Antibacterial Effect of Xanthone from Mangosteen Pericarp Extract (*Garcinia mangostana Linn*.) against *Porphyromonas gingivalis*

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Abstract

Most of bacteria involved in primary endodontic infection are anaerobic bacteria, one of them is Porphyromonas gingivalis. Porphyromonas gingivalis is a non–spore-forming gram-negative anaerobic rod. Mangosteen (Garcinia mangostana) is a fruit that originated from Southeast Asia, especially Indonesia. Several studies have shown that xanthones (active substance) from mangosteen pericarp have remarkable biological activities as an antibacterial agent.

The aim of this study was to find out the MIC (Minimum Inhibitory Concentration) of xanthones from mangosteen pericarp extract against Porphyromonas gingivalis biofilm.

Porphyromonas gingivalis ATCC 33277 was cultured in Brain Heart Infusion (BHI) medium enriched with vitamin K1 and hemin, on anaerobic atmosphere. Porphyromonas gingivalis was diluted according to Mc. Farland standard 8 x 106 CFU/mL. The microtiter plate was incubated for 2 x 24 hour. Xanthones was isolated from ethanolic extract of pericarp of Garcinia mangostana, generated various concentrations from 0,32% to 1,91%. The suspension was read with ELISA Reader with wavelength 600 nm.

At the concentration of 1,59% xanthone of mangosteen pericarp extracts showed lowest optical density. At the concentration of 0,32% showed highest optical density, also higher than bacteria control group.

The mangosteen pericarp extract has antibacterial effect on Porphyromonas gingivalis. The MIC was at 1.59%.

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Introduction

Caries is one of the most common tooth decay on society from a variety of economic and age groups. Untreated caries can reach the dentin and lead to pulp inflammation. Bacteria in carious tissue will enter onto the pulp and root canal through the dentinal tubules or open pulp chamber floor. Inflammation of the pulp will get worse if left untreated, disturbances of blood circulation in the tooth pulp lead to necrosis.[1]

Pulpal inflammation is a poly-microbial inflammation.[1] In the primary root canal infections, the most isolated bacteria are obligate anaerobes, one of which is *Porphyromonas gingivalis*[2]. *Porphyromonas gingivalis* is a black-pigmented bacteria non-motile gram-negative obligate anaerobes.[3] This kind of bacteria are often found in symptomatic or asymptomatic root canal infections and can be aspirated from acute periapical abscess.[4]

Irreversible pulp inflammation requires root canal treatment. Root canal treatment is aimed to eliminate bacteria in the root canal. Medicaments existing material for pulp canal sterilization are known to potentially cause side effects because this material is a therapeutic agent or chemically active and toxic.[5] This condition leads the researcher to discover another alternative made from herbs. Several studies have shown the use of herbal medicine is considered less toxic than modern medicine (synthesis). Herbal medicine has relatively minor side effects if used precisely accurate dose.[6]

Mangosteen (*Garcinia mangostana L.)* is one of the plants originate from Indonesia, easily

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grown in Indonesia and Southeast Asian countries such as Malaysia, Thailand, and Myanmar. Phytochemical study shows that the mangosteen pericarp has the most active ingredient, such as xanthones, flavonoids, saponins, and tannin.[7] Xanthones has been reported to have pharmacological activity as antibacterial, antifungal, and anti-inflammatory.[8] Plant *Cratoxylum formosum ssp. pruniflorum* that contains the largest number of xanthones is able to inhibit the growth of black-pigmented anaerobic bacteria (*Porphyromonas gingivalis* and *Prevotella intermedia*).[9] Research by Kawilarang et al. states that the mangosteen pericarp includes xanthones can inhibit Porphyromonas gingivalis bacteria growth.[10] From both of those studies, we hypothesize that xanthones have potential effect as an antibacterial agents, especially against *Porphyromonas gingivalis*.

Materials and methods

The study was conducted according to the Human Research Ethics Committee of Universitas Airlangga Faculty of Dental Medicine. The pericarp of mangosteen was collected from UPT MATERIA MEDICA, Malang, East Java Province, Indonesia in April 2014. Air-dried and powdered *Garcinia mangostana* (0.5 kg) was thoroughly extracted through ethanol 96% for 24h. The combined extracts were concentrated *in vacuo* to gain crude extract which stored at room temperature.

Hexane extract was soaked with alcohol acetate and then evaporated. Immersion and evaporation process was repeated once more, then extract was soaked in hexane alcohol acetate and evaporated to obtain 31.82% xanthones isolation of 50 ml. Isolation of xanthone was diluted with sterile distilled water to obtain a concentration of xanthones 0.32%, 0.64%, 0.96%, 1.28%, 1.59%, and 1.91%.

The next step was to prepare the bacterial suspension of *Porphyromonas gingivalis*. These bacteria were pure-cultured bacteria derived from Microbiology Laboratorium Universitas Brawijaya, Malang, Indonesia. *Porphyromonas gingivalis* were cultured in *brain heart infusion* (BHI) medium enriched with vitamin K and hemin, then cultured in an anaerobic atmosphere (using anaerobic jar) for 2x24 hours. *Porphyromonas gingivalis* concentration was adjusted to $8x10^6$ CFU/ml. Various concentration of xanthones isolated from ethanolic extract of Garcinia mangostana pericarp was added by 1 ml of bacterial suspension. Blood agar was used as a negative control and the culture of *Porphyromonas gingivalis* was used as a positive control. Microplate was incubated anaerobically for 24 hour. The culture is read with an ELISA reader at a wavelength of 600 nm.

Results

The xanthones extract was divided into 6 group concentrations, 0.32%, 0.64%, 0.96%, 1.28%, 1,59%, and 1.91%. The highest amount of optical density (OD) xanthones against *Porphyromonas gingivalis* was in 0.32%. The graph decreased at a concentration of 0.64%, 0.96%, 1.28% respectively with an optical density of 2.258, 2.045 and 1.198. The graph increased at concentrations of 1,59%, and 1.91% respectively with an optical density of 1.109 and 1.630. Concentration of 1,59% has the lowest optical density.

Collected data from each examination were analyzed statistically with significance level of α=0.05. The statistical test to determine the distribution of the data One-Sample Kolmogorov-Smirnov Test, while the homogeneity test data using Levene Test. Furthermore, the ANOVA and Tukey test were used to compare the significant difference between the optical densities of each concentration.

Normal distribution test checked by using one-sample Kolmogorov-Smirnov test. The p value (*P*=0.275) obtained from the results of one-sample Kolmogorov-Smirnov test indicated the data had a normal distribution (*P*>0.05).

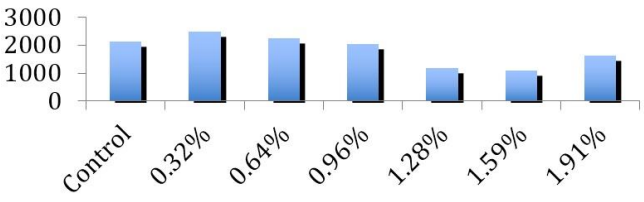

Figure 1. The Data Showed the Average Percentage of Xanthone Mangosteen Pericarp Optical Density Against *Porphyromonas Gingivalis* Culture.

Homogeneity of variance test showed that the data were significantly different with *P*=0.365

(P>0.05). ANOVA p value is 0.004 (P<0.05), and then H0 is rejected so that the data has a significant difference.

Discussion

One of the bacteria in root canal infections able to form biofilms is *Porphyoromonas gingivalis,* which present in infected root canals.[11] Therefore, all ingredients used for root canal treatment must have antibacterial ability. Mangosteen pericarp consists much xanthones than the other natural sources.[12] Xanthones through its chemical structures, tricyclic aromatic system, has antibacterial properties.[8] Active compound content of xanthones from mangosteen pericarp extract has the potential to support the success of root canal treatment.

The higher the value of optical density, the lower the inhibition of xanthones against *Porphyromonas gingivalis* bacteria. This could be seen at a concentration of 0.32% which exceeds the value of optical density positive control. Despite of that condition, the lower the value of optical density, the higher the inhibition of xanthones against *Porphyromonas gingivalis* bacteria. The antibacterial capability presents in a concentration of 1.59%.

Xanthones, the largest component of the mangosteen pericarp, has the ability as anti-tyrosinase and antibacterial, since a carbonyl group content in xanthones can react with the amino acid residues on the proteins cell membrane, extracellular enzymes, and proteins cell wall therefore the proteins of biofilm matrix polymer lead to damage.[13] Xanthones are also effective in eliminating anaerobic gram-negative bacteria which are more resistant to antimicrobial agents.[9]

Antibacterial activity of xanthones against *Porphyromonas gingivalis* bacteria was at concentration of 5% (equivalent to 1.59% xanthones extract). This is consistent with the results of preliminary toxicity tests which states that the proliferation of fibroblasts present in concentrations of 0.99% and 1.99%. From both of these things, allegedly xanthones mangosteen pericarp can be safely used as a dental materials because is not only able to inhibit the growth of *Porphyromonas gingivalis*, but also safe for fibroblasts cell.

Conclusions

The mangosteen pericarp extract has antibacterial effect on *Porphyromonas gingivalis*. The MIC was at 1.59%.

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Declaration of Interest

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