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Full Length Research Paper

Antimicrobial activity against periodontopathic bacteria and cytotoxic study of *Cratoxylum formosum* and *Clausena lansium*

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***Cratoxylum formosum* ssp. *pruniflorum* and *Clausena lansium* are among edible plants that commonly used for various ethnomedical conditions. This study investigated antimicrobial activity against periodontopathic bacteria using agar diffusion technique. These plant extracts demonstrated comparable antimicrobial efficacy against black-pigmented bacteria strains to standard drug chlorhexidine. The CD_{50} of *C. formosum* and *C. lansium* on HGF cells were 0.37 and 0.16 mg/ml, respectively, demonstrated using the MTT assay.**

Key words: Antimicrobial activity, cytotoxic activity, *Cratoxylum formosum ssp. pruniflorum*, *Clausena lansium*.

INTRODUCTION

As emerging decade of antibiotic resistance, plants, especially higher plants which produce hundreds to thousands of diverse chemical compounds with different biological activities, have been evaluated for possibility to use as alternative source in manufacturing new antimicrobial substances. To date phytochemical studies have been shown that chemical constituents in plants exhibited antimicrobial activity against various bacterial species both Gram-positive and Gram-negative (Iauk et al., 2003; Sasikumar et al., 2005).

Cratoxylum formosum ssp. pruniflorum (Pink mempat) known in Thai as “Tiew” is a shrub or small tree which can be up to 20 m tall. It belongs to the family *Clusiaceae*. This plant distributes in several Southeast Asian countries with at least six species of this genus found in Thailand (Smitinand, 2001). It has been traditionally known for its tonic, stomachic and diuretics effects (Grosvenor et al., 1995a). Its therapeutic use was also on treatment of diarrhea and flatulence (Aderson, 1986) and for food poisoning and internal bleeding (Grosvenor et al., 1995b). Main bioactive compounds from this plant were xanthones (Duan et al., 2010), triterpenoids (Bennett et al., 1993) and anthroquinones (Boonnak et al., 2006). *Clausena lansium* (Lour.) Skeels known in Thai as “Mafai jean” is native plant to Southeast Asia region. It is a slow growing, strongly scented evergreen plant belonging to Rutaceae family which reached 3 to 8 m tall. Its parts have evidently been used for various ethnomedical conditions including bronchitis, malaria, viral hepatitis and gastro-intestinal inflammation (Adebajo et al., 2009). Isolated compound from various parts of the plant which exhibited antimicrobial activity included triterpene (Lakshmi et al, 1989) and amide (Ming-He et al., 1989; Jagessar and Rampersaud, 2008).

Plants consist of triterpene compounds have been reported to effectively inhibit growth of periodontopathic bacteria (Rodanant et al., 2010). This might be beneficial in the treatment of periodontal disease since the eradication of bacteria might cut off the overt inflammatory reaction which mediated destruction of the periodontium (Kornman et al., 1997). In order to limit the disease progression, not only the removal of the etiologic factors (eg. bacterial infection) but also the maintenance and remodeling processes have to be initiated. The periodontal tissue repair is developed by the mechanisms

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Table 1. Zone of growth inhibition (mm) showing antimicrobial activity of plant extracts against periodontal pathogens.

Plant extracted	% yield	Diameter of inhibition zone (mm.)				
		Pi	***Aa***	***Pg 381***	***Pg 33277***	***Fn***
C. formosum ssp. *pruniflorum* (Stem bark)	0.58	12.8±3.42	8.8±2.25	16±1.63	16.3±4.64	9.3±0.47
C. lansium (trunk)	1.91	18.2±7.53	0	14.7±2.27	15±5.72	8.8±0.24
Chlorhexidine 2%		18.5±1.54	30.7±0.61	17.5±0.5	19.3±2.52	20±0
DMSO		0	0	0	0	0

of fibroblastic cells. These cells produce collagen, the major structure component of the gingiva and periodontal ligament, which is necessary in the process to regain the normal architecture and probably function of the periodontiium (Ten Cate, 1994). Therefore, it is important to know the cytotoxicity of these plants on human gingival fibroblast, cell type that play a pivotal role in the integrity of the periodontium, if this plant extracts are planned to implement in the treatment of periodontal disease.

The study objective was to investigate the properties of *C. formosum* ssp. *pruniflorum* and *C. lansium* on their antimicrobial effect against periodontopathic bacteria: *Porphyromonas gingivalis (Pg)*; *Prevotella intermedia (Pi)*; *Aggregatibactor actinomycetemcomitans (Aa)* and *Fusobacterium nucleatum (Fn)*, and their cytotoxic effect on human gingival fibroblast (HGF) cells *in vitro*.

MATERIALS AND METHODS

Plant materials

The stem barks of *C. formosum* ssp. *pruniflorum* were collected from Chiangmuan District, Phayao Province and trunks of *C. lansium* were collected from Muang District, Nan Province. Voucher specimen of *C. formosum* ssp. *pruniflorum* (0012677) was deposited at Prince of Songkla University and voucher specimen of *C. lansium* (QBG 23077) was deposited at Queen Sirikit Botanic Garden, Mae Rim District, Chiang Mai Province.

Extraction process

The air-dried powdered plant materials were extracted with the following organic solvents: MeOH and partition with CH_2Cl_2 for the stem barks of *C. formosum* ssp. *pruniflorum*; *n*-hexane for the trunks of *C. lansium* using maceration method. The solutions were filtered and the solvents were removed under vacuum at ca. 40°C using a rotary evaporator to give crude extracts. The percentage yields of the extracts obtained from the above plant materials are presented in Table 1. Each of the plant extracts was dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, USA) to obtain the solutions of 100 mg/ml (w/v) concentration. The stock solutions were kept in a refrigerator at the temperature of 4 to 8°C.

Microorganisms

The microorganisms used in this study were *P. gingivalis* ATCC 33277 (*Pg* ATCC 33277), *P. gingivalis* FDC 381(*Pg* FDC 381), *P. intermedia* ATCC 25611 (*Pi* ATCC 25611), *A. actinomycetemcomitans* ATCC 43718 (Y4) (*Aa* ATCC 43718, Y4) and *F. nucleatum* ATCC 49256 (*Fn* ATCC 49256).

Antibacterial activity

The growth inhibition tests were performed using agar diffusion technique. One hundred microlitre (µl) of each BHI-suspended microorganism was distributed on the agar medium (25 ml/plate) using small-size glass beads. Once the agar surface was dried, paper discs 6 mm diameter (Whatman Internaltional, UK), soaked with 10 µl of the plant extracts solution on each side of the disc, was placed on the agar surface. Chlorhexidine 2% and DMSO were used as positive and negative control, respectively. Each plate cultured with *Pg*, *Pi* and *Fn* was incubated in anaerobic chamber at 37°C for 5 to 7 days, whereas plates cultured with *Aa* were incubated at 37ºC in 5% CO_2 atmosphere for 3 to 4 days. All tests were performed three times and the antibacterial activity was expressed as the mean diameters (mm) of inhibition zone produced by the plant extracts.

Cytotoxicity assay

The stock samples were diluted with Dulbecco's modified eagle's medium (DMEM) to desired concentrations ranging from 5 to 2000 µg/ml. The final concentration of DMSO in each sample did not exceed 1% v / v (Prayong et al., 2008). The cytotoxic activity of the extracts were tested in Human gingival fibroblast (HGF) cell line (ATCC® No. CRL-2014) by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Mosmann, 1983) with minor modification.

Briefly, the cell were seeded in 96 well-plates (200 µl/well at a density of 1×10^5 cells/ml) incubated at 37°C in 5% CO_2 atmosphere for 24 h. At 24 h, cells were washed with sterile phosphate buffer (PBS) 100 µl and treated with 100 µl of plant extract solution at various concentration (five wells were included for each treatment) then incubated at 37°C in 5% CO_2 atmosphere for another 24 h. Washed 96-well plate with sterile PBS 1X and discarded the supernatant concentrations of the sample solutions the supernatant was discarded. 100 µl of MTT solution (0.5 mg/ml) was added to each well and incubated at 37°C for another 2 h. Then the medium was aspirated. In each well, the formed formazan crystals were solubilized with 100 µl of DMSO, leave for 30 min at room temperature. An absorbance of formazan was reported as optical density which was read at 540 nm by ELISA reader (Model series UV 900 Hdi, USA). Three separate experiments were carried out.

RESULTS

The yields of the plant extracts and the antimicrobial

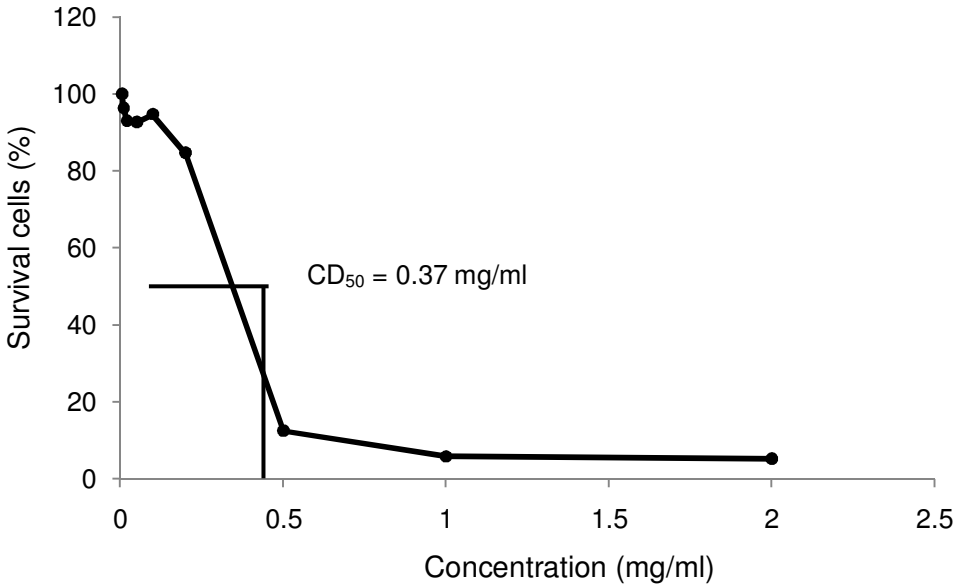

Figure 1. Percentage of HGF survival cells (mean ± standard deviation) after direct exposure to various concentrations of *C. formosum* ssp. *pruniflorum* for 24 h.

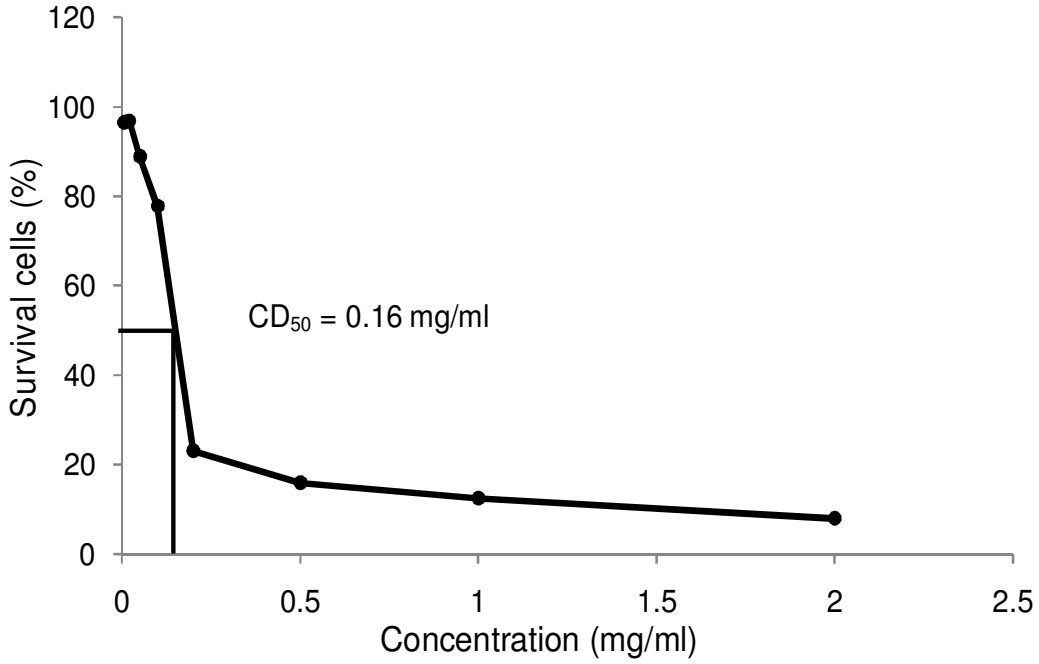

Figure 2. Percentage of HGF survival cells (mean ± standard deviation) after direct exposure to various concentrations of *C. lansium* for 24 h.

activity of *C. formosum* ssp. *pruniflorum* and *C. lansium* extracts against periodontal pathogens (*Pi*, Aa, *Pg* FDC 381, *Pg* ATCC 33277 and *Fn*) was quantitatively assessed by the presence of inhibition zone diameters (Table 1).

The exposure of HGF cells culture to different concentrations of *C. formosum* ssp. *pruniflorum* and *C. lansium* extracts showed that cell survival decreased with an increase in concentration of the plant extracts (Figures 1 and 2).

DISCUSSION

According to the popularities in natural therapies, it is an urgent requirement to evaluate the quantitative and qualitative data in order to confirm the effectiveness and safety of plant extracts even though medicinal plants have traditionally been used to treat some specific medical conditions for long time. To this time, many plant extracts have evidently demonstrated antimicrobial properties (Iauk et al., 2003; Sasikumar et al., 2005; Rodanant et al., 2009).

This study revealed antimicrobial effectiveness of tested plants through the agar diffusion technic. *C. formosum* ssp. *pruniflorum* and *C. lansium* showed inhibiting effects on all strains of tested bacteria (except that *C. lansium* did not inhibit growth of Aa) but were different in the specificity of the spectrum of inhibition. It was noted that both plant extracts exhibited antimicrobial activity against *Pg 381, Pg 33277* and *Pi* comparable to that of the standard drug, chlorhexidine 2% (Table 1). For both plant extracts, the diameter of inhibition zones against *Aa* and *Fn* were smaller than those against *Pg* and *Pi.* Furthermore, the antimicrobial effectiveness of both plant extracts is far less effective than those of Chlorhexidine 2%. According to these result, it could postulate that *C. formosum* ssp. *pruniflorum* and *C. lansium are* more suitable to inhibit growth of black pigmented anaerobic bacteria (*Pg* and Pi) than the other two bacteria (*Aa* and *Fn*). The other postulation could be that chemical constituents within these two plants might be quite similar (Bennett et al., 1993; Lakshmi et al., 1989).

Since there are some disadvantages of the agar diffusion technique (Smith-Palmer et al., 1998; Rios et al., 1988), the result might not demonstrate the full clinical potential of these plants. However, there might be some chemical constituents within these plants which exert microbial inhibitory effect since this study showed their activity against putative periodontopathic bacteria.

Even though these plants demonstrate less antimicrobial effect comparing to chlorhexidine 2%, they might have a significant advantage concerning adverse effects (Flotra et al., 1971). Because of the use of these plants to treat oral disease and its edible use, it might be assure that there is no harmful adverse reaction (Morton, 1987; Suddhasthira et al., 2006) and could be good alternative antimicrobial substances.

The cytotoxicity assay used in this study is the colorimetric MTT assay. The viable cultured cells is calculated by the measuring the amount of formazan product. The advantage of this approach is the capability of the assay to detect very small number of cultured cells with high degree of precision utilizing ELISA reader (Mosmann, 1983). This study demonstrated that viable cell number was decreased when the concentration of the plant extracts were increased which indicated the dose dependent cytotoxic effect of these plant extracts on HGF cells.

The result of this study revealed that the concentration of the plant extracts that showed inhibitory effect is more than the concentration that not toxic to HGF cells. Further study conducts to purify compounds and reveals chemical structure of substance that have antimicrobial potential within plants and are not toxic to the human cells might be useful in the development of new products for human use in treating periodontal disease.

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