Anti-Periodontal Pathogen Porphyromonas Gingivalis and Antioxidant Activities of Thai Medicinal Plant Extracts

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Abstract

Eight plant extracts including rhizomes of *Acorus calamus* (mytle grass), *Alpinia officinarum* (lesser galangal) and *Zingiber officinale* (ginger), fruits of *Carissa carandas* (caranda plum), *Garcinia schomburgkiana* (garcinia) and *Spondias pinnata* (hog plum), fruit peels of *Citrus hystrix* (kaffir lime) and flowers of *Hibiscus sabdariffa* (red sorrel) were determined for their anti-oral pathogen *Porphyromonas gingivalis* and antioxidant activities. Anti-*P. gingivalis* activity was tested by disk diffusion assay and minimum inhibitory concentration (MIC) determination. Garcinia and ginger extracts displayed

The strongest anti-*P. gingivalis* activity (0.39 mg/mL MIC), while the extracts of mytle grass, lesser galangal and hog plum exhibited relatively strong anti-*P. gingivalis* activity (0.78 mg/mL MIC). In addition, the antioxidant activity of these plant extracts was evaluated by ferric reducing antioxidant power (FRAP) assay.

The extracts of hog plum and ginger had strong antioxidant activity with the reducing capacity of 3.60 and 3.49 mM Fe (II) /g extract, respectively, whereas other extracts had lower reducing capacity (0.16-1.06 mM Fe (II) /g extract).

Keywords: Periodontitis, Garcinia schomburgkiana, Zingiber officinale, Spondias pinnata

Introduction

Periodontitis is a prevalent biofilm-mediated inflammatory disease. It destroys gingival tissue and can cause tooth loss. This disease is initiated when the microorganisms embed in subgingival dental plaque. One of the widely accepted as a periodontal pathogen is Porphyromonas gingivalis, an anaerobic Gram-negative bacterium (Sakanaka et al. 2016). There were evidence showing a relationship between destruction of tooth supporting tissue,

inflammatory response and P. gingivalis (Cekici et al. 2014). Normally, antibiotics have been used as therapeutic strategy. However, some antibiotics result in side effects such as liver injury (Björnsson et al. 2010), tooth staining (Claydon et al. 2006) and antibiotic resistance development of bacteria in subgingival sites (Bidault et al. 2007). This represents a threat to public health which affects high health care costs as increased duration of illness, treatment and hospitalisation (Barbieri et al. 2016). Thus, these require the need to search for safer drugs. Medicinal plants as an alternative source of antibacterial agents have recently attracted great interest. A wide variety of plants have been reported to possess useful biological activities (Barbieri et al. 2017). Many researches have been focused on antibacterial activity of plants against oral pathogen such as P. gingivalis (Kraivaphan et al. 2013; Sundaram et al. 2020). Moreover, some medicinal plants are rich in antioxidant compounds including vitamins, minerals and polyphenols (Fierascu et al. 2018). These plants include fruits of garcinia (Garcinia schomburgkiana), hog plum (Spondias pinnata) and caranda plum (Carissa carandas), rhizomes of ginger (Zingiber officinale) and lesser galangal (Alpinia officinarum), flower of red sorrel (*Alpinia officinarum*) and etc. So far, only few plants with both strong anti-*P. gingivalis* and antioxidant activities have been reported. Thus, the aim of this study was to search for new plants with these potential activites.

Table 1. Medicinal plants used in this study

Scientific name	Common name/Thai name	Family	Plant part
Acorus calamus Linn	Mytle grass/ Wan num	Acoraceae	Rhizomes
Alpinia officinarum Hance	Lesser galangal/ Karlek	Zingiberaceae	Rhizomes
Carissa carandas Linn	Caranda plum/ Ma-now-ho	Apocynaceae	Fruits
Citrus hystrix DC.	Kaffir lime/ Makrud	Rutaceae	Fruit peels
Garcinia schomburgkiana Pierre	Garcinia/ Madan	Crusiaceae	Fruits
Hibiscus sabdariffa Linn	Red sorrel/ Krajeabdang	Malvaceae	Flowers
Spondias pinnata (L.f.) Kurz	Hog plum/ Makoknum	Elaeocarpaceae	Fruits
Zingiber officinale Roscoe.	Ginger/ King	Zingiberaceae	Rhizomes

Materials and Methods

Extraction of Plant Materials

Eight species of Thai medicinal plants were used in this study (Table 1). The plant materials were cut, dried and ground to a powder. Fifthteen gram of each was then soaked in 85% ethanol (150 mL) and shaken at 150 rpm, 30 °C for 48 hours. After filtering the mixture, the filtrate was evaporated by vacuum rotary evaporator and air dried. The crude ethanolic extracts were diluted with 10% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 200 mg/mL stock solution.

Microorganism and Culture Preparation

Porphyromonas gingivalis JCM 12257 was cultivated in BHI broth supplemented with 5 mg/L hemin (Sigma-Aldrich, Switzerland) and 1 mg/L menadione (Sigma-Aldrich, Switzerland), and incubated in anaerobic condition at 37°C for 48 h. The turbidity was adjusted to match that of 7 McFarland standard (1.0×10^{8} CFU/mL).

Antimicrobial Susceptibility Testing

Animicrobial activity of all plant extracts was tested against *P. gingivalis* JCM 12257 by disk diffusion assay and minimum inhibitory concentration determination.

Disk diffusion assay

The disk diffusion test against *P. gingivalis* was performed using the procedure as described by Kraivaphan *et al.* (2013). Briefly, cell suspension (100 µL) of *P. gingivalis* was swabbed onto the surface of BHI agar supplemented with 5 mg/L hemin, 1 mg/L menadione and 5% blood. This agar medium surface was aseptically placed with sterile 6-mm filter paper discs (Grade AA DISC, GE Healthcare, UK). Then, 15 µL of 200 mg/mL plant extract was added onto each paper disc. The plates were incubated at 37°C for 10 d under anaerobic condition. Antimicrobial activity was evaluated by measuring diameters of inhibition zones. The 10% DMSO solution was used as a negative control, while ampicillin (1 mg/mL) was used as a positive control. The experiment was done in triplicate.

Determination of Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs)

The MICs of all plant extracts against *P. gingivalis* were evaluated by agar dilution method (Kraivaphan *et al.* 2013). Each plant extract at the final concentrations of 0.02 – 1.56 mg/mL in BHI agar supplemented with 5 mg/L hemin, 1 mg/L menadione and 5% blood was

prepared in a sterile test tube, and examined for the MIC. Then, a loopful of cell suspension was streaked onto the surface of this BHI slant. After incubation at 37°C for 10 d, the growth of *P. gingivalis* at different concentrations of plant extracts was recorded. The lowest concentration of the plant extract that completely inhibited visible growth of *P. gingivalis* was recorded as the MIC. Then, MBCs determination was performed by continuing from BHI agar tube of the MIC test with no visible growth by culture transferring onto a new BHI agar surface. After incubation, the growth of *P. gingivalis* at different concentrations of plant extracts was recorded. The lowest concentration of the plant extract with no visible growth was recorded as the MBC. The MBC is the lowest concentration of plant extracts which was able to inactivate or kill the microorganisms. The 10% DMSO solution was used as a negative control, whereas ampicillin (0.05-5 mg/mL) was used as a positive control.

Determination of Antioxidant Activity

Antioxidant activity of each plant extract was determined by ferric reducing antioxidant power (FRAP) assay according to the method as described by Lado *et al*. (2004). Briefly, 100 µL of each plant extract (1 mg/mL in 30% ethanol) were mixed with 3 mL FRAP reagent, and then left in a water bath at 37°C for 5 min. Working FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer with 2.5 mL of 10 mM TPTZ (2,4,6-tri-2-pyridyl-2-triazine, Fluka, Sigma-Aldrich, Switzerland) in 40 mM HCl solution and 2.5 mL of 20 mM $FeCl_3.6H_2O$. Then, absorbance reading was taken against blank (FRAP reagent) at 594 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). The p-tocopherol (Fluka, Switzerland) was used as a positive control. The absorbance obtained was compared with the standard curve of known Fe(II) concentration (0.047-6.0 mM $FeSO_4.7H_2O$). The reducing capacity of the plant extracts was expressed as Fe^{2+}-TPTZ concentration in the sample (mmol Fe(II)/g extract).

Results and Discussion

Anti- *Porphyromonas gingivalis* activity of Medicinal Plant Extracts

All plant extracts tested could inhibit the growth of *P. gingivalis* with inhibition zone diameter of 8.33-11.33 mm (Table 2). Garcinia and ginger extracts displayed the strongest anti-*P. gingivalis* action with the MIC of 0.39 mg/mL, followed by the extracts of mytle grass, lesser galangal and hog plum (0.78 mg/mL MIC). However, the extracts of caranda plum, kaffir lime

and red sorrel had lesser anti- *P. gingivalis* with 1.56 mg/mL MIC (Table 2). For MBC determination, the MBCs of all extracts against *P. gingivalis* were the same as their MIC values.

Table 2. Anti-*Porphyromonas gingivalis* activity of medicinal plant extracts

Plant extracts (common name)	Diameter of Inhibition zone (mm)[a] ± SD	M inimumInhibitory Concentration (mg/ml)
Mytle grass	9.33 ± 0.28	0.78
Lesser galangal	10.83 ± 0.28	0.78
Caranda plum	8.33 ± 0.28	1.56
Kaffir lime	10.17 ± 0.58	1.56
Garcinia	11.33 ± 0.58	0.39
Red sorrel	9.17 ± 0.28	1.56
Hog plum	10.67 ± 0.28	0.78
Ginger	11.17 ± 0.28	0.39
Ampicillin	28.38 ± 0.58	0.05

[a] Data are mean of three replications.

Note :For disk diffusion test, all plant extracts were tested at200 mg/mL, while ampicillin was tested at 1 mg/mL.

The anti- P. gingivalis of garcinia may be due to the action of its bioflavonoids. Xu et al. (2013) reported that bioflavonoid GB-1 extracted from the plant in the same genus called Garcinnia kola could inhibit the growth of P. gingivalis W83 with the MIC of 64 µg/mL. In addition, antibacterial activity of garcinia may be due to high acid content in garcinia fruit. Suntornsuk et al. (2002) revealed that vitamin C was found in garcinia fruit at 4.6 mg/100 g fresh fruit.

In the current study, the extract of ginger could inhibit the growth of *P. gingivalis* at 0.39 mg/mL MIC. This was in agreement with those reported by EL-Sherbiny (2015). Pathogenic bacterial strains isolated from 33 patients with root canal infection were used as tested microorganisms to evaluate the anti-*P. gingivalis* activity of ginger. They reported that ginger extract had antibacterial action against *P. gingivalis* at 0.6 mg/mL MIC. This may be due to the action of active compounds in ginger. Park *et al.* (2008) reported that ginger extracts from Korea had antibacterial activity against *P. gingivalis*, *Porphyromonas endodontalis* and

Porphyromonas intermedia at 50 μg/mL. The active compounds in ginger were identified by HPLC technique as [10]-gingerol, [12]-gingerol, 5-acetoxy-[6]-gingerol, 3,5-diacetoxy-[6]-gingerdiol and galanonolactone. The compounds with antibacterial activity against *P. gingivalis* were [10]-gingerol and [12]-gingerol. This is in agreement with those reported by Jolad *et. al.* (2005). In addition, red sorrel extract had anti-*P. gingivalis* activity. The results are in agreement with those reported by Sulistyani *et al. (*2016). They reported that red sorrel extract had antibacterial activity against oral pathogenic bacteria such as *Streptococcus mutans*, *Fusobacterium nucleatum* and *P. gingivalis.* This was probably due to the action of organic acids in red sorrel especially protocatechuic acid.

Antioxidant activity of Medicinal Plant Extracts

Antioxidant activity of plant extracts was evaluated by FRAP method. This was performed to determine the capacity of sample to retard oxidation by redox reaction and color change of ferric tripyridyltriazine (Fe^{3+}- TRTZ) complex. When ferric tripyridyltriazine (Fe^{3+}- TRTZ) complex gains electron, it will change to ferrous tripyridyltriazine (Fe^{2+}-TRTZ) compound which is blue violet in color. The more dark in color indicates the more strong reducing capacity (strong antioxidant activity) of the plant extract. Among all extracts, hog plum extract had the strongest antioxidant activity (3.60 mM Fe (II) /g extract) which means that 1 g extract could change ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) complex of 3.60 mM, but it was lower than that of α-tocopherol (5.5 mM Fe (II) /g). Ginger extract had relatively strong antioxidant activity (3.49 mM Fe (II) /g extract). However, other plant extracts had moderately antioxidant activity (0.16 - 1.06 mM Fe (II) /g extract) (Table 3).

Hog plum extract had the strongest antioxidant activity. This is in agreement with those reported by Saikia *et al.* (2016). They evaluated antioxidant activity of hog plum (*Spondias pinnata* L. Kutz) extracted by 80% acetone and found that it contained total phenolics of 1,654.5 mg gallic acid /100 g extract, total flavonoids of 65.63 mg quercetin /100 g extract and reducing capacity of 4,836.81 μmol/ 100 g extract. Moreover, Langyanai *et al.* (2017) also reported strong antioxidant activity of hog plum extract.

In summary, the results of the current study clearly confirmed that ginger, garcinia, hog plum, mytle grass and lesser galangal extracts have therapeutic potential against periodontal pathogen *P. gingivalis*. Hog plum and ginger extracts can potentially be used to develop

formulations in dental care products as anti-*P. gingivalis* and antioxidant agents for prevention and treatment of periodontitis.

Table 3. Antioxidant activity of medicinal plant extracts

Plant extracts (common name)	Antioxidant activity by FRAP assay (mmol fe (II) /g extract)[a] ± SD
Mytle grass	1.06 ± 0.01
Lesser galangal	0.16 ± 0.00
Caranda plum	0.37 ± 0.05
Kaffir lime	0.81 ± 0.05
Garcinia	0.96 ± 0.02
Red sorrel	1.05 ± 0.03
Hog plum	3.60 ± 0.09
Ginger	3.49 ± 0.03
α-tocopherol	5.5 ± 0.07

[a] Data are mean of three replications.

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