Tooth enamel surface micro-hardness with dual species Streplococcus biofilm after exposure to Java turmeric (Curcuma xanthorrhiza Roxb.) extract

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Abstract. Streplococcus biofilm on tooth surfaces can decrease mouth environment pH, thus causing enamel demineralization that can lead to dental caries. Java Turmeric extract has excellent antibacterial effects and can maintain S. mutans biofilm pH at neutral levels for 4 hours. To analyze the effect of Java Turmeric extract on tooth enamel micro-hardness, the Java Turmeric extract was added on enamel tooth samples with Streplococcus dual species biofilm (S. sanguinis and S. mutans). The micro-hardness of enamel was measured by Knoop Hardness Tester. Results showed that Curcuma xanthorrhiza Roxb. could not maintain tooth enamel surface micro-hardness. It is concluded that Java Turmeric extract ethanol could not inhibit the hardness of enamel with Streplococcus dual species biofilm.

1. Introduction
Java Turmeric (Curcuma xanthorrhiza Roxb) is one of the leading medicinal plants identified by the Department of Health in Indonesia. Previous study reported that Java Turmeric has antiviral, antibacterial, antifungal, and anti-metastatic effects [1-7]. Java Turmeric contains pati, fat, proteins, cellulose, minerals, yellow substance (curcumin), and atsiri oil. It also contains essential oils such as camphor, tumerol, sineol, and xanthorrhizol. Xanthorrhizol is reported to have a very good microbial effect [2].

Dental caries is a disease of the hard structures of the tooth; it happens because of demineralization processes in enamel as a result of the amino acid metabolism of bacteria, so that cavities are formed [8-10]. It is caused by the interaction of various factors (multifactorial disease), which consists of: the host factors such as the teeth and saliva, the normal microbial flora of the mouth such as S. mutans, S. sobrinus and Lactobacilli sp., substrate (food), and time [8-11]. The process of dental caries begins with the formation of pellicle from saliva on the surface of the teeth and then the colonization of bacteria (early colonizer), such as Streplococcus sanguinis (S. sanguinis) [12]. S. sanguinis co-aggregates with other bacteria such as to form dental plaque or dental biofilm. The existence of the substrate (sugar) may be metabolized by bacteria such as Streplococcus mutans (S. mutans) and Streplococcus sobrinus (S. sobrinus). It then produces lactic acid which can demineralize enamel [8]. In the demineralization process, ions such as calcium (Ca2) and phosphate (PO43-) dissolve and can cause porosity of enamel and decrease the hardness of teeth [9]. Dental caries occurs when the demineralization process is greater than remineralization [8,13-14].

Although S. sanguinis have been reported to bind with S. mutans, another study reported that early colonization of S. sanguinis has been associated with delayed colonization of S. mutans in children.
[15]. *S. sanguinis* compete with *S. mutans* to colonize on the surface of the teeth. Interaction between *S. sanguinis* and *S. mutans* is influenced by environmental factors such as the density of the colony, the availability of nutrition, and pH. In competition for nutrients, *S. sanguinis* produce hydrogen peroxide (H2O2) that can suppress *S. mutans* growth, and thus survive. This happens because *S. mutans* can survive in an environment of acid while *S. sanguinis* cannot. But in an abundant nutrition environment, hydrogen peroxide is not produced and its energy is used for the growth of the bacteria [16].

Previous research stated that xanthorrhizol and Java Turmeric extract ethanol has the ability to maintain pH in the range of 7.0 for 4 hours on the surface of *S. mutans* [3,17-18]. This research has shown that Java Turmeric extract ethanol has antibacterial efficacy against *S. mutans* and *S. sanguinis*, and inhibits the process of teeth demineralization with a way to inhibit the growth of both the bacteria so that the pH does not decrease. In addition, the influence of Java Turmeric extract ethanol on tooth micro-hardness with *S. mutans* biofilm has been observed but there was no difference between the condition before and after application of the extract ethanol Java Turmeric [18]. Information about the required time for the dual species *S. sanguinis* and *S. mutans* biofilm to reach the critical pH was not known then, nor was the influence of Java Turmeric extract ethanol on enamel micro-hardness with dual species *S. sanguinis* and *S. mutans* biofilm.

2. Materials and Methods

First, the experiment was initiated by making a model of the *Streptococcus dual species* biofilm in a well plate; this model is required to know the time needed for the biofilm model to reach the critical pH. The biofilm model was made as follows: saliva of healthy volunteers was disentrifused (3000 rpm 10 minutes), then filtered with micro filter 0.2μm. Some saliva was cultured on a plate of BHI jell for 24 hours. If no colonies grew, then the saliva production was ready to be used. One mL of the sterile saliva was plugged in a well, and then the well plate was placed on an orbital shaker (80x/minute) for 1 hour and 30 minutes in order to form the pellicle. Two hundred ul *S. sanguinis* and *S. mutans* (1:1), and 600 ul of Brain Heart Infusion-Sucrose (BHIS) 3 % as a nutrient, was added in the well that covered the pellicle. Then the well plate was inserted in an anaerobic jar and was given CO2 gas for 2 minutes, tightly closed; the jar was then inserted into a 37°C incubator. Then the time needed for the biofilm to reach the critical pH was observed from one to twenty-four hours.

The experiment continued in vitro in the laboratory using 24 samples of teeth that had been inserted in resin and were ground and polished until flat and free of scratches. Then samples of the teeth were divided into 4 groups of research: that is, 20 hours, and 24 hours control biofilm groups, and two groups that received the treatment with Java Turmeric extract (15%) for 20 hours, and 24 hours. Each group consisted of 6 samples of teeth; all were measured regarding average enamel surface hardness before treatment, using the Knoop Hardness Tester. In addition, *Streptococcus dual species* biofilm was grown on the surface of the enamel and was incubated for 16 hours for the 20-hour treatment group, and for 20 hours for the 24-hour treatment group; and it was confirmed that they had reached the critical pH for enamel (pH < 5.5). At that point, Java Turmeric extract ethanol was added and the group was returned to the incubator for 4 hours. The biofilm control groups were left in the incubator until they reached the established observation time, while 1 mL of 45% Java turmeric extract (final concentration of the 15% solution) was added to the treatment groups.

The Java Turmeric extract that was used in this research was acquired by the method of maceration extraction using 98% solvent ethanol from Badan Penelitian Tanaman Rempah dan Obat (BALITTRO). The extract was diluted using 10% Dimetil Suloksida (DMSO) and was mixed with the Vortexer for 20 seconds. The data result of enamel surface micro-hardness changes was statistically analyzed using the Shapiro-Wilk normality test. The normality results showed that there were groups that had an abnormal distribution of the micro-hardness before treatment; thus, it would be difficult to interpret the final statistics. Therefore the groups were modified by comparing enamel surface micro-hardness changes in control groups and groups treated with 15% Java Turmeric extract. After that, micro-hardness average showed normal distribution, so that it could be done with
Independent T Test (Parametric tests). Statistical testing was done using a 0.05 (p > 0.05) significance level and 95% (α = 0.05) trust level.

3. Results and Discussion

3.1 Results

*Streptococcus dual species* biofilm pH was 7 at 3 hours, and then the pH decreased to 6 from 4 to 12 hours. It reached 5.5 from 14 to 24 hours, which was the critical pH for enamel. The results of the decrease in biofilm can be seen in Figure 1. Table 1 shows the data regarding enamel surface micro-hardness changes for each group. Enamel surface micro-hardness changes were observed by comparing the average of control groups (teeth groups that were only in the biofilm model) and treatment groups (groups of teeth with added Java Turmeric in the biofilm model). Enamel surface micro-hardness in the 20-hour control group decreased more than enamel surface micro-hardness in the 24-hour treatment group. This may have happened because of the influence of the structure of teeth sample before, and because the biofilm condition was less than optimal. Meanwhile the enamel surface micro-hardness in the treatment groups showed a greater increase during the incubation period. A decrease in enamel for the 20- and 24-hour treatment groups was higher than the control, but differences in the statistics (p < 0.05) were found only the 24-hour treatment group with the group of controls. Thus, treatment with Java turmeric extract ethanol could not maintain enamel surface micro-hardness with *Streptococcus dual species* biofilm.

![Figure 1. pH Changes of Streptococcus dual species biofilm](image)

| pH Scores | Incubation period |
|-----------|------------------|
| 0         | 1-3 hours         |
| 1         | 4 hours           |
| 2         | 12 hours          |
| 3         | 16 hours          |
| 4         | 20 hours          |
| 5         | 24 hours          |

Table 1. Changes of enamel surface hardness with *Streptococcus dual species* biofilm

|                     | 20 hour groups | 24 hour groups |
|---------------------|----------------|----------------|
| Control             | After 20 hours | Control        | After 24 hours |
| 101.33              | 107.33         | 136.67         | 130.67         |
| 151                 | 137.67         | 136.67         | 93             |
| 185.33              | 148            | 68.67          | 173.33         |
| 130                 | 88.67          | 121.67         | 165.33         |
| 133.33              | 189.33         | 159.67         | 172.33         |
| 102.33              | 169.33         | 103.33         | 207.33         |
3.2 Discussion

In this study, the pH of *Streptococcus dual species* biofilm decreased at the 4 hour mark and continued to decrease until it reached the critical pH (pH 5.5) from 16 to 24 hours. In the Handoko research (2012), the *S. mutans* biofilm model could reach pH 4 in 16 hours of incubation, but in this research the *Streptococcus dual species* biofilm model only reached a pH of 5.5 [3,18]. The results of this research were supported by Kreth (2007) who stated that *S. mutans* prevented the growth of *S. sanguinis* because it produced lactic acid while *S. sanguinis* are less aciduric than *S. mutans*, so that it could not survive in acidic conditions and was unable to maintain neutral pH, but was still able to maintain pH until not far below the critical pH [16]. *S. sanguinis* and *S. mutans* have antagonistic biochemistry in the minimum nutrition condition, so *S. sanguinis* can maintain pH neutral biofilm in that condition. But when *S. sanguinis* and *S. mutans* are exposed simultaneously on the pellicle and given the nutrients of 3% BHIS, *S. mutans* metabolizes sucrose to become lactic acid while *S. sanguinis* cannot and is unable to maintain pH biofilm; this is because it cannot divide and produce hydrogen peroxide; this is known to inhibit the growth of *S. mutans*. Otherwise *S. mutans* can also secrete bacteriocin, which may inhibit the growth of *S. sanguinis*.

Test results in the 20-hour incubation groups showed that the enamel surface hardness obtained between the control group and the treatment group was not significantly different. In contrast, the results between groups with the 24 hour incubation showed a significant difference. The control group, with the enamel surface that added Java Turmeric, had a greater difference in the enamel surface hardness. There was a larger decrease in enamel surface hardness for the group with the exposure to Java Turmeric than was found in the control groups. Results of this research are different from Handoko (2012), which showed that Java Turmeric extract exposure can increase enamel microsurface hardness [3,18]. Differences in the results could be caused by the difference in Java Turmeric ethanol extract contents, and Java Turmeric extract has not been well standardized. Besides that, differences in Java Turmeric extract processing methods affect the components of extract. The weakness of this research was that it did not measure biofilm pH after exposure to Java Turmeric extract ethanol. This was due to technical limitations; it was because universal indicator color pH cannot be interpreted after tested on Java Turmeric extract ethanol because the curcumin contains substances that give Java Turmeric its yellow color hue. Besides the preparation factor and measurement of teeth samples can affect the end result [19]. Further research is needed to make a biofilm model that can reach critical pH smaller than 5.5, so the researcher can obtain a better model of demineralization. Pharmaceutical research is needed in order to create a proper preparation for oral use, with attention to the color factor from the curcumin, which that affects the aesthetics.

4. Conclusion

*Streptococcus dual species* biofilm, *S. sanguinis* and *S. mutans* can reach critical pH for enamel (< 5.5) for 16 hours and can survive until 24 hours. Java Turmeric extract ethanol could not inhibit the hardness of enamel with *Streptococcus dual species* biofilm.

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