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学位論文審査並びに最終試験結果報告書

大学院歯学研究科長　　殿

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今般 Herastuti Sulistyani にかかわる学位論文審査並びに最終試験を行い下記の結果を得たので報告する。

記

1　学位論文題目　　Potential Activities of Roselle Calyx Extract (Hibiscus sabdariffa L.) against Oral Bacteria *In Vitro*

2　論文要旨　　別添

3　学位論文審査の要旨　　別添（様式第12号）

4　最終試験の要旨　　別添（様式第13号）

以上の結果 Herastuti Sulistyani は博士（歯学）の学位を授与する資格のあるものと判定する。

様式第 12 号

学位論文審査の要旨

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学位論文題目　Potential Activities of Roselle Calyx Extract (*Hibiscus sabdariffa* L.) against Oral Bacteria *In Vitro*

以下本文

This study were to investigate the potential activities of roselle calyx extract (RCE) against oral bacteria, in particular, the antibacterial activity against target organisms, cytotoxicity, and effect on biofilm formation, gingipain activity, cytokine production, and morphology of oral bacteria.

Eight bacteria were used in this study. The antibacterial activity of RCE was determined by treating the bacterial cells with RCE 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 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 RCE in HGF, Ca 9-22, and KB cells. The effect of RCE on lysine-specific cysteine protease (Kgp) and arginine-specific cysteine protease (Rgp) was evaluated using a synthetic substrate colorimetric assay. Additionally, its effect on cytokine production was quantified using an enzyme-linked immunosorbent assay (ELISA) kitand the morphological alterations in the cells of *Streptococcus mutans* and *Porphyromonas gingivalis* were studied using scanning electron microscopy (SEM). The number of viable bacteria in the RCE-treated group was significantly lower than that in the control group were. 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-dependentmanner. RCE showed less cytotoxicity to human oral cells and inhibited Kgp and Rgp activities. In addition, it inhibited the production of interleukin (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 an irregular shape with enlargement and clumping of cells. In *P. gingivalis*, treatment with RCE caused aggregation and distortion of cellular morphology.

Therefore, because of the favorable bioactivity and the simplicity of the production process of the extract, RCE can be used as a novel agent for the prevention and treatment of oral infectious diseases.

This thesis has been approved by the Examining Committee for the thesis requirement for the Doctor of Philosophy degree.

様式第13号

最終試験（学力の確認）の要旨

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審査委員会において，最終試験を行い申請者の学力の確認を行ったところ，学位論文に関する十分な知識と研究遂行能力を有するとみとめた．以上の結，博士（歯学）の学位を授与するに値するものと判定した．