Journal of Applied and Natural Science
14(2), 543 - 549 (2022)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Teeth bleaching effect and anti-oral microbial activity of water-extracted apple (*Malus asiatica*)

Soon-Jeong Jeong
Department of Dental Hygiene, College of Health Science, Youngsan University, Yangsan 50510, Korea; Institute of Basic Science for Well-Aging, Youngsan University, Yangsan 50510, Korea

Email: jeongsj@ysu.ac.kr

Article Info
https://doi.org/10.31018/jans.v14i2.3470
Received: April 11, 2022
Revised: June 2, 2022
Accepted: June 6, 2022

How to Cite
Jeong, S. J. (2022). Teeth bleaching effect and anti-oral microbial activity of water-extracted apple (*Malus asiatica*). *Journal of Applied and Natural Science*, 14(2), 543 - 549. https://doi.org/10.31018/jans.v14i2.3470

Abstract
Apple contains nutrients such as sugars, dietary fiber, and vitamins as well as bioactive phytochemicals such as organic acid, fatty acid and phenolic compounds. This study was conducted to confirm the applicability of apples as a bleaching agent and functional substances for the prevention of oral infectious diseases and maintenance of oral health by investigating the bovine teeth bleaching effect, anti-oral microbial activity, and inhibitory effect of halitosis of water-extracted apple (WEA).
From the results of quantitative analysis of the surface color of bovine teeth, the application of WEA significantly increased the ΔE value, indicating a bleaching effect. In the case of 100mg/ml, the ΔE value increased as the WEA application time increased, and the bleaching effect was the greatest. Based on the results of the disk diffusion test and selective culture using CRT bacteria test kit, WEA showed anti-oral microbial activity against the dental caries bacteria, *Streptococcus mutans* and *Latobacillus casei*, the periodontal bacteria, *Aggregatibacter actinomycetemcomitans*, and *Eschericha coli*, but showed no antifungal effect against *Candida albicans*, causing oral candidiasis. From the results of colony formation and generation of halitosis from salivary microorganisms, WEA inhibited the growth of salivary microorganisms and generation of components inducing halitosis such as hydrogen sulfide, methyl mercaptan and dimethyl sulfide, that occur during the metabolic process of oral microorganisms. Therefore, WEA is a functional substance derived from a safe and useful natural product that can be used for the prevention of oral infectious diseases and maintenance of oral health

Keywords: Anti-oral microbial activity, Apple, Halitosis, *Malus asiatica*, Teeth bleaching

INTRODUCTION

Most of the patients who visit the dental clinic are for the treatment of oral diseases, including dental caries and periodontitis, but interest in and treatment for teeth bleaching is increasing due to the recent improvement in the desire for beauty and living standards (Jeong, 2021). Teeth are stained by intrinsic factors such as the pathology of the pulp, certain drugs and systemic diseases, and extrinsic factors such as tobacco, coffee and coke (Jeong 2021). Teeth bleaching is to improve the color of teeth while minimizing structural damage to the stained (Hwang *et al*., 2015), and carbamide peroxide composed of urea and hydrogen peroxide, or hydrogen peroxide (H_2O_2) are used for teeth bleaching (Mazilu *et al*., 2019). Hydrogen peroxide is a strong oxidizing agent divided into water and active oxygen (Dahl and Pallesen, 2003). The active oxygen produced causes a chemical reaction with the stained material, resulting in a tooth bleaching effect (Park *et al*., 2006). However, hydrogen peroxide changes the enamel surface into a rough and porous structure that facilitates the adherence of oral microorganisms to the enamel, the first stage of bacterial infection (Sakanaka *et al*., 1996) and has side effects such as dentin hypersensitivity, swelling of the gingiva, inflammation and bleeding (Attin *et al*., 1997). Therefore, it is necessary to develop a material having a tooth bleaching effect that can be applied safely and non-invasively, and related studies are in progress (Watts and Addy, 2001). The oral cavity is the entrance to the digestive and respiratory tract, where many factors inducing teeth stain enter, and it forms the microbiome, which is estimated to be inhabited by about 600 bacterial species, some of which are the cause of oral infectious diseases (Shaheena *et al*., 2019; Jeong *et al*., 2020). In order to maintain oral health and inhibit infectious oral diseases, including dental caries and periodontitis, control of oral microbial

growth is necessary (Pan *et al*., 2020). Antibiotics have been used for a long time to control infectious diseases caused by bacteria (Park et al., 2016), but it is necessary to develop new substances for controlling bacteria due to the increase in antibiotic-resistant strains, decrease in the therapeutic effect, and side effects. Recently, research on substances for controlling bacteria using natural products that effectively replace antibiotics, relatively low development cost and period, and safety are increasing (Park *et al*., 2016). In addition, studies on the use of natural products in oral hygiene products and cavity prevention such as dentifrices, mouthwash rinse solution, restorative materials, periodontal dressing and materials for endodontics (Jeon *et al*., 2011; Palombo, 2011), and xerostomia (Villa *et al*., 2011) are also increasing.

Apple is the fruit of *Malus asiatica* belonging to the Rosacease family, and contains nutrients such as sugar, dietary fiber, and vitamins as well as bioactive phytochemicals such as organic acids, fatty acids and phenolic compounds, and is reported to have antioxidant, anti-inflammatory, cardiovascular disease prevention, obesity prevention and anti-wrinkle effects (Kidon and Grabowska, 2020; Nezbedova *et al*., 2021). The benefits of apples are attributed to the various phytochemicals in large amounts of apples (Nam and Ko, 2020). Apple is an important source of phenolic compounds, accounting for 22 % of phenolic compounds consumed by the human body (Vinson *et al*., 2001; Scalbert and Williamson, 2000). The phenolic compounds contained in apples are mainly in the free form, which is easily bioavailable, and it is different from other fruits that mainly have the conjugated form (McCann *et al*., 2007; Kalinowska *et al*., 2014). Although the health benefits and various efficacy of apple, there are few studies on the effect of water-extracted apple (WEA) on teeth bleaching effect, anti-oral microbial activity and inhibitory effect of halitosis.

This study aimed to confirm the applicability of WEA as the teeth bleaching agent and functional substances for the prevention of oral infectious diseases and maintenance of oral health by investigating the bovine teeth bleaching effect, anti-oral microbial activity inhibitory effect of halitosis of WEA using apple extract.

MATERIALS AND METHODS

Preparation of water extract of apple

Apples were purchased from traditional markets at Busan in Korea and 100 g was used for water extraction. Apples were immersed in 1L DW, and heated for 3h. The solution cooled to room temperature was filtered with filter paper (Whatman Inc, Maidstone, UK) and concentrated at 60 °C with a rotary evaporator (Eyela A-1000, Eyela, Tokyo, Japan) and freeze-dried at -70 °C. Freeze-dried WEA was dissolved in DW to the appropriate concentration and filtered through a syringe filter (Minisart®, sartorius, Göttingen, Germany).

Preparation of bovine teeth and quantitative analysis of the bovine teeth color

Bovine teeth without caries, fractures and cracks were cut to appropriate size with diamond bur and high-speed handpiece. Bovine teeth before and 1, 2, 4, 7 day after WEA treatment were washed with DW twice and excess moisture was removed. The color of bovine teeth was measured with a colorimeter (Konica minolta, Tokyo, Japan) 3 times. The value of color was determined by the previously reported CIE L*A*B* colorimetric method (Lee and Kim, 2017). L* is the contrast, a* is the red-green color, b* is the yellow-blue color, and the following formula was used for quantitative measurement of color.

$$\Delta E = \sqrt{(L^*)^2 + (a^*)^2 + (b^*)^2} \quad \text{Eq. 1.}$$

(Formula for determining the value of a color).

Preparation of oral microorganisms and measurement of microbial growth

S. mutans (KCCM 40105), *A. actinomycetemcomitans* (KCTC 2581), *L. casei* (KCCM 12452), *E. coli* (KCTC 1039), and *C. albicans* (KCCM 11282) were purchased from the Korea Microbiological Conservation Center (KCCM) and the gene bank (KCTC) for evaluation of anti-oral microbial activity of WEMA. *S. mutans* were cultured in BHI (MB cell Ltd., Seoul, Korea) agar and broth, *A. actinomycetemcomitans* and *L. casei* in MRS (MB cell Ltd.) agar and broth, *E. coli* in LB (MB cell Ltd.) agar and broth, and *C. albicans* in PDB, PDA (MB cell Ltd.). Cultured oral microorganisms in an incubator (Daihan Scientific Co., Daegu, Korea) for 24 h at 36.5 °C were diluted to $5X10^6$ CFU/ml according to standardized method from Bauer *et al.* (1966) and used. The absorbance in the culture medium of *S. mutans* and *L. casei* for quantitative measurement of microbial growth was measured at 600 nm wavelength using UV-Vis spectrophotometer (X-ma 1200, Human Corp., Seoul, Korea).

Disk diffusion test

Oral microbial cultures were diluted according to the standardized method and 100 µl of them were smeared on the agar plate. Sterilized paper discs (Φ6 mm, Advantec Toyo Kaisha Ltd., Tokyo, Japan) with 50, and 100 mg WEA and ampicillin antibiotic discs (10 IU, Oxoid Ltd., Hampshire, United Kingdom) used as control were placed on agar plates smeared with oral microorganisms. After culturing in the incubator for 24 h at 36.5 °C, the formed clear zone was measured with a vernier calliper (Mitutoyo Co., Kamagawa, Japan).

Caries risk test (CRT) bacteria test kit

Fifteen people without dental caries and periodontal disease participated in the experiment. This study was approved by the Ethics Committee (YSUIRB-202112-HR-051-01) of Youngsan University. Saliva was collected from participants who were applied with 10ml of 100 mg WEA after the same meal. Collected saliva was smeared on CRT® bacteria test kit (Invoclar Vivadent, NY, USA) according to the manufacturer's instructions, and *S. mutans* and *L. casei* in saliva were selectively cultured for 24h.

Standard plate count (SPC) of salivary oral microorganisms

SPC is a representative indicator of the degree of contamination by the microorganism, and it is possible to confirm the effect on oral microorganisms by WEA. Changes of oral microorganisms in saliva by WEA were determined by the number of colonies formed after smearing diluted saliva on a plate count agar and culturing for 24h.

Measurement of halitosis

According to the manufacturer's instructions, the halitosis was collected from 15 participants without dental caries and periodontal disease who applied 10ml of 100mg WEA after the same meal. Hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide ($(CH_3)_2S$) which are volatile sulfur compounds causing halitosis were measured using oral chroma (Nissha FIS Inc., Osaka, Japan).

Statistical analysis

All experiments were performed at least in triplicate. The collected data were represented as means±standard deviation (SD) and analyzed using SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Significant difference (*p < 0.05, **p < 0.01, ***p < 0.001) was determined by independent samples t-test.

RESULTS

Bovine teeth bleaching effect of WEA

The results of the teeth bleaching effect of WEA on bovine teeth maintained at 50mg/ml, 100mg/ml and 200mg/ml of WEA for 1, 2, 4 and 7 days are shown in Fig. 1. The ΔE values of teeth maintained in 50mg/ml and 100mg/ml of WEA from 2 to 7 days were significantly increased compared to DW (p < 0.05, p < 0.01, p < 0.001), and showed a bleaching effect. In particular, ΔE value of 100mg/ml increased as the treatment period increased and was significantly larger than the value of 50 mg/ml. ΔE value of 200mg/ml was similar to the value of 50mg/ml at 2 and 3 days, but decreased similarly to the value in DW on 7th day.

Anti-oral microbial activity of WEA against oral microorganisms

The disk diffusion test was performed using oral bacteria (*S. mutans, A. actinomycetemcomitans, L. casei* and *E. coli*) and fungi (*C. albicans*) and the created clear zone was measured (Fig. 2). WEA showed greater anti-oral microbial activity at 100mg/ml than at 50 mg/ml, but this effect was not greater than that of antibiotics (Table 1). WEA showed anti-oral microbial activity against bacterial species but had no effect on fungi.

The results on the effect of WEA on *S. mutans* and *L. casei*, which are representative bacteria related to dental caries among salivary microorganisms, determined by selectively culturing *S. mutans* and *L. casei* in saliva after applying 100 mg/ml WEA using the CRT bacteria test kit are shown in Fig. 3A. 100mg/ml of WEA inhibited the colony formation of *S. mutans* and *L. casei* more than these of 50 mg/ml. Absorbance values to quantitative analyze the effect of WEA on *S. mutans* and *L. casei* were significantly decreased in the medium treat-

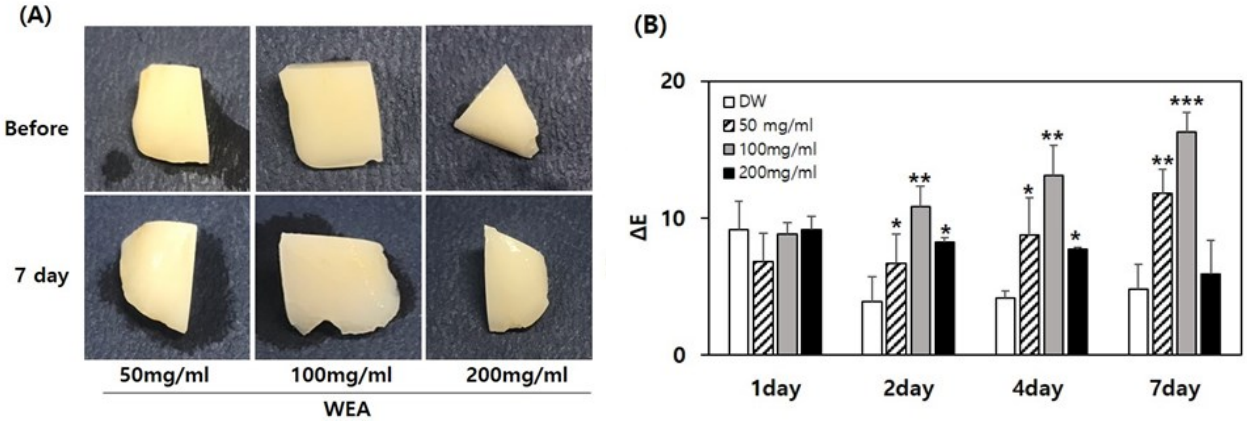


Fig. 1. *Bleaching effect of water-extracted apple (WEA) on bovine teeth according to the time. (A) Bovine teeth treated with WEA. (B) Quantitative analysis of the whitening effect of WEA according to the time. The results are represented in mean±S.D. of results from independent three times experiments. *<0.05, **<0.01, ***<0.001 compared with the DW group.*

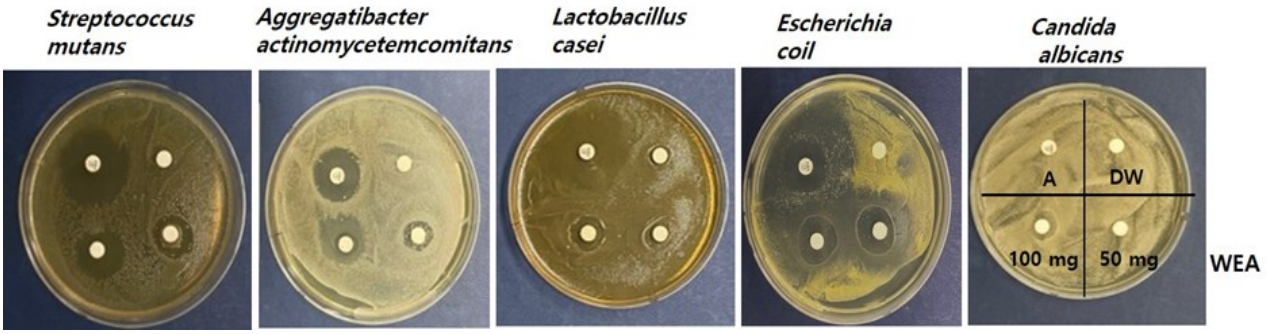


Fig. 2. *Anti-oral microbial activity of water-extracted apple (WEA)*

ed with 100 mg/ml of WEA than that of 50 mg/m (Fig. 3B) ($p < 0.05$, $p < 0.01$).

Inhibitory effects of WEA on colony formation and growth of salivary microorganisms and generation of halitosis

Changes in CFU/ml of salivary microorganisms according to 100 mg/ml WEA application are shown in Fig. 4A. CFU/ml of salivary microorganisms immediately after DW application was slightly decreased, but 3h after application significantly increased compared to before application (Fig. 4B) ($p < 0.01$). CFU/ml of salivary microorganisms immediately after application of 100mg/ml WEA was significantly decreased, and that of 3h after the application was similar to that before application (Fig. 4B).

Changes in components inducing halitosis such as H_2S, CH_3SH and $(CH_3)_2S$ according to 100 mg/ml WEA application are shown in Fig. 5. Measured components significantly decreased after application compared to before application of 100mg/ml WEA ($p < 0.05$, $p < 0.01$, $p < 0.001$), and the decrease in measurements was maintained until 3h after application. Among them, $(CH_3)_2S$ was significantly decreased immediately after application with 100mg/ml WEA, and the effect of WEA persisted until 3h after application.

DISCUSSION

Accumulating evidence shows that apple contains large amounts of bioactive phytochemicals such as organic acids, fatty acids, phenolics and polyphenols (Nam and Ko, 2020; Nezbedova *et al*., 2021).

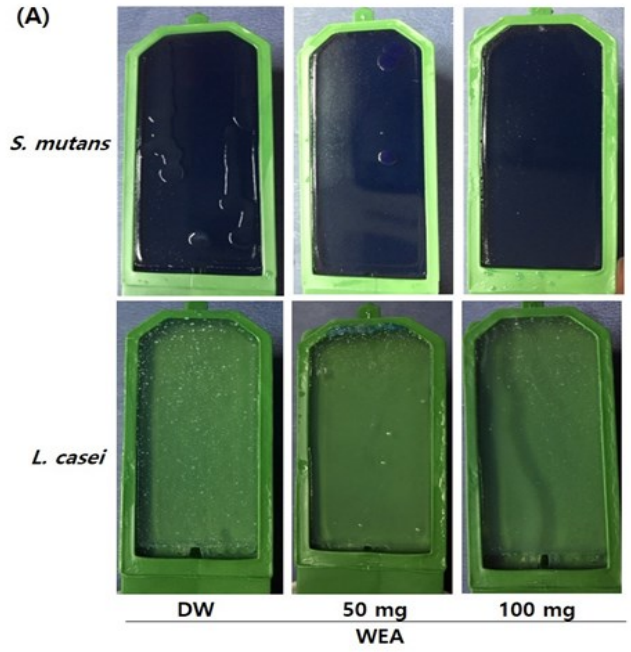
Organic acids are used as preservatives, drug absorption modifiers, antioxidants, and acidulants (Raybaudi-Massilia *et al*., 2009; Ma *et al*., 2018). In addition, organic acids used as active agents play an essential role in teeth bleaching and stain removal (Mazilu *et al*., 2019). Organic acids contained in fruits are divided into three types: malic acid, citric acid, and tartaric acid (Ma *et al*., 2018). Malic acid accounts for 90 % of the organic acids contained in apples (Ma *et al*., 2018). In this study, bovine teeth maintained in WEA of each concentration showed a significant increase in the ΔE value of bovine tooth surface (Fig. 1) ($p < 0.05$, $p < 0.01$, $p < 0.001$). In particular, the ΔE value of bovine teeth treated with 100 mg/ml of WEA increased as the treatment period increased, and the teeth bleaching effect was large. It is estimated that the bovine teeth bleaching effect in this study were induced by malic acid contained in large amounts in WEA. Therefore, WEA having a large amount of malic acid, can be used as a safe and non-invasive natural functional material for teeth bleaching.

Phenolics and polyphenols, belonging to phenolic compounds, are well known as one of the phytochemicals with useful antimicrobial activity (Cowan, 1999; Xu *et al*., 2011). Several studies have reported that polyphenols can enhance the antimicrobial effect when used together with antibiotics, leading to a reduction in the prescribed dosage of antibiotics and a reduction of the side effects of antibiotics (Cho *et al*., 2017; Eumkeb *et*

Table 1. Anti-oral microbial effects of water-extracted apple (WEA)

Oral micoorganisms	WEA			Ampicillin (10 IU)
	0 mg/ml	50 mg/ml	100 mg/ml	
Streptococcus mutans	-	+	++	+++
Aggregatibacter actinomycetemcomitans	-	+	++	++
Lactibacillus casei	-	+	+	+++
Escherichia coli	-	++	++	+++
Candida albicans	-	-	-	-

resistrant (<5 mm), +: susceptible (5-14 mm), ++: more susceptible (15-24 mm), +++: most susceptible (>25 mm).



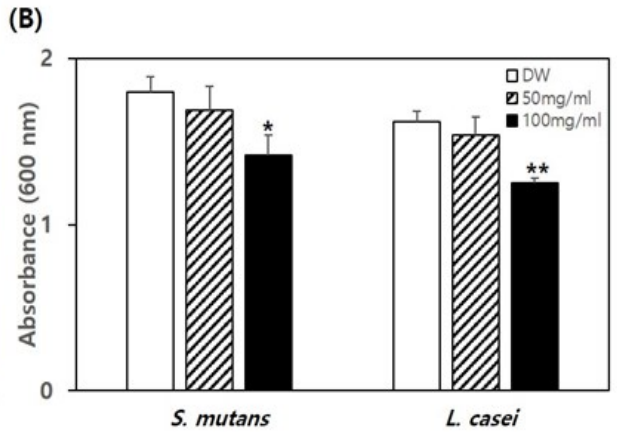
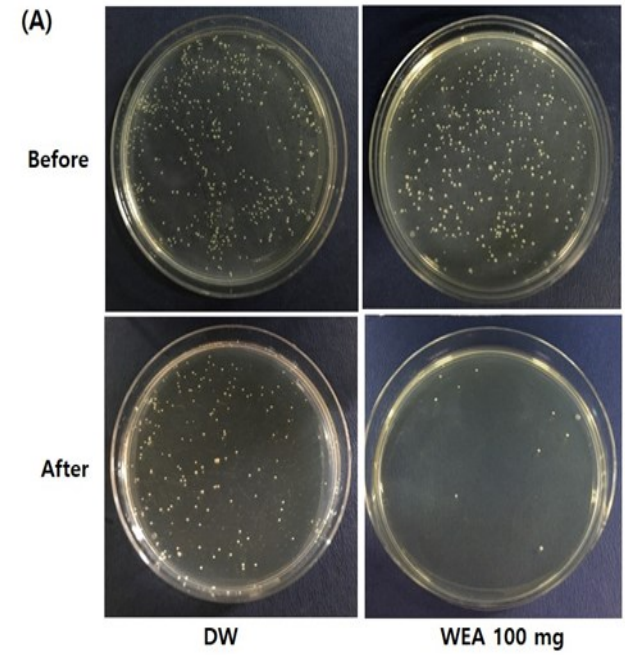


Fig. 3. *Inhibitory effects on growth of Streptococcus mutans and Lactobicillus casi of water-extracted apple (WEA). (A) Changes of colony on CRT bacteria test kit according to application of WEA. (B) Changes of absorbance in culture medium of S. mutans and L. casei according to application of WEA. The results are represented the mean±S.D. of results from independent three times experiments. *<0.05, **< 0.01, ***<0.001 compared with the sterile DW group*



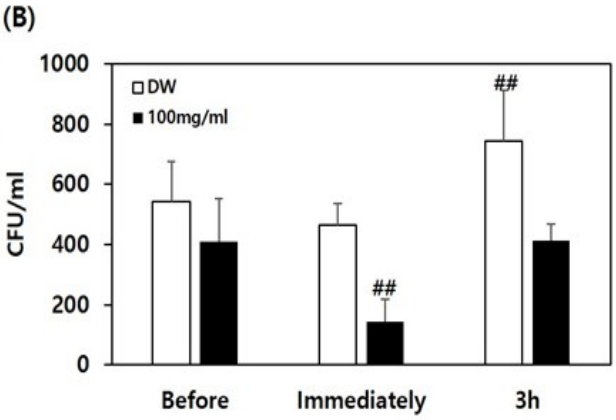


Fig. 4. *Inhibitory effects on growth of oral microorganisms of water-extracted apple (WEA). (A) Changes in the colony of oral microorganisms after application of WEA. (B) Changes in colony-forming unit (CFU)/ml of oral microorganisms after application of WEA according to the time. The results are represented in mean±S.D. of results from independent three times experiments. ##< 0.01 compared with the group before WEA application*

al., 2010). Phenolic toxicity to microorganisms is shown relative to the number and position of hydroxy groups on the phenolic ring and is due to the inhibition of enzyme activity by oxidized compounds (Cowan, 1999). Green tea polyphenols show an antimicrobial effect by strongly inhibiting the adhesion of oral epithelial cells of *S. mutans* (Xu *et al*., 2011) and *Porphylomonas gingivalis* (Sakanaka *et al.,* 1996). In this study, *S. mutans*, *A. actinomycetemcomitans*, *L. case* and *E. coli* belonging to bacteria, and *C. albicans* belonging to the fungi were used to confirm the anti-oral microbial activity of WEA. *S. mutans* is a major bacterium causing early dental caries by forming dental plaque (Jeong *et al*., 2020). *L. casei* is known as the bacterium causing advanced dental caries, which leads to further progression of dental caries (Byun *et al*., 2004). *A. actinomucetemcomitans* is a bacterium causing aggressive periodontitis characterized by rapid destruction of periodontal tissues (Eswar *et al.,* 2016; Kim *et al.,* 2018). Although *E. coli* is a representative bacterium of Enterobacteriaceae, it accounts for 15 % of oral bacterial flora, and the rate of colonization in the oral cavity in-

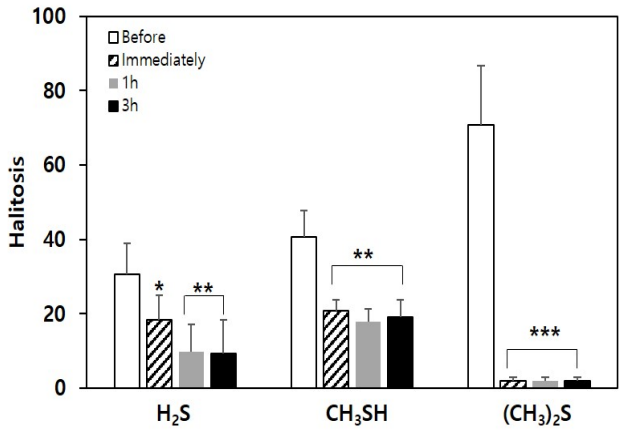


Fig. 5. *Inhibitory effect on components inducing halitosis of water-extracted apple (WEA)*

creases with age and is known to be related to xerostomia (Jeong *et al.,* 2020). *C. albicans* is a representative of oral opportunistic fungus and causes oral candidiasis (Jeong *et al*., 2020). WEA showed anti-oral microbial activity against bacterial species (Fig. 2) and inhibited the forming colony and growth of salivary microorganisms (Fig. 3 and 4). Therefore, WEA was confirmed to have anti-oral microbial activity on bacteria causing oral infectious diseases, and this result is similar to that of green tea polyphenols for *Porphyromonas gingivalis* (Sakanaka *et al.* 1996) and tea catechin for *Streptococcus mutans* (Xu *et al*., 2011). The anti-oral microbial activity of WEA in this study is also thought to be due to the phenolic compounds abundantly contained in the apple. In this study, WEA also inhibited the generation of H_2S, CH_3SH and $(CH_3)_2S$, which are components of halitosis (Fig. 5). Halitosis generates as a result of the metabolism of microorganisms and is closely related to the growth of microorganisms in the oral cavity, and control of oral microorganisms is essential to inhibit the generation of halitosis (Jeon *et al.,* 2015). Therefore, WEA can control oral microorganisms as well as inhibit the generation of halitosis through anti-oral microbial activity and inhibition of oral microbial growth.

Conclusion

WEA of 100 mg/ml showed a bleaching effect on bovine teeth related to malic acid contained in WEA. It can control oral microorganisms as well as inhibit the generation of halitosis, through anti-oral microbial activity and inhibitory effect on the growth of *Streptococcus mutans, Latobacillus casei*, *Aggregatibacter actinomycetemcomitans*, and *Eschericha coli*. The anti-oral microbial activity and inhibition of oral microbial growth in the saliva of WEA were related to phenolic compounds. Therefore, WEA is a functional substance derived from a safe and useful natural product that can be used for the prevention of oral infectious diseases and maintenance of oral health.

ACKNOWLEDGEMENTS

This work was supported by the Youngsan University Research Fund of 2021 from Youngsan University, the Republic of Korea.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Attin, T., Kielbassa, A. M., Schwanenberg, M. & Hellwig, E. (1997). Effect of floride treatment on remineralization of bleached enamel. *J. Oral Rehabil*. 24, 282-286. https://doi.org/10.1111/j.1365-2842.1997.tb00327.x
2. Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turcj, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology,* 45 (4), 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493
3. Byun, R., Nadkarni, M. A., Chhour, K. L., Martin, F. E. & Jacques, N. A., Hunter N. (2004). Quantitative analysis of diverse Lactobacillus species present in advanced dental caries. Journal of Clinical Microbiology, 42 (7), 3128-3136. https://doi.org/10.1128/JCM.42.7.3128-3136.2004
4. Cho, J. J., Kim, H. S., Kim, C. W. & Cho, S. J. (2017). Interaction with polyphenols and antibiotics. *Journal of Life Science*, 27 (4), 476-482. https://doi.org/10.5352/JLS.2017.27.4.476
5. Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12 (4), 564-582. https://doi.org/10.1128/CMR.12.4.564
6. Dahl, J. E. & Pallesen, U. (2003). Tooth bleaching-a critical review of the biological aspects. *Crit. Rev. Oral Biol. Med.* 14, 292-304. https://doi.org/10.1177/154411130301400406.
7. Eswar, P., Devaraj, C. G. & Agarwal P. (2016). Antimicrobial activity of Tulsi {Ocimum sanctum (Linn.)} extract on a periodontal pathogen in human dental plaque: an invitro study. *J. Clin. Diagn Res.* 10, ZC53-ZC56. https://doi.org/10.7860/JCDR/2016/16214.7468
8. Eumkeb, G., Sakdarat, S. & Siriwong, S. (2010). Reversing β-lactam antibiotic resistance of Staphylococcus aureus with galangin from Alpinia officinarum Hance and synergism with ceftazidime. *Phytomedicine,* 18, 40-45.
9. Hwang, G. W., Choi, M. S. & Lim, S. A. (2015). Improvement of bleaching effect on the color of enamel surface with TiO_2 catalysis convergence bleaching agent. *J. Korea Convergence. Soc.* 6, 165-171. http://dx.doi.org/10.15207/JKCS.2015.6.5.165
10. Jeon, J.-G. Rosalen, P. Falsetta, M. & Koo H. (2011). Natural Products in Caries Research: Current (Limited) Knowledge, Challenges and Future Perspective. Caries Res. 45, 243–263.
11. Jeon, Y. M., Cho, J. W., Lee, J. Y. & Woo, S. H. (2015). The effects of oral microorganism on oral malodor. Int J Clin Prev Dent. 11 (3), 171-176.
12. Jeong, M. J., Lim, D. S., Lee, M. H, Heo, K., Kim, H. H. &

Jeong, S. J. (2020). Inhibitory Effects on oral microbial activity and production of Lipopolysaccharides-Induced Pro-Inflammatory mediators in Raw264.7 Macrophages of Ethanol Extract of Perilla flutescens (L.) Britton. *J Dent Hyg Sci*. 20 (4), 213-220. https://doi.org/10.17135/jdhs.2020.20.4.213

13. Jeong, S. J. (2021). Effects of *Citrus limon* extract on oxidative stress-induced nitric oxide generation and bovine teeth bleaching. *J Dent Hyg Sci*. 21 (2), 96-103. https://doi.org/10.17135/jdhs.2021.21.2.96.
14. Kalinowska, M., Bielawska, A., Lewandowska-Siwkiewicz, H., Priebe, W. & Lewandowski, W. (2014). Apples: Content of phenolic compounds vs. variety, part of apple and cultivation model, extraction of phenolic compounds, biological properties. *Plant Physiol.* Biochem. 84, 169–188.
15. Kidon, M. & Grabowska, J. (2020). Bioactive compounds, antioxidant activity, and sensory qualities of red-fleshed apples dried by different methods. LWT. 136, 110302. https://doi.org/10.1016/j.lwt.2020.110302
16. Kim, M. Y., Choi, E. O. Kwon, D. H., Kim, H. J., Ahn, K. I., Ji, S. Y., Jeong, J. W., Park, S. H., Hong, S. H., Kim, G. Y., Park, C., Kim, H. W., Moon, S. K., Yun, S. J., Kim, W. J. & Choi, H. H. (2018). Induction of apoptosis by Citrus unshiu peel in human breast cancer MCF-7 cells: involvement of ROS-dependent activation of AMPK. *Biol. Pharm Bull*. 41, 713-721. https://doi.org/10.1248/bpb.b17-00898
17. Lee, K. H. & Kim, S. (2017). Utilization of resin infiltration for prolonging of tooth whitening effects. *J Korean Acd Pediatr Dent*. 44, 1-9. https://doi.org/10.5933/JKAPD.2017.44.1.1
18. Ma, B., Yuan Y., Gao, M, Ogutu, C., Li, M. & Ma, F. (2018). Determination of predominabt organic acid components in Malus species: correlation with apple domestication. Matabolites, 8 (4), 74. doi: 10.3390/metabo8040074.
19. Mazilu(Moldovan), A., Sarosi, C., Moldovan, M., Miuta, F., Prodan, D., Antoniae, A., Prejmerean, C., Dumitrescu, L. S., Popescu, V., Raicu, A. D. & Saceleanu, V. (2019). Preparation and characterization of natural bleaching gels used in cosmetic dentistry. *Material*, 12 (13). https://doi.org/10.3390/ma12132106.
20. McCann, M., Gill, C., Brien, G.O., Rao, J., McRoberts, W., Hughes, P., McEntee, R. & Rowland, I. (2007) Anti-cancer properties of phenolics from apple waste on colon carcinogenesis in vitro. Food Chem. *Toxicol*. 45, 1224–1230.
21. Nam, S. S. & Ko, K. S. (2020). Antioxidants of apple leaf extract. *Journal of the Korean Applied Science and Technology*, 37 (5), 1116-1124. https://doi.org/10.12925/jkocs.2020.37.5.1116
22. Nezbedova, L., McGhie, T., Christensen, M, Heyes, J, Nasef, N. A. & Mehta, S. (2021). Onco-Preventive and chemo-protective effects of apple bioactive compounds. Nutrients, 13, 4025. https://doi.org/10.3390/nu13114025
23. Palombo, E. A. (2011). Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evidence Based Complement. *Altern. Med.* 2011, 1–15.
24. Pan, H., Wang, D. & Zhang, F. (2020). In vitro antimicrovial effect of curcumin-based photodynamic therapy on porphyromonas gingivalis and aggregatibacter actinomycetemcomitans. Photodiagnosis and Photodynamic Therapy, 32, 102055. https://doi.org/10.1016/j.pdpdt.2020.1020 5 5
25. Park, E. S., Seong, S. R., Hong, S. T., Kim, J. E. & Lee, S. T. (2006). A clinical evaluation of a bleaching strip containing 2.09% hydrogen peroxide. *Korean Acad. Conserv. Dent.* 31, 269-281. https://doi.org/10.5395/JKACD.2006.31.4.269
26. Park, J. W., Wendt, M. & Heo, G. J. (2016). Antimicrobial activity of essential oil of Eucalyptus globulus against fish phathogenic bacteria. Lab. Anim. Res. 32 (2), 87-90. DOI: https://doi.org/10.5625/lar.2016.32.2.87
27. Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., Martin-Belloso, O. (2009). Antimicrobial activity of malic acid against Listeria monocytogenes, Salmonella Enteritidis and Escherichia coli o157:H7 in apple, pear and melon juices. *Food control,* 20, 105-112.
28. Sakanaka, S., Aizawa, M., Kim, M & Yamamotto, T. (1996). Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, Porphyromonas gingivalis. *Bioscience Biotechnology and Biochemistry*, 60 (5), 745-749. https://doi.org/10.1271/bbb.60.745
29. Scalbert, A. & Williamson, G. (2000). Dietary Intake and Bioavailability of Polyphenols. *J. Nutr.* 130, 2073S–2085S.
30. Shaheena, S., Chintagunta, A. D., Dirisala, V. R. & Kumar, N. S. S. (2019). Extraction of bioactive compounds from Psidium guajava and their application in dentistry. *AMB Express*, 9, 208. https://doi.org/10.1186/s13568-019-0935-x
31. Villa, A., Polimeni, A., Strohmenger, L., Cicciù, D., Gherlone, E. & Abati, S. (2011). Dental patients' self-reports of xerostomia and associated risk factors*. J. Am. Dent. Assoc.* 142, 811–816.
32. Vinson, J. A., Su, X., Zubik, L. & Bose, P. (2001). Phenol antioxidant quantity and qQuality in foods: Fruits. *J. Agric. Food Chem*. 49, 5315–5321. https://doi.org/10.1021/jf0009293
33. Watts, A. & Addy, M. (2001). Tooth discolouration and staining: Tooth discolouration and staining: A review of the literature. *Br. Dent. J.* 190, 309–316.
34. Xu, X., Zhou, X. D. & Christine D. W. (2011). The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of streptococcus mutans. Antimicrovial Agents and Chemotherapy, 55 (3), 1229-1236. https://doi.org/10.1128/AAC.01016-10