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ANTI-PERIODONTAL BACTERIAL ACTIVITY OF OXYRESVERATROL

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INTRODUCTION

Periodontal disease is one of the most crucial global oral health problems. More than two-thirds of the world's population suffers from some form of periodontitis. Periodontitis is an inflammatory disease caused by group of anaerobic gram negative bacteria such as and *Porphyromonas gingivalis*, *Actinobacillus* sp., *Prevotella* sp. and *Fusobacterium* sp.^{1,2} The disease is clinically differentiated from gingivitis by the loss of the connective tissue attachment to the teeth.

Presently, the periodontitis treatment generally used by the dentists is scaling and root planning, which will remove bacteria and bacteria deposits from the gingival and tooth surface. In addition, chemotherapeutic agents or antibiotics are also used in the treatment. However, there are several disadvantages including causing brown stains on the teeth and tongue and altering taste perception these treatments might cause some side effects for patients. Thus, the search for alternative agents based on herbal extracts or natural compound is necessary.

The natural compound focused in this study is oxyresveratrol (2,3',4,5'-tetrahydroxystilbene) from Thai medicinal plant, *Artocarpus lakoocha*³. Oxyresveratrol has been found in various plants such as heartwood or fruits of *A. heterophyllus*, *A. lakoocha*, *A. gomezianus*, *A. dadah*, wood extracts or fruits of mulberry trees (*Morus australis*, *M. alba L.*), fruits of *Melaleuca leucadendron*, rhizome of *Smilacis chinae*, as well as Egypt herb *Schoenocaulon officinale*⁴⁻⁷. Water-soluble oxyresveratrol is known for its antiviral and hepato-protective activities. Importantly, oxyresveratrol also showed anti-inflammatory⁸ and antibacterial activities previously. Thus, Oxyresveratrol has potential to be a good target for the periodontal disease treatment.

In this study, the anti-periodontal bacterial activity of oxyresveratrol will be tested in two major periodontal pathogens, *P. gingivalis* and *A. actinomycetemcomitans*. Moreoever, we test the antibacterial activity of oxyresveratrol in *Streptococcus mutans*, and *Streptococcus sobrinus*. which are the two major dental caries pathogen. We aim to develop oxyresveratrol to use as product for control periodontitis such as mouthwash or irrigating solution.

MATERIALS AND METHODS

Disc Diffusion Assay. Antibacterial activity was evaluated by the agar disc diffusion method on nutrient agar medium. *P. gingivalis* and *A. actinomycetemcomitans* were grown in anaerobic blood-agar plates in anaerobic environment at 37 °C for 3-5 days. The test compound was introduced on the disc and allowed to dry. Then the disc was impregnated on the seeded agar plate. Dimethyl sulfoxide (DMSO) was used as a negative control whereas 2% chlorhexidine was used as positive control. The plates were done in triplicates and were incubated for 48 h at 37°C for antibacterial activity. The antimicrobial activity was taken on the basis of diameter of zone of inhibition, and the mean of three readings is presented.

Determination of MIC and MBC values.

Minimal inhibitory concentration (MIC) value of oxyresveratrol was determined for A. actinomycetemcomitans by using twofold serial dilution in 96-well plates. 100 μ l of 10 μ g/ml oxyresveratrol was added and the dilution was performed. Subsequently, 100 μ l dilute bacterial suspension with final inoculum of 10⁵ bacteria/ml was added to the microtiter plate. The experiment was

performed in triplicate. The bacterial suspension with 10% DMSO was used as negative control and 2% chlorhexidine was used as positive control. Following incubation under anaerobic conditions, the MIC was determined by the last concentration of oxyresveratrol with bacterial growth.

The minimum bactericidal concentration (MBC) value was determined by subculture of the wells which showed no bacterial growth on the sterile agar plate. The MBC was determined by the least concentration which showed no visible growth on agar plate.

RESULTS

The anti-bacterial activity of oxyresveratrol on anti-periodontal pathogen

Antibacterial activity of oxyresveratrol was tested against *P. gingivalis, A. actinomycetemcomitans, Streptococcus mutans,* and *Streptococcus sobrinus.* Clear inhibition zones were found a: 48 h and 72 h after incubation at 37 °C for *A. actinomycetemcomitans* and *P. gingivalis* respectively. However, there is no clear zone for *S. mutans* and *S. sobrinus.* The MIC and MBC for *A. actinomycetemcomitans* were 0.08 and 0.16 μ g/mL, respectively. No bioactivity was observed in the solvent control, 2% DMSO. The results obtained from three repetitions for each bacterium were similar. No bioactivity was observed in the solvent control, 2% DMSO.

Oral Pathogen	Zone of Inhibition(mm)	
	10 mg/ml oxyresveratrol	2% chlorhexidine
Porphyromonas gingivalis	15	17
Aggregatibacter actinomycetemcomitans	17	19
Streptococcus mutans	-	
Streptococcus sobrinus		+

Table 1. Anti-oral bacterial activity of oxyresveratrol

DISCUSSION

From the disc diffusion result, oxyresveratrol can cause clear zones only with the periodontal pathogen, not bacteria for dental caries (table 1). This implied that oxyresveratrol is able to inhibit the periodontal pathogen specifically. However, the Minimal Inhibition Concentration and the Minimum Bactericidal Concentration for *P. gingivalis* is determined in the future study. Moreover, the toxicity of oxyresveratrol will be further studied in oral cells.

CONCLUSION

The results of present study indicate oxyresveratrol as a potential candidate to be used as antiperiodontitis agent because of its anti-bacterial activity against main oral pathogen related to periodontal disease.

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