance was $P < 0.05$. Correlations between bacteria killing and the incubation time or extract concentration were evaluated by calculating Spearman's rank correlation coefficient (Spearman's rho). The statistical analysis was performed using the Software Package for Social Sciences (SPSS, version 20.0, IBM Inc., USA).

RESULTS

MICs and MBCs

The MICs of ethanol extracts of *X. strumarium* leaf for oral streptococci were between 250 and 500 µg/ml and MICs of fruit extracts were between 125 and 500 µg/ml (Table 1). The MBCs for streptococci were between 250 and 2,000 µg/ml. *A. naeslundii*, *A. odontolyticus* and *A. actinomycetemcomitans* showed similar MICs and MBCs as oral streptococcal strains used in this study. The Gram-negative oral anaerobic bacteria which are related to periodontal diseases showed much lower MICs and MBCs for extracts of *X. strumarium* leaf and fruit. The MICs of leaf extracts were 62.5 and 15.62 µg/ml for *F. nucleatum* and *P. gingivalis*, respectively. Fruit extracts also has similar MICs and MBCs as leaf extracts for both species of bacteria. The MICs for oral *C. albicans* were 250 and 125 µg/ml for leaf and fruit extracts, respectively. MBC for *C. albicans* was 2,000 µg/ml for both extracts. The MICs of ethanol were also determined for checking the suppressive effects of ethanol in extracts of *X. strumarium* leaf and fruit. The MICs of ethanol for microbial strains used in this study were between 6.25 and 12.5% which were much higher than the concentration of ethanol (2%) in the highest concentration of extracts (2,000 µg/ml) used in this study.

Effects of the extract concentration and incubation

Table 1. Antimicrobial activity of extracts of *Xanthium strumarium* L. against oral microorganisms

Microorganism	<i>X. strumarium</i> L. Leaf		<i>X. strumarium</i> L. Fruit	
	MIC ^a	MBC ^a	MIC ^a	MBC ^a
<i>S. mitis</i> KN156	500	500	250	1000
<i>S. mutans</i> KN405	250	500	125	250
<i>S. sobrinus</i> 6715	500	2000	500	2000
<i>A. naeslundii</i> CCUG 35333	250	2000	250	2000
<i>A. odontolyticus</i> ATCC 17929	250	2000	125	1000
<i>A. actinomycetemcomitans</i> ATCC 33384	500	1000	500	1000
<i>F. nucleatum</i> ATCC 23726	62.5	62.5	62.5	62.5
<i>P. gingivalis</i> ATCC 33277	15.62	62.5	31.25	62.5
<i>C. albicans</i> KCTC 7270	250	2000	125	2000

^aµg/ml.**time on bacterial killing**

S. mutans KN405 was killed by leaf extracts of *X. strumarium* L. (Fig. 1A, B). At 1 and 1.5 mg/ml of extract, the number of live *S. mutans* cell was reduced by 36 and 51%, respectively (Fig. 1A). Extract of 0.25 and 0.5 mg/ml suppressed 9 and 33% of this strain of bacteria, respectively. The difference in the number of live bacteria as a function of the concentration of the extracts was statistically significant ($P < 0.05$) (Fig. 1A). The number of live *S. mutans* had significant negative correlations with the concentration of the extract ($R = -0.837$, $P < 0.01$). In the experiment for examining the effect of incubation time for killing of bacteria,

the killing of *S. mutans* was dependent on incubation time (Fig. 1B). The incubation with leaf extracts of *X. strumarium* L. for 3h and 4h at 500µg/ml of extract inhibited 33% and 38% of *S. mutans* KN405, respectively. About 48% bacteria were killed after 5 h incubation. The killing of *S. mutans* was dependent on the incubation time (Fig. 1B). The number of live bacteria differed significantly depending on the incubation time for *S. mutans* ($P < 0.05$) (Fig. 1B). There was significant negative correlations between the number of live bacteria and the incubation time ($R = -0.779$, $P < 0.01$).

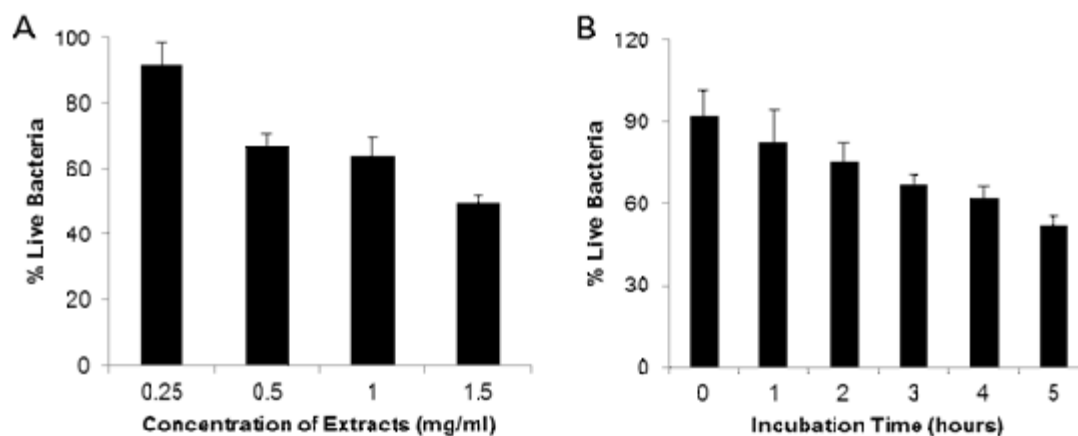


Fig. 1. Effects of (A) concentrations of extracts and (B) incubation time on killing of bacteria. Serially diluted ethanol extracts of *X. strumarium* leaf were added in wells containing *S. mutans* KN405 and wells were incubated at 37°C. The effect of incubation time for killing of bacteria by extracts of *X. strumarium* was investigated by increasing incubation time with 500µg/ml of final concentration of extracts. After incubation, Live/Dead® BacLight™ Bacterial viability Kits was used for enumeration of viable bacteria. Values indicate means of triplicate determinations; standard deviations of the mean (error bars) are indicated by vertical lines

SEM examination

After *S. mutans* KN405 and *P. gingivalis* ATCC 33277 were treated with 500 µg/ml of leaf extracts of *X. strumarium* for 4 h at 37°C, bacteria were observed with SEM (Fig. 2). The treatment of bacteria with extracts of leaf changed the cell surface texture of both bacteria. The surface of bacteria treated with extract was rougher than untreated bacteria and rugged in part of bacterial surface. Small bumps appeared on the surface of both bacteria after treatment with extract. The size of bumps of *P. gingivalis* was larger than *S. mutans*.

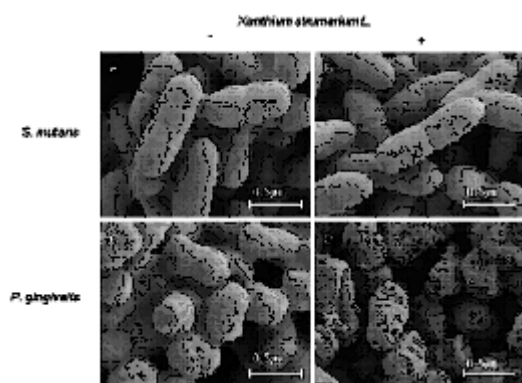


Fig. 2. Scanning electron microscopy (SEM). *S. mutans* KN405 and *P. gingivalis* ATCC 33277 were treated with 500 µg/ml of extracts of *X. strumarium* leaf for 4 h at 37°C. The bacterial suspensions after treatment were centrifuged and bacterial pellets were fixed in glutaraldehyde in PBS. The fixed samples were then washed and dehydrated in a graded series of ethanol. After critical point drying, the samples were mounted on stub, coated with gold and observed with SEM.

DISCUSSION

In this study, we examined the antimicrobial activity of ethanol extract of *X. strumarium* against oral microbial species which are known as etiological agents of dental caries (*S. mutans* and *S. sobrinus*), root caries (*A. naeslundii* and *A. odontolyticus*), and periodontal diseases (*A. actinomycetemcomitans*, *F. nucleatum*, and *P. gingivalis*). The early colonizer to tooth surface in the process of plaque formation (*S. mitis*) and fungus that was found frequently in oral cavity (*C. albicans*) were also examined. The data presented in this study have shown that extracts of *X. strumarium* leaf and fruit possess

antimicrobial activity against a wide variety of oral microbial species. Especially, the MICs and MBCs for oral anaerobic bacteria such as *F. nucleatum* and *P. gingivalis* which have been known as causative agents for inducing periodontal diseases were examined as low as 62.5 µg/ml. Anti-inflammatory activity and analgesic property of extracts of *X. strumarium* has been well disclosed¹⁵. In addition to these biological activities, antimicrobial activities of extracts of *X. strumarium* against etiological bacteria for dental caries and pathogens related to periodontal disease shown in this study may provide the possibility of use of extracts of *X. strumarium* in dental care products.

Yoo *et al* recently reported the feasibility of tea, cranberry, and other select plant derivatives as a potential basis for alternative therapeutic agents against *S. mutans*⁴. Sharma *et al.*¹⁶ have shown that hydroxychavicol isolated from the extract of Piper betle leaves showed inhibitory activity against oral cavity pathogens. It exhibited an inhibitory effect on oral cavity pathogens such as *S. mutans*, *Actinomyces viscosus*, *Enterococcus faecalis*, *F. nucleatum* and *P. gingivalis* (MICs of 62.5 to 500 µg/ml). *P. gingivalis* has the lowest MIC among bacterial species used in that study, which is similar as in our present study.

Two major oral diseases, dental caries and periodontal disease, were caused by oral biofilm known as dental plaque composed with oral pathogenic bacteria. The suppression of oral bacteria resulting in the decrease of dental plaque accumulation is the most effective method to prevent dental caries and periodontal diseases. A number of previous studies have shown that plant extracts can inhibit formation of dental plaque¹⁷⁻²¹. For example, the extracts from *Rosmarinus officinalis* were utilized for preventing dental caries²¹⁻²³. In our study, the antimicrobial activity of extracts of *X. strumarium* was examined against planktonic bacteria, not against biofilm bacteria. The antibacterial activity of extracts of *X. strumarium* to biofilm might be different from the data obtained in this study using planktonic bacteria, therefore, further study to investigate the suppressive activity of extracts of *X. strumarium* against oral biofilm will be necessary. The effects of extracts of *X. strumarium* on the virulence factors of oral pathogens, for example, bacterial adhesion

on tooth surface, coaggregation with other bacteria, water-insoluble glucan formation and eventually formation of dental plaque, should be investigated in further studies.

The changes of bacterial surface by treatment of bacteria with extract of *X. strumarium* were observed by SEM. The bacterial surface changes might be related to the mechanisms of bactericidal activities of extract of *X. strumarium* in these bacterial species used in this study. However, the further investigations for revealing the exact mechanism of antibacterial activity of extract of *X. strumarium* will be needed.

Natural products of plant extracts may be good alternatives to current treatments for oral health problems. The data of our present study suggested that extracts of *X. strumarium* would be a useful compound for the development of antimicrobial agents against oral pathogens and it has great potential for use in dental care products such as mouthwash and toothpaste for preventing and treating oral infections.

ACKNOWLEDGEMENTS

This work was supported by grant No. RTI05-01-02 from the Regional Technology Innovation Program of the Ministry of Knowledge Economy (MKE).

REFERENCES

- Hamada, S., Slade, H.D. Biology, immunology, and cariogenicity of *Streptococcus mutans*. *Microbiol. Rev.*, 1980; **44**(2): 331-84.
- Socransky, S.S., Haffajee, A.D. Periodontal microbial ecology. *Periodontol.* 2000., 2005; 38:135-87.
- Cowan, M.M. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 1999; **12**(4): 564-82.
- Yoo, S., Murata, R.M., Duarte, S. Antimicrobial traits of tea- and cranberry-derived polyphenols against *Streptococcus mutans*. *Caries Res.*, 2011; **45**(4): 327-35.
- Taheri, J.B., Azimi, S., Rafieian, N., Zanjani, H.A. Herbs in dentistry. *Int. Dent. J.*, 2011; **61**(6): 287-96.
- Bark, K.M., Heo, E.P., Han, K.D., Kim, M.B., Lee, S.T., Gil, E.M., Kim, T.H. Evaluation of the phototoxic potential of plants used in oriental medicine. *J. Ethnopharmacol.*, 2010; **127**(1): 11-8.
- Kuo, Y.C., Sun, C.M., Tsai, W.J., Ou, J.C., Chen, W.P., Lin, C.Y. Chinese herbs as modulators of human mesangial cell proliferation: preliminary studies. *J. Lab. Clin. Med.*, 1998; **132**(1): 76-85.
- Mehta, P., Chopra, S., Mehta, A. Antimicrobial properties of some plant extracts against bacteria. *Folia Microbiol. (Praha)*, 1983; **28**(6): 467-9.
- Jin, H.J., Kim, J.H., Sohn, C.H., DeWreede, R.E., Choi, T.J., Towers, G.H.N., Hudson, J.B., Hong, Y.K. Inhibition of *Taq* DNA polymerase by seaweed extracts from British Columbia, Canada and Korea. *J. App. Phycol.*, 1997; 9383-8.
- Clinical and Laboratory Standards Institute *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, M07-A8, Vol.29, No.2*; CLSI: Wayne, PA, 2009; .
- Boulos, L., Prevost, M., Barbeau, B., Coallier, J., Desjardins, R. LIVE/DEAD BacLight : application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. *J. Microbiol. Methods.*, 1999; **37**(1): 77-86.
- Bunetel, L., Guerin, J., Agnani, G., Piel, S., Pinsard, H., Corbel, J.C., Bonnaure-Mallet, M. In vitro study of the effect of titanium on *Porphyromonas gingivalis* in the presence of metronidazole and spiramycin. *Biomaterials.*, 2001; **22**(22): 3067-72.
- Stocks, S.M. Mechanism and use of the commercially available viability stain, BacLight. *Cytometry A.*, 2004; **61**(2): 189-95.
- Kim, Y.H., Lee, S.Y. Comparison of LIVE/DEAD® BacLight™ Bacterial Viability Test and alamarBlue® Method for Enumeration of Live and Dead Bacteria for Oral Bacterial Species. *Int. J. Oral Biol.*, 2012; **37**(4): 197-201.
- Han, T., Li, H.L., Zhang, Q.Y., Han, P., Zheng, H.C., Rahman, K., Qin, L.P. Bioactivity-guided fractionation for anti-inflammatory and analgesic properties and constituents of *Xanthium strumarium* L. *Phytomedicine.*, 2007; **14**(12): 825-9.
- Sharma, S., Khan, I.A., Ali, I., Ali, F., Kumar, M., Kumar, A., Johri, R.K., Abdullah, S.T., Bani, S., Pandey, A., Suri, K.A., Gupta, B.D., Satti, N.K., Dutt, P., Qazi, G.N. Evaluation of the antimicrobial, antioxidant, and anti-inflammatory activities of hydroxychavicol for its potential use as an oral care agent. *Antimicrob. Agents Chemother.*, 2009; **53**(1): 216-22.
- Homer, K.A., Manji, F., Beighton, D. Inhibition of peptidase and glycosidase activities of *Porphyromonas gingivalis*, *Bacteroides intermedius* and *Treponema denticola* by plant

- extracts. *J. Clin. Periodontol.*, 1992; **19**(5): 305-10.
18. Lee, D.H., Seo, B.R., Kim, H.Y., Gum, G.C., Yu, H.H., You, H.K., Kang, T.H., You, Y.O. Inhibitory effect of *Aralia continentalis* on the cariogenic properties of *Streptococcus mutans*. *J. Ethnopharmacol.*, 2011; **137**(2): 979-84.
 19. Wu-Yuan, C.D., Green, L., Birch, W.X. In vitro screening of Chinese medicinal toothpastes: their effects on growth and plaque formation of mutans streptococci. *Caries Res.*, 1990; **24**(3): 198-202.
 20. Yu, H.H., Lee, D.H., Seo, S.J., You, Y.O. Anticariogenic properties of the extract of *Cyperus rotundus*. *Am. J. Chin. Med.*, 2007; **35**(3): 497-505.
 21. Smullen, J., Finney, M., Storey, D.M., Foster, H.A. Prevention of artificial dental plaque formation in vitro by plant extracts. *J. Appl. Microbiol.*, 2012; .
 22. Bernardes, W.A., Lucarini, R., Tozatti, M.G., Flauzino, L.G., Souza, M.G., Turatti, I.C., Andrade e Silva, M.L., Martins, C.H., da Silva Filho, A.A., Cunha, W.R. Antibacterial activity of the essential oil from *Rosmarinus officinalis* and its major components against oral pathogens. *Z. Naturforsch. C.*, 2010; **65**(9-10): 588-93.
 23. Bernardes, W.A., Lucarini, R., Tozatti, M.G., Souza, M.G., Silva, M.L., Filho, A.A., Martins, C.H., Crotti, A.E., Pauletti, P.M., Groppo, M., Cunha, W.R. Antimicrobial activity of *Rosmarinus officinalis* against oral pathogens: relevance of carnosic acid and carnosol. *Chem. Biodivers.*, 2010; **7**(7): 1835-40.