Original Article

DOI: 10.5582/ddt.2017.01055

Effects of *Caesalpinia sappan* on pathogenic bacteria causing dental caries and gingivitis

Rinrampai Puttipan[1,2], Penpicha Wanachantararak[3], Sakornrat Khongkhunthian[2,4], Siriporn Okonogi[1,2,*]

[1] *Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand;*
[2] *Research Center of Pharmaceutical Nanotechnology, Chiang Mai University, Chiang Mai, Thailand;*
[3] *Central Laboratory of Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand;*
[4] *Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand.*

Summary **The present study explores antimicrobial activities of *Caesalpinia sappan* extracts against three strains of oral pathogenic bacteria; *Streptococcus mutans* DMST9567 (*Smu9*), *Streptococcus mutans* DMST41283 (*Smu4*), and *Streptococcus intermedius* DMST42700 (*Si*). Ethanol crude extract of *C. sappan* (Cs-EtOH) was firstly compared to that of other medicinal plants using disc diffusion method. Cs-EtOH showed significantly higher effective inhibition against all tested strains than other extracts and 0.12% chlorhexidine with the inhibition zone of 17.5 ± 0.5, 18.5 ± 0.0, and 17.0 ± 0.0 mm against *Smu9*, *Smu4*, and *Si*, respectively. Three fractionated extracts of *C. sappan* using hexane, ethyl acetate, and ethanol, respectively, were further investigated. The fractionated extract from ethanol (F-EtOH) presented the strongest activities with the minimum bactericidal concentration (MBC) of 125-250 μg/mL. Killing kinetics of F-EtOH was depended on the bacterial species and the concentration of F-EtOH. Two-fold MBC of F-EtOH could kill all tested strains within 12 h whereas its 4-fold MBC showed killing effect against *Si* within 6 h. Separation of F-EtOH by column chromatography using chloroform/methanol mixture as an eluent yielded 11 fractions (F1-F11). The fingerprints of these fractions by high-performance liquid chromatography at 280 nm revealed that F-EtOH consisted of at least 5 compounds. F6 possessed the significantly highest antimicrobial activity among 11 fractions, however less than F-EtOH. It is considered that F-EtOH is the promising extract of *C. sappan* for inhibiting oral pathogenic bacteria and appropriate as natural antiseptic for further develop of oral hygiene products.**

Keywords: Oral pathogens, antimicrobial activity, extracts, medicinal plants, *Ceasalpinia sappan*

1. Introduction

Dental caries, a scientific term for tooth decay, is the localized destruction of a susceptible tooth surface whereas gingivitis is one of the periodontal diseases which is characterized by bleeding, plaque formation and gum inflammation (*1*). Both dental caries and gingivitis play an impressive impact on the human health and welfare. These health problems and the consequent cost of dental treatment leads to costing billions of dollars each year (*2*). Oral microorganisms are one of the most important factors responsible for dental carries and gingivitis (*3*). The most insidious bacteria are oral pathogenic strains particularly *Streptococcus spp*. Although this genus is the normal flora microorganisms in oral cavity, some species such as *Streptococcus mutans* and *Streptococcus intermedius* are the pathogenic bacteria. *S. mutans* is considered to be the most cariogenic bacteria initiate to dental caries whereas *S. intermedius* displays the significant role to generate gingivitis (*4,5*). They can early colonized on tooth surface (*6*) and form organized microorgamism communication as biofilm or dental plaque (*7*). They can also produce acid that destroys the tooth's enamel layer. Many evidences show that elimination of these

**Address correspondence to:*
Dr. Siriporn Okonogi, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.
E-mail: okng2000@gmail.com

pathogenic bacteria and microbial dental plaque biofilm can prevent gingivitis and dental caries (*8,9*). However, these oral pathogens cannot be easily eliminated completely from oral cavity with common daily sanitation. Antiseptics appear to be the efficient tools for this prevention, however the prolong use of these agents has led to emergence of multidrug-resistant pathogens (*10*).

Recently, much attention has been given to natural products with health-promoting benefits. Novel therapeutic products from plants have been extensively reported (*11-13*). Plants produce various active molecules that are able to fight bacterial infections successfully (*14,15*). Many potential plant extracts have been reported on their antimicrobial activity against oral pathogens (*16,17*). Moreover, the synergistic interactions of secondary plant metabolites with antibiotics or certain bioactive compounds were reported (*18,19*).

Caesalpinia sappan is a member in family Leguminosae. The heartwood of *C. sappan* has been used as coloring agent in many products of food, beverage, and cosmetics. Importantly, *C. sappan* has long been used as traditional medicine in treatment of many kinds of infectious diseases. Some phenolic compounds existing in this plant have been isolated as well as the finding on their pharmacological activities such as antioxidant (*20*) and anti-inflammation (*21*). For antimicrobial activity, it has been reported that *C. sappan* extracts can inhibit the growth of several aerobic and facultative bacteria such as *Salmonella ebony*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella faecalis*, *Enterobacter aerogenes*, *Pseudomonas aerogenosa*, and *Staphylococcus aureus* (*22*). Moreover, the extract showed antifungal activity against *Aspergillus niger* and *Candida albicans* (*23*). However, up to our best knowledge, there is no scientific evaluation of *C. sappan* extracts on inhibition of oral pathogenic bacteria such as *S. mutans* and *S. intermedius* that are the major cause of dental carries and gingivitis, respectively. This study was designed to elucidate their potential effects on those pathogens.

2. Materials and Methods

2.1. *Chemicals*

Chloroform, *n*-hexane, ethyl acetate, ethanol, methanol, and dimethyl sulfoxide (DMSO) were from RCI Labscan Ltd. (Bangkok, Thailand). Difco™ Brain heart infusion (BHI) broth and agar were from Bacton, Dickinson and Company (Maryland, USA). Silica gel 60 (0.040-0.063 mm) for column chromatography (230-400 mesh ASTM) was from Merck KGaA (Darmstadt, Germany). The other chemicals and solvents were of the highest grade available.

2.2. *Plant materials and preparation of ethanol crude extracts*

The fresh samples of *C. sappan* and other medicinal plants presented in Table 1 were collected from the local area in Chiang Mai Province, Thailand. All plants were identified by the botanist in the botanical herbarium of Faculty of Pharmacy, Chiang Mai University to obtain the reference numbers. The plant materials were dried and pulverized into fine powder before macerating in 95% ethanol (24 h × 3 at room temperature). After maceration, the mixtures were filtered and the solvent was removed from the filtrates to obtain the ethanol crude extracts. The obtained ethanol crude extract of *C. sappan* (Cs-EtOH) and those of other plants were kept in the refrigerator until use.

2.3. *Fractionated extracts preparation*

The dried powder of *C. sappan* was subjected to fractionated extraction previously described (*24*) using 3 different solvents; *n*-hexane, ethyl acetate, and ethanol in orderly to obtain the fractionated extracts namely F-Hexane, F-EtOAc, and F-EtOH, respectively.

2.4. *Bacteria strains and culture*

Three strains of Gram-positive facultative anaerobic pathogenic bacteria including *S. mutans* DMST9567 (*Smu9*), *S. mutans* DMST41283 (*Smu4*), and *S. intermedius* DMST42700 (*Si*) were cultured and incubated under anaerobic condition at 37°C in 5% CO_2. Blood agar plates were prepared from 5% human blood in BHI agar.

2.5. *Comparing antimicrobial activity of ethanol crude extracts*

The antibacterial activity of Cs-EtOH in comparison with the ethanol crude extracts of other medicinal plants, which their antibacterial activity has been previously reported, was investigated using disc diffusion method. Before testing, the amount of the cultures was adjusted by adding BHI broth until the turbidity was equivalent to 0.5 McFarland standard which referred to 1×10^8 CFU/mL. Each blood agar plates were streaked with bacterial stock suspension. The extracts were dissolved in DMSO to a final concentration of 200 mg/mL before adding onto the sterile paper discs of 5 mm in diameter, and then put them on the top layer of the blood agar plates. Chlorhexidine (CHX) 0.12% solution and DMSO were used as positive and negative controls, respectively. The plates were incubated at 37°C in 5% CO_2 anaerobic chamber for 24 h. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone.

Table 1. Inhibition zone of ethanol crude extracts of various medicinal plants against oral pathogenic bacteria

Plant species	Reference No.	Diameter of inhibition zone (mm)		
		Smu9	*Smu4*	*Si*
Acacia catechu	009208	NZ	NZ	NZ
Caesalpinia sappan	002276	17.5 ± 0.5	18.5 ± 0.0	17.0 ± 0.0
Cajanus cajan	023173	10.0 ± 0.0	11.0 ± 0.0	15.5 ± 0.0
Cassia alata	023179	10.7 ± 0.6	NZ	NZ
Phyllanthus amarus	006540	NZ	14.7 ± 0.3	15.0 ± 0.0
Piper sarmentosum	006283	NZ	NZ	NZ
Psidium guajava	008610	NZ	7 ± 0.0	7.3 ± 0.3
Sesbania grandiflora	023176	NZ	NZ	NZ
Syzygium aromaticum	023230	10.0 ± 0.0	10.3 ± 0.3	10.3 ± 0.6
Syzygium cumini	023177	NZ	NZ	8.8 ± 0.3
0.12% CHX		16 ± 0.0	15 ± 0.0	10.0 ± 0.0

NZ = No zone.

2.5. *Antimicrobial susceptibility of C. sappan extracts*

The antimicrobial susceptibility of F-Hexane, F-EtOAc, F-EtOH, and Cs-EtOH was evaluated by comparing the value of minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts against the tested pathogenic strains using broth microdilution method. The sample stock solutions (in 25% DMSO) were diluted to the serial concentrations of 7.8-2,000 µg/mL and added into the suspension of 1×10^6 CFU/mL of the bacterial strains in the 96-well plates. The plates were then incubated at 37°C in 5% CO_2 anaerobic chamber for 24 h. The lowest concentration that inhibited the bacteria was considered as the MIC of the extract. The cultures were further investigated for MBC by streaking on blood agar plates. The plates were incubated at 37°C in 5% CO_2 anaerobic chamber for 24 h. The lowest concentration in the plates that bacterial growth could not be visible was considered as the MBC.

2.6. *Separation of F-EtOH and fingerprint preparation*

F-EtOH was separated using column chromatography. Silica gel 60 was used as the stationary phase and the mixture of chloroform/methanol at the ratios of 15:1, 10:1, and 5:1 was used as the mobile phase. Thin layer chromatography (TLC) was used for characterized the spots of the collected fractions. The fractions having similar TLC pattern were pooled and concentrated by rotary evaporator. The yield of each fraction was recorded. The fingerprint of all fractions were prepared by means of high performance liquid chromatography (HPLC) using HPLC Shimadzu CLASS-VP™ model (Kyoto, Japan) and the reversed phase Eurospher 100, i.d. 4 mm, C18 column, Knauer (Berlin, Germany). The system was conducted with isocratic at room temperature. The mixture of 1 % v/v acetic acid in DI water (A) and methanol (B) at A:B ratio of 75:25 was used as the mobile phase. HPLC procedure was operated with the mobile flow rate of 1 mL/min, injection volume of 10 µL, running time of 30 min, and monitored with UV/VIS detector at 280 nm.

2.7. *Killing kinetics of F-EtOH*

The suspensions of the tested bacterial strains (1×10^6 CFU/mL) were prepared in BHI broth in 96-well plates. F-EtOH was diluted with BHI broth and added into the bacterial suspension and gently mixed to have the final concentrations of 2 and 4 folds of its MBC. The plates were incubated at 37°C in 5% CO_2 anaerobic chamber for 24 h. The surviving bacteria were monitored at 0, 1, 2, 4, 6, 12, and 24 h by collecting the culture at each time point and cultured on the blood agar plates. The number of viable bacterial colonies was counted. CHX 0.12% solution was used as a positive control and the BHI broth was used as a negative control. Killing kinetic profiles were generated between log colonies of the survival bacteria and time.

2.8. *Statistical analysis*

The experiments were conducted in triplicate. The results were expressed as mean ± SD and statistically analyzed *via* SPSS statistic 17.0 software. ANOVA and Turkey's Multiple test have been determined the significant at $p < 0.05$.

3. Results

3.1. *Antimicrobial activity of crude ethanol extracts*

As shown in Table 1, among various ethanol crude extracts of the tested plants, Cs-EtOH showed the highest potential against the tested oral pathogens with the widest inhibition zone against *Smu9*, *Smu4*, and *Si* of 17.5 ± 0.5, 18.5 ± 0.0, and 17.0 ± 0.0 mm, respectively. Its inhibition activity was significantly higher than a positive control, CHX solution, which showed the inhibition zone of only 16 ± 0.0, 15 ± 0.0, and 10.0 ± 0.0 mm, respectively. Followed Cs-EtOH was the extract of flowers of *Syzygium aromaticum* which showed the

Table 2. MIC/MBC of the fractionated and ethanol crude extracts of *C. sappan* against oral pathogenic bacteria

Oral pathogenic bacteria	MIC/MBC (µg/mL)							
	F-Hexane		F-EtOAC		F-EtOH		Cs-EtOHs	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Smu9	> 2,000	> 2,000	125	250	62.5	125	125	250
Smu4	> 2,000	> 2,000	250	500	125	250	250	500
Si	> 2,000	> 2,000	125	250	125	125	125	250

Table 3. Yield and MIC/MBC of the fractions of F-EtOH separated by column chromatography

Fraction	Yield (% w/w)	MIC/MBC (µg/mL)					
		Smu9		*Smu4*		*Si*	
		MIC	MBC	MIC	MBC	MIC	MBC
F1	1.0	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000
F2	1.2	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000
F3	3.0	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000
F4	2.0	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000	> 2,000
F5	1.4	1,000	> 2,000	1,000	> 2,000	500	1,000
F6	8.6	125	500	125	500	125	250
F7	14.6	125	500	125	1,000	125	500
F8	19.0	125	1,000	250	2,000	250	1,000
F9	17.2	250	2,000	250	2,000	250	1,000
F10	3.4	500	2,000	250	2,000	250	2,000
F11	3.6	500	2,000	250	2,000	250	2,000

inhibition zone against *Smu9*, *Smu4*, and *Si* of 10.0 ± 0.0, 10.3 ± 0.3, and 10.3 ± 0.6 mm, respectively. The extract from the aerial part of *Phyllanthus amarus* showed the inhibition zone against *Smu4*, and *Si* wider than that of *S. aromaticum*, but it could not inhibit *Smu9*. The extracts from some common medicinal plants such as *Cassia alata* and *Psidium guajava* showed slight inhibition and against only one strain whereas the extracts from some plants such as *Piper sarmentosum* and *Sesbania grandiflora* showed no activity against the tested oral pathogens.

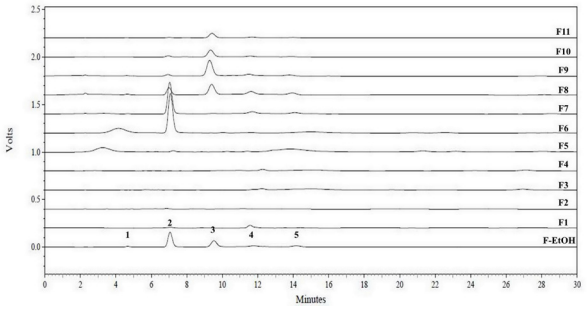

Figure 1. HPLC fingerprints of F-EtOH in comparison with its fractions obtained from the separation by column chromatography and detected at 280 nm.

3.2. *Antimicrobial susceptibility of C. sappan extracts*

Antimicrobial susceptibility against the 3 strains of oral pathogenic bacteria; *Smu9*, *Smu*, and *Si*, was expressed as their MIC and MBC values. The results are showed in Table 2. It was found that among Cs-EtOH and the three fractionated extracts of *C. sappan*, F-Hexane presented MIC and MBC values higher than 2,000 µg/mL for all strains. Meanwhile, F-EtOAc, F-EtOH, and Cs-EtOH revealed lower MIC and MBC values. It was clearly seen that F-EtOH possessed the lowest MIC of 62.5 and 125 µg/mL against *Smu9* and *Smu4*, respectively indicating its highest antibacterial activity. The results on MBC values also confirmed that this fractionated extract of *C. sappan* possessed the highest antibacterial activity against the tested oral pathogenic bacteria as it showed the least MBC values. Therefore, F-EtOH was selected for further studies on killing kinetics patterns against those pathogens.

3.3. *Separation of F-EtOH and fingerprint preparation*

Separation of F-EtOH by column chromatography yielded 11 fractions (F1-F11). After removing solvents, the yield of each fraction was recorded. It was found that the yield of each fraction was different as shown in Table 3. It was found that approximately 25% of the extract was loss during separating process since a summation of the yield of all fractions was only 75%. The yields of F6-F9 were relatively higher than the others. Optimal HPLC conditions previously reported for *C. sappan* extracts (*25*) were used to prepare HPLC fingerprints of the extracts.

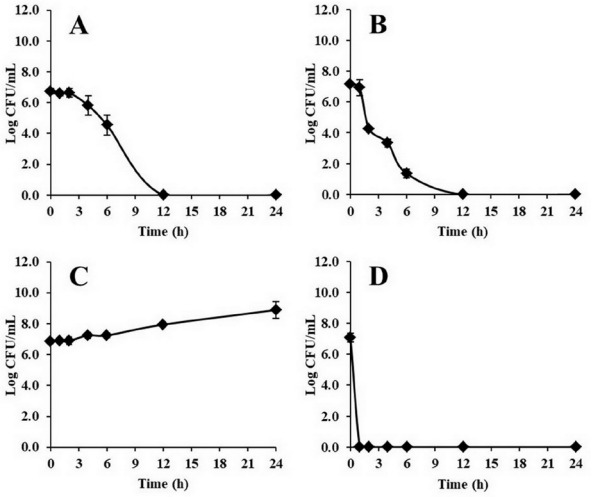

Figure 2. Killing kinetics of F-EtOH at the concentrations of 2-fold MBC (A) and 4-fold MBC (B) in comparison with negative control (C) and positive control (D) against *S. mutans* DMST9567.

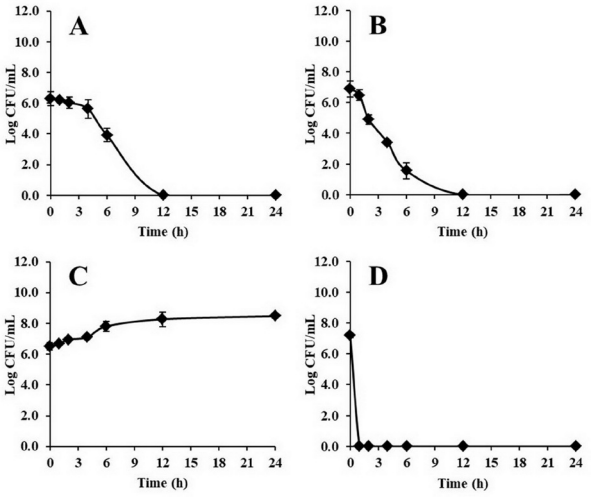

Figure 3. Killing kinetics of F-EtOH at the concentrations of 2-fold MBC (A) and 4-fold MBC (B) in comparison with negative control (C) and positive control (D) against *S. mutans* DMST41283.

The HPLC fingerprints of 11 fractions isolated from F-EtOH in comparison with that of F-EtOH were shown in Figure 1. The results revealed that F-EtOH consisted of at least 5 compounds which exhibited at different retention times. Peak 2 is the largest one followed by peaks 3, 5, 4, and 1, respectively. Comparing the HPLC fingerprints of 11 fractions, F1-F4 did not exhibit any peak at the same retention times as peaks 1-5 of F-EtOH. F6 revealed the highest pure fraction that contained mostly peak 2 and the very less of one more peak at the position prior to peak 2.

3.4. *Antimicrobial susceptibility of F-EtOH fractions*

Serial dilutions of 11 fractions separated from F-EtOH were prepared and determined for MIC and MBC. The results as shown in Table 3 revealed that F1-F4 had no

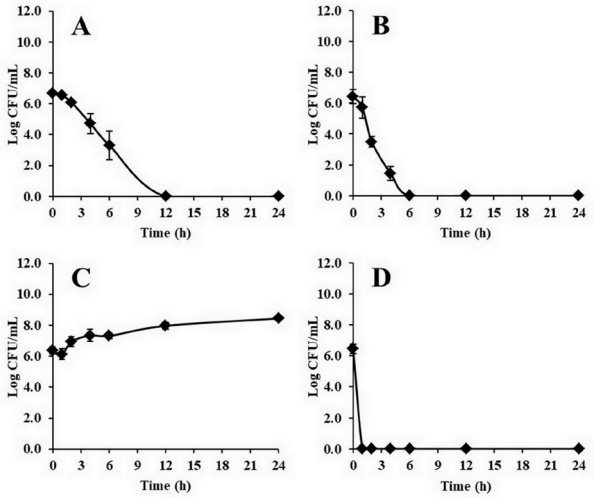

Figure 4. Killing kinetics of F-EtOH at the concentrations of 2-fold MBC (A) and 4-fold MBC (B) in comparison with negative control (C) and positive control (D) against *S. intermedius*.

antibacterial activity against all tested bacterial strains. Interestingly, F5-F11 that one of their HPLC peaks was at the same retention time as peak 2 (of F-EtOH) had antibacterial activity against all tested oral pathogenic strains. It was noted that F8-F11 that HPLC peaks were the same retention time as peaks 3, 4, and 5 were also able to inhibit the bacterial strains. Among 11 fractions, F6 was the most effective fraction, however, its ability was less than F-EtOH. It was considered that the compound at peak 2 was the major active antibacterial agent of F-EtOH. The high antibacterial activity of F-EtOH was considered to be due to the synergism of this active compound and the minor constituents existing in this fractionated extract. F-EtOH was therefore selected for further study.

3.5. *Killing kinetics of F-EtOH*

The killing kinetic profiles of F-EtOH against both strains of *S. mutans*, *Smu9* and *Smu4*, were shown in Figures 2 and 3, respectively. It was found that the killing kinetic profiles of 2-fold MBC and 4-fold MBC against these strains during the first 3-6 h were slightly different. F-EtOH of 4-fold MBC showed higher potential than that of 2-fold MBC. However, the bacteria were completely killed by both concentrations within 12 h. The killing kinetic profiles of F-EtOH against *S. intermedius* were shown in Figure 4. It was found that the bacterial inhibition activity of F-EtOH against this strain was significantly higher than against *S. mutans* and it was clearly dose dependent. *Si* could be completely killed within 12 h by 2-fold MBC whereas it was only 6 h that the pathogen was completely killed by 4-fold MBC.

4. Discussion

Currently, there is a trend to use the active ingredient

from natural sources such as plants. Many plants that used to be the ingredients in the traditional medicinal remedies for oral health care have been researched. The secondary metabolite compounds of plants have been reported as bioactives for several activities including antimicrobial activity against many pathogens and many of them are purposed to use instead of chemicals (*26*). *C. sappan* has been extensively studied and reported on its bioactivities such as antioxidant, anti-inflammatory, and antimicrobial activities. Previous study reported the antimicrobial activities of *C. sappan* extract obtained from water extraction of its wood (*27*). It is found that the main active compounds from its aqueous extraction is brazilin (*28,29*). Unfortunately, this compound is unstable. Therefore, it is less possible to use this pure compound for clinical applications. For some plants, the whole extracts show higher benefits than their pure compounds regarding to the synergistic effects on stability and activity (*15,18,19,30*). However, for aqueous extract, it is difficult to remove water from the aqueous solution of the extract by common rotary evaporator. Ethanol is easier to remove than water. Ethanol has been reported to be safe and effective for extraction of many bioactive compounds, especially phenolic compounds and volatile oils (*31,32*). Therefore, ethanol was chosen as extracting solvent for preparation of the crude extract of *C. sappan* and the other plants in the present study. Although, extensive researches have widely reported on biological activities of *C. sappan* (*21-23,28,29,33,34*), the researches on inhibition of oral pathogens are still limited. The current article provides more scientific data of *C. sappan* extracts on the oral pathogenic bacteria. The results show many interesting issues. It is found that not all of the extracts, which were previously reported to have inhibitory activity against some other bacterial species, could inhibit the oral pathogenic bacteria. Among the crude extracts from 10 medicinal plants, there are only 4 plants that possess the antibacterial activity against all tested strains of oral pathogens. Among them, Cs-EtOH is the most effective extract on inhibition of all tested strains of oral pathogenic bacteria. More interestingly, when the fractionated extracts of *C. sappan* from three different extracting solvents, *n*-hexane, ethyl acetate, and ethanol are compared, it is found that only F-EtOH has the highest potential on inhibition of those tested pathogens. This result indicated that the polarity of the active ingredients of *C. sappan* are relatively high. Our result was in accordance with the previous report which presented that the most active extract of *C. sappan* on antimicrobial activity against *Salmonella ebony*, *Klebsiella pneumonia*, *Escherichia coli*, and *Bacillus subtilis* was found in the polar solvent fraction (*22*). It has also been reported that *C. sappan* contains many active ingredients which mostly are phenolic compounds that can be categorized into several structural groups such as brazilin, chalcone, protosappanin, and homoisoflavonoids (*21*). Brazilin was reported to have strong antimicrobial activity (*35*). Recently, it has been reported that brazilein which is the oxidizing form of brazilin could be easily occurred during extraction or storage, and separation from brazilin is quite difficult (*36*). Therefore, it might be possible that either brazilin or brazilein or both of them possessed high potential for the antibacterial activity against the test oral pathogens in the present study.

In addition, after separation of F-EtOH by column chromatography, F-EtOH demonstrates its activity significantly higher than its purified fractions. This result is considered to be due to the synergistic effect of the major active compounds and the other minor compounds inside the extracts.

Kinetic inhibitory activity against oral pathogenic bacteria of F-EtOH reveals its high effects on killing the tested bacteria. F-EtOH can completely kill the tested oral pathogenic bacteria within 6-12 h. The killing activity is depended on the type of the pathogenic species and the dose of F-EtOH. It is concluded that F-EtOH is the most effective extract of *C. sappan* and it is worth for further study in animal or clinical trial.

Acknowledgements

This study was supported by a grant from the Thailand Research Fund (TRF) through the Research and Researcher for Industry (RRi), Grant No. PHD5710050.

References

1. Wade WG. The oral microbiome in health and disease. Pharmacol Res. 2013; 69:137-143.
2. Listl S, Galloway J, Mossey PA, Marcenes W. Global economic impact of dental diseases. J Dent Res. 2015; 94:1355-1361.
3. Sbordone L, Bortolaia C. Oral microbial biofilms and plaque-related diseases: microbial communities and their role in the shift from oral health to disease. Clin Oral Investig. 2003; 7:181-188.
4. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 2005; 43:5721-5732.
5. Kreth J, Merritt J, Qi F. Bacterial and host interactions of oral streptococci. DNA Cell Biol. 2009; 28:397-403.
6. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res. 1994; 8:263-271.
7. Kolenbrander PE, Andersen RN, Blehert DS, Egland PG, Foster JS, Palmer RJ. Communication among oral bacteria. Microbiol Mol Biol Rev. 2002; 66:486-505.
8. Lauten JD, Boyd L, Hanson MB, Lillie D, Gullion C, Madden TE. A clinical study: Melaleuca, Manuka, Calendula and green tea mouth rinse. Phyther Res. 2005; 19:951-957.
9. Rao NJ, Subash KR, Kumar KS. Role of phytotherapy in gingivitis: A review. Int J Pharmacol. 2012; 1:1-5.
10. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med. 2004;

10:S122-S129.
11. Okonogi S, Holzer W, Unger FM, Viernstein H, Mueller M. Anti-inflammatory effects of compounds from *Polygonum ordoratum*. Nat Prod Commun. 2016; 11:1651-1654.
12. Puttipan R, Okonogi S. Antioxidant activity of *Rafflesia kerrii* flower extract. Drug Discov Ther. 2014; 8:18-24.
13. Rungrojsakul M, Saiai A, Ampasavate C, Anuchapreeda S, Okonogi S. Inhibitory effect of mammea E/BB from *Mammea siamensis* seed extract on Wilms' tumour 1 protein expression in a K562 leukaemic cell line. Nat Prod Res. 2016; 30:443-447.
14. Okonogi S, Prakatthagomol W, Ampasavate C, Klayraung S. Killing kinetics and bactericidal mechanism of action of *Alpinia galanga* on food borne bacteria. African J Microbiol Res. 2011; 5:2847-2854.
15. Anantaworasakul P, Hamamoto H, Sekimizu K, Okonogi S. *In vitro* antibacterial activity and *in vivo* therapeutic effect of *Sesbania grandiflora* in bacterial infected silkworms. Pharm Biol. 2017; 55:1256-1262.
16. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evid Based Complement Alternat Med. 2011; 2011:680354.
17. Chelli-Chentouf N, Tir Touil Meddah A, Mullie C, Aoues A, Meddah B. *In vitro* and *in vivo* antimicrobial activity of Algerian Hoggar *Salvadora persica* L. extracts against microbial strains from children's oral cavity. J Ethnopharmacol. 2012; 144:57-66.
18. Issam AA, Zimmermann S, Reichling J, Wink M. Pharmacological synergism of bee venom and melittin with antibiotics and plant secondary metabolites against multi-drug resistant microbial pathogens. Phytomedicine. 2015; 22:245-255.
19. Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. 2000; 31:247-256.
20. Sasaki Y, Hosokawa T, Nagai M, Nagumo S. *In vitro* study for inhibition of NO production about constituents of *Sappan Lignum*. Biol Pharm Bull. 2007; 30:193-196.
21. Min BS, Cuong TD, Hung TM, Min BK, Shin BS, Woo MH. Compounds from the heartwood of *Caesalpinia sappan* and their anti-inflammatory activity. Bioorg Med Chem Lett. 2012; 22:7436-7439.
22. Bukke AN, Hadi FN, Produtur CS. Comparative study of *in vitro* antibacterial activity of leaves, bark, heart wood and seed extracts of *Caesalpinia sappan* L. Asian Pacific J Trop Dis. 2015; 5:903-907.
23. Srinivasan R, Selvam GG, Karthik S, Mathivanan K, Baskaran R, Karthikeyan M, Gopi M. *In vitro* antimicrobial activity of *Caesalpinia sappan* L. Asian Pac J Trop Biomed. 2012; 2:S136-S139.
24. Tachakittirungrod S, Okonogi S, Chowwanapoonpohn S. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. Food Chem. 2007; 103:381-388.
25. Yan-yan J, Yan L, Ying S, Jinyi Z, Fang D, Yuan S, Ai-dong W. A simple high-performance liquid chromatographic method for the determination of brazilin and its application to a pharmacokinetic study in rats. J Ethnopharmacol. 2014; 151:108-113.
26. Teanpaisan R, Kawsud P, Pahumunto N, Puripattanavong J. Screening for antibacterial and antibiofilm activity in Thai medicinal plant extracts against oral microorganisms. J Tradit Complement Med. 2017; 7:172-177.
27. Yim NH, Jung YP, Cho WK, Kim T, Kim A, Im M, Ma JY. Screening of aqueous extracts of medicinal herbs for antimicrobial activity against oral bacteria. Integr Med Res. 2013; 2:18-24.
28. Mueller M, Weinmann D, Toegel S, Holzer W, Unger FM, Viernstein H. Compounds from *Caesalpinia sappan* with anti-inflammatory properties in macrophages and chondrocytes. Food Funct. 2016; 7:1671-1679.
29. Wang YZ, Sun SQ, Zhou YB. Extract of the dried heartwood of *Caesalpinia sappan* L. attenuates collagen-induced arthritis. J Ethnopharmacol. 2011; 136:271-278.
30. Naksuriya O, Okonogi S. Comparison and combination effects on antioxidant power of curcumin with gallic acid, ascorbic acid, and xanthone. Drug Discov Ther. 2015; 9:136-141.
31. Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T. A comparison of the antibacterial efficacies of essential oils against oral pathogens. Mol Oral Microbiol. 2004; 19:61-64.
32. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005; 26:343-356.
33. Nirmal NP, Rajput MS, Prasad RGSV, Ahmad M. Brazilin from *Caesalpinia sappan* heartwood and its pharmacological activities: A review. Asian Pac J Trop Med. 2015; 8:421-430.
34. Shen J, Zhang H, Lin H, Su H, Xing D, Du L. Brazilein protects the brain against focal cerebral ischemia reperfusion injury correlating to inflammatory response suppression. Eur J Pharmacol. 2007; 558:88-95.
35. Xu HX, Lee SF. The antibacterial principle of *Caesalpina sappan*. Phyther Res. 2004; 18:647-651.
36. Kim DS, Baek NI, Oh SR, Jung KY, Lee IS, Lee HK. NMR assignment of brazilein. Phytochemistry. 1997; 46:177-178.

(Received October 23, 2017; Revised November 20, 2017; Accepted December 3, 2017)