CHALLENGES AND OPPORTUNITIES IN APPLYING TEMULAWAK (*Curcuma xanthorrhiza* Roxb.) FOR INDUSTRIAL ORAL CARE PRODUCTS

Jae-Kwan Hwang[1*], Yaya Rukayadi[1,2]
[1]*Department of Biotechnology, Yonsei University*
[2]*Biopharmaca Research Center, Bogor Agricultural University*

ABSTRACT

Curcuma xanthorrhiza Roxb., commonly known as temulawak or java turmeric, is an indigenous plant from Java, Bali, and Mollucas, Indonesia. It has been used by Indonesian ancestors for food, medicinal purposes, and as a tonic. *C. xanthorrhiza* has been traditionally used to treat stomach diseases, liver disorders, constipation, bloody diarrhea, dysentery, children's fevers, haemorrhoid, and skin eruptions. Recently, it has been reported that temulawak confers various biological activities such as antitumor, hypotriglyceridaemic, anti-inflammatory, hepatoprotective, and antibacterial. In our research, we found that xanthorrhizol (1,3,5,10-bisabolatetraen-3-ol), isolated from the rhizome of *C. xanthorrhiza*, possessed remarkable anticariogenic activity against oral pathogens. Xanthorrhizol exhibited the highest antibacterial activity against *Streptococcus* species causing dental caries and also demonstrated antibacterial potential against *Actinomyces viscosus* and *Porphyromonas gingivalis* which are responsible for periodontitis. Xanthorrhizol killed completely *Streptococcus mutans* in a minute. This bactericidal activity is of practical significance, since applications of xanthorrhizol in mouthwash or toothpaste should be effective within a few minutes. Xanthorrhizol also showed a promising activity as an antibacterial agent for inhibiting and removing *S. mutans* biofilms. Clinical test analysis showed that xanthorrizol was active *in vivo* and was not toxic to the host cells. In Korea, temulawak oil has been developed and marketed as xanthorrhizol-containing toothpaste. Thus, there are some challenges and opportunities in applying temulawak for industrial oral care products.

INTRODUCTION

Java turmeric is classified to the kingdom *Plantarum*, division *Spermatophyta*, sub-division *Angiospermae*, class *Monocotyledonae*, order *Zingiberales*, family *Zingiberacea*e, genus *Curcuma*, and species *Curcuma xanthorrhiza* Roxb., synonym *Curcuma javanica*. Vernacular names of java turmeric are koneng gede (Sundanese), temu lawak (Javanese), temo labak (Madurese) (Indonesia); temu lawas, temu raya (Malaysia); and wan chakmotluk (Thailand). *C. xanthorrhiza* is native to Java, Bali, and the Moluccas. It is commonly cultivated in Java, Peninsular Malaysia, Philippines, and Thailand, occasionally also in India.

C. xanthorrhiza Roxb. is a herb with branched rhizome, dark yellow to reddish-brown at the exterior, orange or orange-red in the interior; leaf sheaths up to 75 cm long, blades elliptical-oblong to oblong-lanceolate, 25–100 cm × 8–20 cm, green with reddish-brown band along the midrib; inflourescence on a separate shoot, bracts pale green, coma bracts purple; corolla 4–6 cm long, pale red; labellum 2–2.5 cm × 1.5–2 cm, yellowish with a darker yellow median band, other staminodes longitudinally folded, yellowish-white, anther with long spurs. *C. xanthorrhiza* Roxb. is found in thickets

* Correspondence author:
Jae-Kwan Hwang, Department of Biotechnology, Yonsei University, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-749, South Korea. Tel: +82-2-2123-5881, Fax: 82-2-362-7265. E-mail: *jkhwang@yonsei.ac.kr*

and teak forest, mainly on moist, fertile, humus-rich soils, up to 750 m altitude (Wardini & Prakoso 1999).

C. xanthorrhiza has been traditionally used in Indonesia for food and medicinal purposes (Lin *et al*. 1995). For food, temulawak produces starch and a yellow dye. Young stem and rhizome parts are eaten as a vegetable, either raw or cooked. The inflourescences are eaten cooked. In Java, a soft drink called 'bir temulawak' is prepared by cooking dried pieces of the rhizome. For medicinal purposes, rhizomes of temulawak are used to treat various abdominal complaints and liver disorders (jaundice, gall-stones, promoting the flow of bile). A decoction of the rhizome is also used as a remedy for fever and constipation, and is taken by women as a galactagogue and to lessen uterine inflammation after giving birth. Others applications are against bloody diarrhea, dysentery, inflammation of the rectum, haemorrhoids, stomach disorders caused by cold, infected wounds, skin eruptions, acne vulgaris, ecxema, smallpox, and anorexia. In Indonesia, rhizome is used as an important ingredient into many "jamus" (Wardini & Prakoso 1999). Modern researches done by various overseas researchers found that temulawak has the following effects: antihepatotoxic, antioxidant, antitumor, anti-inflammation, etc.

PYTHOCHEMISTRY OF TEMULAWAK

The fresh rhizomes of *C. xanthorrhiza* Roxb. contain terpenoids, one monoterpenoid, and curcuminoids. There are 9 sesquiterpenoids (α-curcumene, arturmerone, xanthorrhizol, germacrone, β-curcumene, β-sesquiphellandrene, curzerenone, α-turmerone, and β-turmerone) and 3 curcuminoids (curcumin, mono-demethoxycurcumin, and bis-demethoxycurcumin) (Uehara *et al*. 1992). Dried rhizomes of *C. xanthorrhiza* Roxb. contain on average 3.8% of essential oil, with ar-curcumene, xanthorrhizol, α-, β-curcumene, and germacrene as major constituents. Cyclo-isopren-emyrcene and *p*-tolylmethylcarbinol, which are often mentioned as essential oil constituents in older literature, are artifacts which originated from distillation and fractionation of oils at higher temperatures. The phenolic sesquiterpene xanthorrhizol is species specific: its presence can thus be used to distinguish *C. xanthorrhiza* Roxb. from e.g. *Curcuma longa*. Three nonphenolic diarylheptanoids isolated from *C. xanthorrhiza* Roxb. have been identified as *trans*, *trans*-1,7-diphenyl-2,3-heptadien-4-one (alnustone), *trans*-1,7-diphenyl-1,3-hepten-5-ol, and *trans*, *trans*-1,7-diphenyl-1,3-heptadien-5-ol (Wardini & Prakoso 1999).

XANTHORRHIZOL ISOLATED FROM THE RHIZOME OF TEMULAWAK

Xanthorrhizol, a natural sesquiterpenoid (Figure 1), can be isolated as a pure form from ethyl acetate fraction of the methanol extract of *C. xanthorrhiza* Roxb., according to the method of Hwang *et al.* (2000b). Briefly, the rhizomes (100 g) were ground and extracted with 400 ml methanol 75% (v/v), and further fractionations were carried out consecutively with ethyl acetate (4.8 g), *n*-butanol (1.7 g), and water (1.1 g). Xanthorrhizol (0.2 g) was isolated from the ethyl acetate fraction by using a silica gel column chromatography (Merck; 70–230 mesh; 5 $\times$ 43 cm; *n*-hexane/ethyl acetate, 10:1). Xanthorrhizol was identified by direct comparison of the ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and electron ionization (EI)-mass spectral results with the published data (Itokawa *et al*. 1985). The specific rotation of xanthorrhizol was determined as $[\alpha]_D^{20}$: –50.2^{o} (*c* = 0.65, $CHCl_3$).

Our study has led to the isolation of xanthorrhizol, and the compound had a powerful activity against oral pathogens and *Streptococcus mutans* biofilms (Hwang *et al.* 2000a,b; Rukayadi & Hwang 2006a,b). Other results related to xanthorrhizol have been published by our group and showed an anticandidal activity of this compound (Rukayadi *et al*. 2006), a suppressive effect of natural

sesquiterpenoids on inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) activities in mouse macrophage cells (Lee *et al.* 2002), an effect of cisplatin-induced hepatotoxicity abrogation on the regulation of gene transcription in mice (Kim *et al*. 2004), a potential to attenuate the high dose cisplatin-induced nephrotoxicity in mice (Kim *et al*. 2005a), a natural sesquiterpenoid and an anti-metastatic potential in experimental mouse lung metastasis model (Choi *et al*. 2005). Our findings demonstrated that xanthorrhizol is a compound with multibioactive functions. In the future, a single drug for variously therapeutic will be very important in order to reduce drug-drug interactions.

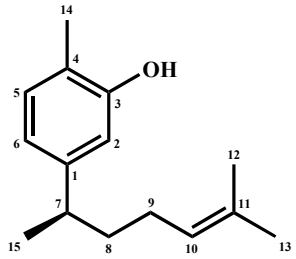

Figure 1 Structure of xanthorrhizol.

ANTIBACTERIAL ACTIVITY OF XANTHORRHIZOL AGAINST ORAL PATHOGENS

The antibacterial activity of xanthorrhizol was evaluated against oral microorganisms in comparison with chlorhexidine. Antibacterial activity was determined in terms of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Xanthorrhizol exhibited the highest antibacterial activity against *Streptococcus* species causing dental caries and also demonstrated antibacterial potential against *Actinomyces viscosus* and *Porphyromonas gingivalis* which are responsible for periodontitis (Table 1).

Table 1 Antibacterial activity of xanthorrhizol[a] (Hwang *et al*. 2000a)

Bacteria	Xanthorrhizol		Chlorhexidine	
	MIC	MBC	MIC	MBC
Actinomyces viscosus KCTC 9146	16	16	4	4
Candida albicans ATCC 10231	125	250	32	32
Lactobacillus casei ATCC 4646	250	500	32	64
Lactobacillus acidophilus ATCC 4356	500	500	32	32
Porphyromonas gingivalis W50	32	32	8	16
Streptococcus mutans ATCC 25175	2	4	1	2
Streptococcus salivarius ATCC 13419	4	8	2	2
Streptococcus sobrinus ATCC 27351	4	4	4	4
Streptococcus sanguis ATCC 35105	4	8	2	4

[a] MIC, MBC as μg ml.

MIC of xanthorrhizol against *S. mutans*, which is known to be a major causative organism for dental plaque and can also be a source of infective endocarditis (Banas 2004), was determined to be 2 μg/ml, which was much lower than other natural anticariogenic agents such as 16 μg/ml of sangunarine, 125 μg/ml of tea polyphenol, 125 μg/ml of carvacrol, 250 μg/ml of isoeugenol, 500 μg/ml of eucalyptol, and 500 μg/ml of thymol. Figure 2 shows that 5 μg/ml treatment of xanthorrhizol killed completely *S. mutans* in a minute. This bactericidal activity is of practical significance, since applications of xanthorrhizol in mouthwash or toothpaste should be effective within a few minutes. These results suggested that xanthorrhizol could be employed as a potential anticariogenic agent.

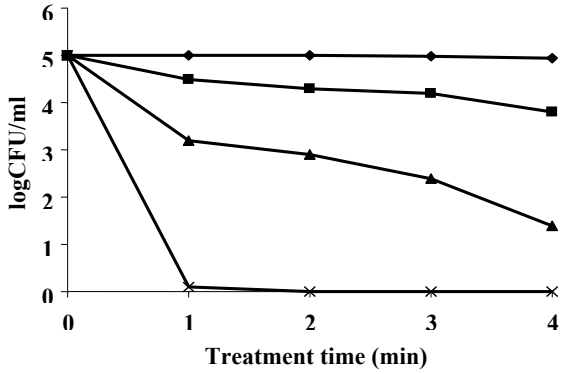

Figure 2 Bactericidal activity of xanthorrhizol against *S. mutans* (-♦-: control; -■-: 1 μg/ml; -▲-: 3 μg/ml; -×-: 5 μg/ml).

ANTI-BIOFILM ACTIVITY OF XANTHORRHIZOL

S. mutans and other oral streptococci are colonizers of the surface of the tooth, where, together with many other bacterial species, they build a biofilm also known as dental plaque. Plaque development commences with the adhesion of microorganisms to acquired salivary pellicles on the enamel's surface. Therefore, inhibition of pathogenic bacterial adherence can yield benefits in controlling caries and periodontal disease (Liljemark & Bloomquist 1996). Sharma *et al.* (2005) reported that prevention of microbial adhesion and detachment of adhering microorganisms from the surface of the tooth is important. Moreover, microbial adhesion in the oral cavity occurs under highly unfavorable conditions, and flow of saliva and movement of the tongue, lips, and cheeks, for instance, cannot prevent adhesion. This indicates that the forces involved in microbial adhesion to the surface of the tooth are quite strong. Hence, interest has increased in studying prevention of microbial adhesion and reducing plaque development.

A widely adopted approach to reduce and remove plaque development is the topical application of bactericides, e.g. triclosan, chlorhexidine, and cetylpyridinium chloride, by inhibiting the plaque development and lowering the number of microorganisms in saliva (Eley 1999). In general, they are nonselective in their efficacy, and their frequent use can potentially lead to a change in the oral microbiota and the occurrence of resistant strains (McMurry *et al.* 1998). An alternative approach for controlling plaque formation is to select molecules that can block or reduce bacterial adherence. Coating of the substratum surfaces by biomaterial is highly effective in the prevention of bacterial adhesion (Roosjen *et al.* 2004). Vaccines against major cariogenic oral bacteria and chemical agents for coating the tooth surface or interfering with bacterial binding, have been investigated (Wade *et al*. 1994; Hajshengallis & Michalek 1999; Kato *et al*. 1999). In principle, the vaccine approach is more efficient, but its limitations are high in cost. Chemical agents can offer an alternative means of reducing plaque formation. However, these chemicals possess many adverse effects such as microorganisms building tolerance, vomiting, diarrhea, and teeth staining (Chen *et al.* 1989). We have evaluated the effect of coating the wells of a polystyrene plate with xanthorrhizol on *S. mutans* biofilm formation. Coating with xanthorrhizol resulted in significant (up to 60%) reduction of adherent cells compared to that of cells in uncoated wells, and similar result was found in the chlorhexidin-coated wells (Figure 3).

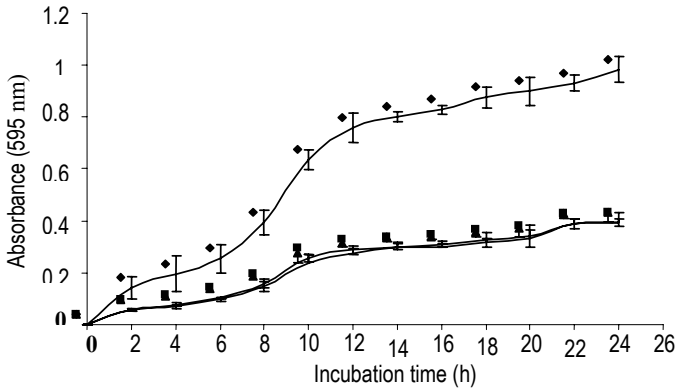

Figure 3 Effect of xanthorrhizol impregnation on subsequent biofilm formation by *S. mutans* ATCC 27351 on the wells of uncoated- (♦), xanthorrhizol-coated- (■), and chlorhexidine-coated (▲) microtiter plates at 37 °C during 24 h. The concentration of xanthorrhizol or chlorhexidine was 5 μg/ml. *Significantly different ($p < 0.05$) (Rukayadi & Hwang 2006b).

We determined the effect of xanthorrhizol on the *S. mutans* biofilms *in vitro*. The biofilms of *S. mutans* at different phases of growth were exposed to xanthorrhizol at different concentrations (5, 10, and 50 μmol/ml) and for different time exposures (1, 10, 30, and 60 min). The results demonstrated that the activity of xanthorrhizol in removing *S. mutans* biofilm depended on the concentration, exposure time, and growth phase of biofilm (Table 2). A concentration of 5 μmol/ml of xanthorrhizol completely inhibited biofilm formation by *S. mutans* at adherent phases of growth, whereas 50 μmol/ml of xanthorrhizol removed 76% of biofilm at plateau accumulated phase when exposed to *S. mutans* biofilm for 60 min (Table 2).

Table 2 The effect of xanthorrhizol at different concentrations and exposure time on biofilms of *S. mutans* at different phases of growth (Rukayadi & Hwang 2006a)

Xanthorrhizol Exposure time (min)	Percentage of mean remaining cells of *S. mutans* biofilm after treatment with xanthorrhizol (% ± SD)†			
	Adherent phase (4 h)	Active accumulated phase (12 h)	Beginning plateau accumulated phase (20 h)	Plateau accumulated phase (24 h)
5 μmol l^{-1}				
1	0	77 ± 6	86 ± 6*	92 ± 8*
10	0	61 ± 9	77 ± 7	79 ± 9*
30	0	39 ± 2	60 ± 7	63 ± 1
60	0	23 ± 7	34 ± 4	36 ± 4
10 μmol l^{-1}				
1	0	71 ± 8	76 ± 4	84 ± 9
10	0	54 ± 3	57 ± 2	72 ± 2
30	0	13 ± 3	42 ± 9	59 ± 6
60	0	8 ± 2	28 ± 6	44 ± 5
50 μmol l^{-1}				
1	0	46 ± 7	68 ± 8	70 ± 6
10	0	10 ± 1	31 ± 9	54 ± 5
30	0	0	23 ± 1	39 ± 9
60	0	0	11 ± 4	24 ± 4

†Values are expressed as the percentage of absorbance (595 nm) of cells in treated wells compared with that in untreated wells (considered to be 100%), and SD is standard deviation (errors) of the mean percentage of absorbance (595 nm) of cells derived from four times of experiment and four replicates (wells) per experiment.

*Not significantly different from the control treatment. All entries in the table are significantly different ($P < 0.05$) from the controls unless otherwise indicated.

CLINICAL TEST OF XANTHORRHIZOL

Some clinical tests of oral hygiene products containing *C. xanthorrhiza* Roxb. extract (Cx) have been determined and published. Gingivalis suppression effect of the *de novo* dentifrice containing *C. xanthorrhiza* Roxb., bamboo salt, and various additives was investigated (Hwang *et al.* 2005). The results showed that the dentifrice containing bamboo salt, *C. xanthorrhiza* Roxb., ursodeoxycholic acid, and various additives could be a useful dentifrice in reducing gingivalis. Other test was a highly selective antibacterial effect of Cx extract against oral pathogens and clinical effectiveness of a dentifrice containing Cx in controlling bad breath (Kim *et al.* 2005b). The summary of this test showed that Cx with its highly selective antibacterial activity appeared to be an attractive candidate to replace chemicals, and oral hygiene products with Cx will be a new paradigm delivering natural benefit for consumers.

CHALLENGES AND OPPORTUNITIES IN APPLYING TEMULAWAK FOR INDUSTRIAL ORAL CARE PRODUCTS

Some plant extracts clearly demonstrate antibacterial properties although the mechanism of their activities are still poorly understood (Dorman & Deans 2000). The spice extracts, cinnamon bark oil, papua-mace extract, and clove bud oil were all reported to inhibit the growth of many oral bacteria (Saeki *et al.* 1989). Sanguinarine, an alkaloid extract from the rhizome of *Sanguinaria canadensis*, has been reported to possess a broad spectrum against a wide variety of oral bacteria (Joann & Sigmund 1985). In particular, green tea extract, which is customarily drunk after every meal in Japan, is known to contain several polyphenols that inhibit the growth of *S. mutans* (Sakanaka *et al.* 1989). However, they have relatively mild effects against oral pathogens compared to that of commercial synthetic anticariogenic agents. Our study showed that temulawak extract or xanthorrhizol has more powerful activity against oral pathogens compared to that of anticariogenic agents. In Korea, temulawak extract has been developed and marketed as xanthorrhizol-containing toothpaste (Figure 4). Thus, there are some challenges and opportunities in applying temulawak for industrial oral care products.

Figure 4 Temulawak extract-containing toothpaste.

REFERENCES

Chen CP, Lin CC, Namba T. 1989. Screening of Taiwanese crude drugs for antibacterial activity against *Streptococcus mutans*. *J Ethnopharmacol* 27:285-295.

Choi MA, Kim SH, Chung WY, Hwang JK, Park KK. 2005. Xanthorrhizol, a natural sesquiterpenoid from *Curcuma xanthorrhiza*, has an anti-metastatic potential in experimental mouse lung metastasis model. *Biochem Biophys Res Commun* 326:210-217.

Dorman HJD, Deans SG. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J Appl Microbiol* 88:308-316.

Eley BM. 1999. Antibacterial agents in the control of supragingival plaque-A review. *Br Dent J* 186:286-296.

Hajshengallis G, Michalek SM. 1999. Current status of a mucosal vaccine against dental caries. *Oral Microbiol Immunol* 14:1-20.

Hwang JK, Shim JS, Pyun YR. 2000a. Antibacterial activity of xanthorrhizol from *Curcuma xanthorrhiza* against oral pathogens. *Fitoterapia* 71:321-323.

Hwang JK, Shim JS, Baek NI, Pyun YR. 2000b. Xanthorrhizol: a potential agent from *Curcuma xanthorrhiza* against *Streptococcus mutans*. *Planta Med* 66:196-197.

Hwang SJ *et al*. 2005. Gingivalis suppression effect of the *de novo* dentifrice containing *Curcuma xanthorrhiza*, bamboo salt and various additives. *J Korean Acad Dent Health* 29:222-236.

Itokawa H, Hirayama F, Takeya K. 1985. Studies on the antitumor bisabolane sesquiterpenoids isolated from *Curcuma xanthorrhiza*. *Chem Pharm Bull* 33:3488-3492.

Joann LD, Sigmund SS. 1985. Comparative *in vitro* activity of sanguinarine against oral microbial isolates. *Antimicrob Agents Chemother* 27:663-665.

Kato H *et al*. 1999. The immunogenicity of various peptide antigens inducing cross-reacting antibodies to a cell surface protein antigen of *Streptococcus mutans*. *Oral Microbiol Immunol* 14:213-219.

Kim SH, Hong KO, Chung WY, Hwang JK, Park KK. 2004. Abrogation of cisplatin-induced hepatotoxicity in mice by xanthorrhizol is related to its effect on the regulation of gene transcription. *Toxicol Appl Pharmacol* 196:346-355.

Kim SH, Hong KO, Hwang JK, Park KK. 2005a. Xanthorrhizol has a potential to attenuate the high dose cisplatin-induced nephrotoxicity in mice. *Food Chem Toxicol* 43:117-22.

Kim BI *et al*. 2005b. A highly selective antibacterial effect of *Curcuma xanthorrhiza* extract against oral pathogens and clinical effectiveness of a dentifrice containing *Curcuma xanthorrhiza* extract for controlling bad breath. *J Korean Acad Dent Health* 35:1053-1069.

Lee SK *et al*. 2002. Suppressive effect of natural sesquiterpenoids on inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) activity in mouse macrophage cells. *J Environ Pathol Toxicol Oncol* 21:141-148.

Liljemark WF, Bloomquist C. 1996. Human oral microbial ecology and dental caries and periodontal diseases. *Crit Rev Oral Bio Med* 7:180-198.

Lin SC, Lin CC, Lin YH, Supriyatna S, Teng CW. 1995. Protective and therapeutic effects of *Curcuma xanthorrhiza* on hepatotoxin-induced liver damage. *Am J Chin Med* 23:243-254.

McMurry LM, Oethiger M, Levy SB. 1998. Triclosan targets lipid synthesis. *Nature* 394:531-532.

Rukayadi Y, Hwang JK. 2006a. *In vitro* activity of xanthorrhizol against *Streptococcus mutans* biofilms. *Lett Appl Microbiol* 42:400-404.

Rukayadi Y, Hwang JK. 2006b. Effect of coating the wells of a polystyrene microtiter plate with xanthorrhizol on the biofilm formation of *Streptococcus mutans*. *J Basic Microbiol* 46:411-416.

Rukayadi Y, Yong D, Hwang JK. 2006. *In vitro* anticandidal activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. *J Antimicrob Chemother* 57:1231-1234.

Roosjen A, de Vries J, Mei HC van der, Busscher HJ. 2005. Stability and effectiveness against bacterial adhesion of poly(ethylene oxide) coatings in biological fluids. *J Biomed Mater Res Part B: Appl Biomater* 73B:347-354.

Saeki Y, Ito Y, Okuda K. 1989. Antibacterial action of natural substances on oral bacteria. *Bull Tokyo Dent Coll* 30:129-135.

Sakanaka S, Kim M, Taniguchi M, Yamamoto T. 1989. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a cariogenic bacterium. *Agric Biol Chem* 53:2307-2311.

Sharma PK, Gibcus MJ, Mei HC van der, Busscher HJ. 2005. Influence of fluid shear and microbubbles on bacterial detachment from a surface. *Appl Environ Microbiol* 71:3668-3673.

Wardini TH, Prakoso B. 1999. *Curcuma* L. In: de Padua LS, Bunyapraphatsara N, Lemmens RHMJ, editors. *Plant Resources of South-East Asia 12. (1) Medicinal and Poisonous Plants 1*. 1st Volume. Bogor: Prosea. pp. 210-219.

Uehara S, Yosuda I, Takeya K, Itokawa H. 1992. Terpenoids and curcuminoids of the rhizome of *Curcuma xanthorrhiza* Roxb. *Yakugaku Zasshi* 112:817-823.