Potential Activities of Roselle Calyx Extract (*Hibiscus sabdariffa* L.) against Oral Bacteria *In Vitro*

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北海道医療大学大学院歯学研究科

Herastuti Sulistyani

Introduction

Oral bacteria are major etiologic agents of oral infectious diseases. Bacterial species can form oral biofilms by initial attachment to the tooth surface, followed by coaggregation and coadhesion. Previous studies have demonstrated associations between the presence of oral biofilms and oral infectious diseases, such as dental caries and periodontitis (Samaranayake, 2002; Song et al., 2007). Chlorhexidine (CHX) is one of the standard antibiofilm agents in the field of dentistry. However, the use of CHX has adverse effects, including staining of teeth, detrimental effects on oral cells, and development of hypersensitivity reactions (Chang et al., 2001; Song et al., 2007; Pemberton & Gibson, 2012). Therefore, development of novel agents for inhibiting the growth and ability of biofilm formation of bacteria is required as one of the strategies for the prevention of oral infectious diseases.

The use of plant extracts as alternative medical treatments has become popular in recent years. The term “plant products” usually refers to secondary metabolites produced by plants. These subtances serve as the defense mechanism for the plant against predation by microorganisms, insects, and herbivores (Cowan, 1999). *Hibiscus sabdariffa* L. (family Malvaceae), commonly known as roselle or red sorrel in English, is widely grown in Central and West Africa, Southeast Asia, and in other regions. Roselle is an annual, erect, bushy, 2-4 m tall herbaceous subshrub. The thick, red and fleshy, cup-shaped part of the flower is known as calyx; the calyx has been used worldwide in cold and hot beverages, puddings, and jellies (Morton, 1987).

The roselle calyx is rich in secondary metabolites, which have medicinal properties. Previous studies have shown that the calyx contain flavonoids, such as gossypetine, hibiscetin, and sabdaretin and has been used in folk medicine (Hirunpanich et al., 2005).

The extract has antihypertensive, hepatoprotective, antihyperlipidemic, antioxidant, anticancer, anti-inflamatory, and antimicrobial properties. Although some studies reported the effects of roselle as an herbal medicine, to date, only a few studies examined the effects of roselle calyx extract (RCE) as an antibacterial agent, particularly in the field of dentistry. Thus, the purpose of this study was to investigate the potential activities of RCE against oral bacteria, in particular, the antibacterial activity against, cytotoxicity and effect on biofilm formation, gingipain activity, cytokines production, and morphology of oral bacteria.

Materials and Methods

Roselle calyx powder was soaked in ethyl alcohol for 24 h at room temperature. After centrifugation, the extract was lyophilized and dissolved in phosphate-buffered saline (PBS). The pH was adjusted, and the extract was aseptically filtered. The bacteria used in this study were *Streptococcus mutans*, *Streptococcus sanguinis*, *Lactobacillus casei*, *Actinomyces naeslundii*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Prevotela intermedia*. The antibacterial activity of the RCE was determined by treating the bacterial cells with the extract for 10–20 min at room temperature. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the micro-dilution method, and the effect of the RCE on biofilm formation was determined using a polystyrene micro plate assay. In addition, we used the WST-1 assay to determine the cytotoxicity of the RCE on HGF, Ca 9-22, and KB cells. The effect of RCE on Kgp and Rgp was evaluated using a synthetic substrate colorimetric assay. Additionally, its effect on cytokine production was quantified using ELISA kit and the morphological alterations

in the cells of *Streptococcus mutans* and *Porphyromonas gingivalis* were studied using scanning electron microscopy (SEM).

Results

The number of viable bacteria in the RCE-treated group was significantly lower than that in the control group were. In particular, significant antibacterial activity was observed against *F. nucleatum*, *P. intermedia*, and *P. gingivalis*. The MIC ranged from 7.2 to 28.8 mg/mL, while the MBC ranged from 14.4 to > 57.6 mg/mL. RCE at the sub-MIC level inhibited biofilm formation by all eight bacterial species in a dose-dependent manner. RCE showed less cytotoxicity to human oral cells and inhibited Kgp and Rgp activities. In addition, it inhibited the production of IL-6 and IL-8 from KB cells induced by *P. gingivalis* in a dose-dependent manner. After treatment with RCE, some part of *S. mutans* showed irregular shape such as enlargement and clumping of cells. In *P. gingivalis*, treatment with RCE caused aggregation and distortion of cellular morphology.

Discussion

We found that RCE had bactericidal activity against both cariogenic and periodontopathic bacteria. RCE showed the strongest inhibitory activity against *F. nucleatum*, *P. intermedia*, and *P. gingivalis*, which indicated that RCE was more effective against gram-negative bacteria than gram-positive bacteria. The antibacterial activity observed in our study may be because of the main compound in RCE, such as flavonoids, which have the ability to bind to bacterial cell walls, disrupts the membrane integrity, resulting in death (Cowan, 1999). In addition, our results showed that RCE at sub-MIC levels could inhibit the formation of biofilm by eight bacteria in a dose-dependent manner. The inhibitory effects of the extract on biofilm formation may be

depend on the phenolic compounds present in the extract, because these compounds bind strongly to proteins and the enzymes, thus bacteria are unable to attach to the tooth surface (Song et al., 2007).

When considering the development of novel agents as oral care products, their toxic effects on human oral mucosal cells should be carefully examined. An ideal oral care product should be an efficient antimicrobial agent but should not be toxic to human oral cells. Results from our study indicated that RCE is safe to be used as an oral care product.

Our study reported that RCE inhibited the activity of Kgp and Rgp at a sub-MIC concentration. This activity may be due to polyphenol compounds as reported in previous study (Yamanaka et al., 2007). Moreover, the RCE showed an inhibitory effect on IL-6 and IL-8. The mechanism of inhibition may be via a down-regulation of the activator protein-1 activity (Verri et al., 2001). From the SEM observations, there was distortion of cellular morphology of *S. mutans* and *P. gingivalis* that would result in bacterial death, after treatment with RCE.

Conclusion

Because of the favorable bioactivity and the simple process involved in producing the extract from the plant, the RCE has a high potential to be used as a novel agent for preventing oral infectious diseases.

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