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Research Article

EFFICACY OF JAVANESE TURMERIC ETHANOL EXTRACT IN ERADICATING *STREPTOCOCCUS SANGUINIS* AND *PORPHYROMONAS GINGIVALIS* BIOFILM

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ABSTRACT

Objective: Javanese turmeric is an Indonesian native medicinal plant with antibacterial activities. This study aimed to analyze the efficacy of identified Javanese turmeric ethanol extract (IJTEE) in eradicating *Streptococcus sanguinis* and *Porphyromonas gingivalis* biofilms.

Methods: Biofilm assay: Single and combination biofilms formed at different phases were exposed to IJTEE in 0.5–25% concentrations for 1 h. The percentage of eradication was tested using the microtetrazolium assay.

Results: The efficacy of IJTEE in eradicating the biofilm was equal to that of chlorhexidine against the early phase of biofilm formation. IJTEE is more effective against *S. sanguinis* biofilm formation than against *P. gingivalis* biofilm formation.

Conclusion: IJTEE can eradicate *S. sanguinis* and *P. gingivalis* biofilms.

Keywords: Identified Javanese turmeric ethanol extract, *Streptococcus sanguinis*, *Porphyromonas gingivalis*, Biofilm eradication.

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INTRODUCTION

Chlorhexidine is an antiseptic mouthwash reported to reduce dental plaque formation and is considered as an antiseptic gold standard in maintaining oral health [1]. Chlorhexidine has a broad-spectrum antimicrobial activity that is effective against Gram-positive, Gram-negative, and fungal bacteria [2]. However, chlorhexidine has some unexpected effects, such as staining on tooth surfaces, metal flavor, irritating the oral mucosa, and increased calculus formation [2]. These side effects have led to increased research on the development of other drugs, particularly herbal medicinal plants, to determine an alternative antiseptic mouthwash that is efficacious, relatively safe, and developed using natural-based ingredients.

Javanese turmeric (*Curcuma xanthorrhiza* Roxb.) is an original Indonesian herb that needs to be developed and utilized as herbal medicine owing to its health benefits [3]. Javanese turmeric is among the nine leading species of herbs in Indonesia. Research on turmeric as herbal plants has been conducted since 2003 [3]. Javanese turmeric is traditionally used as medicine for digestive disorders, liver disease, constipation, dysentery, fever in children, hemorrhoids, and reddish skin. Turmeric has also been reported to contain antitumor, hypotriglyceride, anti-inflammatory, and hepatoprotection properties [4].

The rhizome of the Javanese turmeric is the plant part used in herbal medicine [3,5]. The rhizome contains active ingredients, such as xanthorrhizol, essential oils, and curcuminoids, which promote health benefits [5]. Xanthorrhizol from turmeric rhizome has an antibacterial activity toward oral health bacterial pathogens [4]. It is a specific component that has been found in essential oil derivatives from *Curcuma* groups. An *in vitro* study by Hwang *et al.* has proven that xanthorrhizol isolated from a methanol extract of turmeric had an antibacterial effect against *Streptococcus sanguinis* and *Porphyromonas gingivalis* at a certain concentration [4].

However, obtaining a xanthorrizol isolate from the ginger rhizome requires considerable technology and cost. Another disadvantage of using isolates of turmeric active substances is the non-utilization of other nutritious biological compounds contained in the ginger rhizome. Extracts are relatively easy forms of preparation made to determine the efficacy of the turmeric rhizome [6]. Some solvents that can be used for the extraction of turmeric rhizome are aquadest, ethanol, methanol, hexane, and dichloromethane. Based on research, the yield of turmeric rhizome extract was the highest when using aquadest as the solvent [7].

S. sanguinis is a Gram-positive, facultative aerobe with the largest population in the oral cavity and is one of the earliest bacteria to colonize the tooth surface. *S. sanguinis* biofilms can cause dental caries and periodontal disease. The bacteria produce extracellular polysaccharides (EPSs) in the form of soluble or insoluble glucan and contribute to plaque formation. *S. sanguinis* is also known to produce proteases that can divide secretory immunoglobulin A [8,9].

P. gingivalis is a black-pigmented, Gram-negative, anaerobic, asaccharolytic rod, indicating that it uses proteins and peptides to survive [8]. *P. gingivalis* is commonly found in subgingival areas, particularly in the subgingiva of patients with periodontal tissue disorders [10]. *P. gingivalis* produces various proteins associated with tissue destruction, such as active proteases, hemolysin, collagenase, cytotoxic metabolites, and capsules [8].

A dental plaque or an oral biofilm is a community of microorganisms that attach to a surface in the oral cavity and is embedded in an extracellular matrix and microbial polymer [11,12]. Oral biofilm formation occurs through several stages, including pellicle formation on the tooth surface, pioneer bacterial colonization, bacterial coaggregation/coadhesion, and biofilm maturation [12]. The bacteria present in a biofilm are reported to be more resistant to antibacterial agents. Some resistance mechanisms include limited penetration of antibiotic agents, general stress response induction, decreased metabolism, increased expression of multidrug resistance pumps, activation of the quorum-sensing system, and changes in bacteria membrane protein [13].

In a previous study by Hwang *et al.*, xanthorrhizol isolated from the methanol extract of turmeric has been reported to have a minimum inhibitory concentration (MIC) of 4 μg/ml and a minimum kill rate

(MBC) of 8 µg/ml against *S. sanguinis* bacteria [4]. The MIC and MBC were 32 µg/ml against *P. gingivalis* [4]. This study showed that xanthorrizol is more sensitive to *S. sanguinis* than to *P. gingivalis.* Other studies have also shown that xanthorrhizol has an inhibitory effect toward *S. sanguinis* biofilm formation. The present study aimed to analyze the efficacy of indentified Javanese turmeric ethanol extract in eradicating the synthesis of *S. sanguinis* biofilm and *P. gingivalis* biofilm as individual biofilms or in combination.

METHODS

Eppendorf tubes and pipette tips (10 µl, 200 µl, and 1000 µl) in sufficient quantities were prepared in container, labeled, and subsequently sterilized using an autoclave at 121°C for 2 h. Sterile tools were stored at room temperature.

The seeding mediums used were brain heart infusion (BHI) agar and BHI broth. The BHI media were prepared by mixing 52 g of BHI powder in an Erlenmeyer tube and then diluting with distilled water to 1000 ml. The Erlenmeyer tube containing BHI agar and BHI broth was sealed with a cotton plug and covered with aluminum foil. Subsequently, the media were sterilized in an autoclave at 121°C for 2 h. After the BHI media cooled to 50°C, 20 ml of BHI solution was poured into a petri dish and then placed at room temperature for solidification. Growth media were stored at 4°C in a refrigerator until use.

P. gingivalis was cultured in BHI media enriched with Vitamin K. The BHI media were prepared by mixing 52 g of BHI agar powder and 10 µl Vitamin K in an Erlenmeyer tube and then diluting using aquadest water up to 1000 ml. The Erlenmeyer tube containing BHI agar and BHI broth was sealed with a cotton plug, covered with an aluminum foil, and sterilized in the autoclave at 121°C for 2 h. After sterilization, 10 µl of Vitamin K was added to the BHI agar and BHI broth. When the BHI agar was cooled to 50°C, 20 ml of BHI solution was poured into a petri dish and then placed at room temperature for solidification. Both media were stored at 4°C in a refrigerator until use.

S. sanguinis and *P. gingivalis* were obtained from the bacterial stock at the Oral Biology Laboratory Faculty of Dentistry University of Indonesia. A total of 10 µl of *S. sanguinis* from the bacterial stock was inoculated into the BHI agar medium, whereas as much as 10 µl of *P. gingivalis* stock was inoculated into the Brucella media, and Vitamin K was added to the BHI agar. Subsequently, the slider was heated using a Bunsen burner until it turned red and was cooled by attaching it to the agar edge. The slider was evenly scratched on the agar and its whole surface. The petri dish was then placed into the anaerobic jar and incubated anaerobically for 48 h at 37°C.

Main solvent prepared through *S. sanguinis* and *P. gingivalis* culture with an Eppendorf sterile tube. The main solvent was prepared by obtaining as much as a single colony of each bacterium from their cultures using a loop; this was then inserted into the Eppendorf tube containing 1 ml of BHI broth. The Eppendorf tubes were centrifuged at 13,000 rpm for 60 s, and the supernatant was discarded. Subsequently, the pellet was resuspended in 1 ml of BHI broth and homogenized using a vortex mixer for ± 20 s.

The concentration of bacteria used was determined by preparing bacterial suspensions of *P. gingivalis* and *S. sanguinis* from 10^{-1} to 10^{-8}. Eppendorf tubes with sequential numbers were prepared for bacterial suspensions from 10^{-1} to 10^{-8}. Each Eppendorf tube was filled with 900 µl of BHI liquid. A total of 100 µl of the main suspension were added to the Eppendorf tube (10^{-1}) and then homogenized using the vortex to produce a bacterial suspension of 10^{-1}. The same procedure was performed from the first to the second Eppendorf tubes, and so on, to obtain a bacterial suspension by dilution from 10^{-1} to 10^{-8}. A total of 10 µl of each suspension (10^{-1}–10^{-8}) were cultured in BHI medium agar for 48 h in anaerobic environment at 37°C. The bacterial count was calculated after incubation.

In this study, the turmeric ethanol extract concentration was tested at 0.5%, 1%, 5%, 10%, 15%, and 25%. The turmeric ethanol extract was diluted in a 15-ml centrifuge tube with 10% DMSO to obtain turmeric ethanol extracts of various desired concentrations. Subsequently, the tubes were stored in the refrigerator at 4°C until use.

The negative controls in this study were biofilm models of *S. sanguinis*, *P. gingivalis*, and a combination of both added to 100 µl of BHI broth. The positive controls were biofilm models of *S. sanguinis*, *P. gingivalis*, and the combination of both in 100 µl of 0.2% chlorhexidine.

In a single biofilm model, 100 µl of *S. sanguinis* or *P. gingivalis* from the 10^{-5} bacterial suspension in a growth medium with 0.2% sucrose was added to each well (96-well plate). The well plates were then incubated at different biofilm-forming phases: adhesion phase (4 h), active-phase accumulation (12 h), and maturation (24 h). The biofilm formed on a well base was rinsed with 100 µl of phosphate-buffered saline (PBS) solution to remove planktonic bacteria.

In each group, 100 ml of turmeric ethanol extract was added at various concentrations. In the positive control group, 100 µl of 0.2% chlorhexidine was added, whereas the biofilm in the negative control group was not treated. The well plate was incubated for 60 min at an anaerobic environment at 37°C. This procedure was performed thrice, with as many as two independent evaluations.

The incubated well plate was rinsed once with 100 µl of PBS suspension. Then, 5 mg/ml microtetrazolium (MTT) solution was freshly prepared based on the amount required in a 15-ml centrifuge tube wrapped with aluminum foil. To each well, 10 µl of the MTT solution was introduced and then the well-plate was incubated for 3 h at 37°C. Furthermore, as much as 100 µl of acidified isopropanol was added to each well. The well plate was placed above the shaker at room temperature for 1 h. The optical density was measured using a microplate reader at a 490-nm wavelength. Biofilm eradication was expressed in percent against the control using the following formula [14,15]:

$$= \left(1 - \left(\frac{OD\ sample - OD\ blank\ sample}{OD\ control - OD\ blank\ control}\right)\right) \times 100\%$$

Minimal biofilm eradication rate (MBEC) was determined based on the eradication value reaching 50% ($MBEC_{50}$) and 90% ($MBEC_{90}$).

ANOVA with *post hoc* analysis was used to analyze data obtained from the percentage measurement of biofilm eradication. Data were analyzed to determine whether there was a difference in the eradication percentage between the turmeric ethanol biofilm extract and chlorhexidine. The correlation test was used to determine whether there is a correlation between increasing turmeric ethanol extract concentrations and biofilm eradication percentages.

RESULTS

In this study, *S. sanguinis* and *P. gingivalis* were used at a bacterial concentration of 10^{-5}. The bacterial count of *S. sanguinis* and *P. gingivalis* at a 10^{-5} concentration was 63 and 40 colonies, respectively.

In the 4-h and 12-h phases of the *S. sanguinis* biofilm model, the percentage eradication of *S. sanguinis* biofilm due to turmeric ethanol extract was <50%. The 24-h biofilm model had concentrations of 0.5%, 20%, and 25%. In this study, the $MBEC_{50}$ of the turmeric ethanol extract in the 4-h and 12-h biofilms of *S. sanguinis* was 0.5%, whereas the $MBEC_{50}$ for the 24-h biofilm of *S. sanguinis* was 20%. In this study, the $MBEC_{90}$ of the turmeric ethanol extract in the 4-h biofilm of *S. sanguinis* was 15% whereas that for the 12-h and 24-h biofilm could not be established.

In the 12-h and 24-h biofilm models, the percentage of biofilm eradication due to the Javanese turmeric ethanol extract was smaller than that due to the positive control. In the 4-h biofilm model, the percentage of biofilm eradication due to the turmeric ethanol extract

was identified as equivalent to the positive control at 15–25% concentration. ANOVA showed no significant difference between the eradication percentage of 4-h biofilm after exposure to the turmeric ethanol extract (concentration, 10–25%) and the positive control ($P > 0.05$). In the 12-h and 24-h biofilm models, the percentage of biofilm eradication due to the turmeric ethanol extract was significantly different from that of the positive control ($p<0.05$).

A significant correlation was found between the concentration of the turmeric ethanol extract and percentage of biofilm eradication after 4 h and 12 h ($p<0.025$). Both correlations are strong and positive (Pearson correlation coefficient >0.5), whereas no significant correlation was found for the 12-h biofilm ($p<0.025$). Table 1 shows that an increase in biofilm formation time resulted in decreased efficacy of the turmeric ethanol extract in eradicating *S. sanguinis* biofilms.

In the 4-h and 12-h *P. gingivalis* biofilm models, the percentage of biofilm eradication due to the turmeric extract ethanol at all concentrations was more than 50%. However, for the 24-h biofilm model, the percentage of biofilm eradication at all concentrations of the turmeric extract ethanol did not reach 50%. Therefore, in this study, it was found that the $MBEC_{50}$ of the turmeric extract ethanol against *P. gingivalis* biofilms at 4 h and 12 h was 0.5%. However, the $MBEC_{50}$ against *P. gingivalis* biofilm at 24 h could not be determined. The $MBEC_{90}$ of the turmeric ethanol extract against *P. gingivalis* biofilm at 4 h was 0.5% whereas that against the biofilm model at 12 h and 24 h could not be established.

In biofilm models at 12 h and 24 h, the percentage of biofilm eradication due to the turmeric extract ethanol was smaller than that due to the positive control. However, in the biofilm model at 4 h, the percentage of biofilm eradication due to the turmeric ethanol extract was equal to that due to the positive control at all concentrations. ANOVA showed no significant difference between the percentage of biofilm eradication at 4 h due to the turmeric ethanol extract (at all concentrations) and the positive control ($P > 0.05$). However, for the 12-h and 24-h biofilm models, there was a significant difference between the percentage of biofilm eradication due to the turmeric ethanol extract and the positive control ($p<0.05$).

There was no significant correlation between increasing concentrations of the turmeric ethanol extract and the percentage of biofilm eradication at 4, 12, and 24 h ($P > 0.025$). Table 2 shows that the increase of biofilm formation time led to decreased efficacy of the turmeric ethanol extract in eradicating *P. gingivalis* biofilm.

In the combination biofilm model of *S. sanguinis* and *P. gingivalis* (4, 12, and 24 h), the percentage of biofilm eradication due to the turmeric ethanol extract at all concentrations was >50%. In this study, the $MBEC_{50}$ of the turmeric ethanol extract against the combined biofilm formation of *S. sanguinis* and *P. gingivalis* was 0.5% (4, 12, and 24 h). The $MBEC_{90}$ of the turmeric ethanol extract against the combined biofilm formation of *S. sanguinis* and *P. gingivalis* was 15% (4 h) whereas that in the 12-h and 24-h biofilms could not be established.

In the 12-h and 24-h biofilm models, the percentage of biofilm eradication due to the turmeric ethanol extract was smaller than that due to the positive control. In the 4-h biofilm model, the percentage of biofilm eradication in the turmeric ethanol extract at concentrations of 15–25% has equivalent number to control. ANOVA test result in Table 3 showed no significant difference between eradication percentage of 4-h biofilm from turmeric ethanol extract (all concentrations) with positive control ($P > 0.05$), whereas in the 12-h and 24-h biofilm, a significant difference was found between percentage eradication of biofilm from turmeric ethanol extract with positive control ($p<0.05$).

A significant correlation was found in the correlation test between the increase of turmeric extract ethanol concentration with eradication percentage of 4-h and 12-h biofilm ($p<0.025$). Both correlations were strong and positive (Pearson correlation > 0.5), whereas no significant correlation was found in the 24-h biofilm ($p<0.025$). The results showed that the increase in biofilm formation time led to decreased efficacy of turmeric extract ethanol in eradicating biofilm combination.

Table 1: Percentages of biofilm eradication using the Javanese turmeric ethanol extract toward the 4, 12, and 24-h *Streptococcus sanguinis* biofilms

Groups	Biofilm formation period (h)		
	4	12	24
Negative control	0	0	0
Positive control	95.93±0.45	92.97±3.07	84.25±4.08
IJTEE 0.5%	88.04±1.23[a,*]	77.28±3.41[a,*]	62.37±4.75*
IJTEE 1%	87.02±0.87*	79.28±4.10*	44.89±4.23*
IJTEE 5%	88.14±3.01*	69.29±4.04*	44.59±4.30*
IJTEE 10%	89.58±4.37	74.11±4.34*	47.54±7.57*
IJTEE 15%	92.62±2.00[b]	73.43±5.38*	47.83±4.41*
IJTEE 20%	92.00±3.46	80.92±1.43*	56.36±2.73[a,*]
IJTEE 25%	92.39±2.71	81.07±2.69*	62.61±4.22*

*p<0.05 compared to positive control. [a]$MBEC_{50}$, [b]$MBEC_{90}$. IJTEE: Identified Javanese turmeric ethanol extract, MBEC: Minimal biofilm eradication

Table 2: Percentages of biofilm eradication using the Javanese turmeric ethanol extract toward the 4, 12, and 24-h *P. gingivalis* biofilms

Group	Biofilm formation period (h)		
	4	12	24
Negative control	0	0	0
Positive control	94.41±3.71	91.94±1.58*	86.67±1.22*
IJTEE 0.5%	91.94±1.431	60.61±5.54[b*]	33.76±9.82*
IJTEE 1%	94.07±6.51	59.07±10.49*	32.59±13.58*
IJTEE 5%	92.00±1.47	65.99±7.24*	38.18±4.50*
IJTEE 10%	91.80±5.26	57.93±2.39*	34.74±6.56*
IJTEE 15%	95.70±2.65	56.60±2.78*	28.85±3.49*
IJTEE 20%	93.57±2.88	59.51±7.17*	36.92±3.69*
IJTEE 25%	93.08±2.18	64.48±10.64*	30.30±9.13*

*p<0.05 compared with positive control. [a]$MBEC_{50,}$ [b]$MBEC_{90}$. IJTEE: Identified Javanese turmeric ethanol extract, MBEC: Minimal biofilm eradication

Table 3: Eradication biofilm percentage from turmeric ethanol extract in the combined 4, 12, and 24-h *Streptococcus sanguinis* and *Porphyromonas gingivalis* biofilms

Group	Biofilm formation period (h)		
	4	12	24
Negative control	0	0	0
Positive control	93.85±0.28	89.89±4.06*	89.84±1.46*
IJTEE 0.5%	86.22±2.41[a]	60.15±5.05[a,*]	58.79±7.30[a,*]
IJTEE 1%	86.51±3.42	74.31±5.40*	61.94±7.33*
IJTEE 5%	88.22±0.40	77.16±2.78*	65.69±2.21*
IJTEE 10%	89.17±2.38	73.72±4.01*	67.75±4.18*
IJTEE 15%	91.91±2.05[b]	74.36±3.69*	66.40±3.75*
IJTEE 20%	94.64±4.90	75.07±4.40*	70.01±2.52*
IJTEE 25%	92.69±4.56	80.41±1.23*	64.10±7.07*

*p<0.05 compared with positive control. [a]$MBEC_{50,}$ [b]$MBEC_{90}$. IJTEE: Identified Javanese turmeric ethanol extract, MBEC: Minimal biofilm eradication

DISCUSSION

The oral biofilm is the main cause of various diseases in the oral cavity. The bacteria that make a biofilm are reported to be more resistant to antibacterial agents. One of the mechanisms to eliminate and reduce oral biofilm formation is by using an effective antiseptic mouthwash. Turmeric rhizome is reported to have antibacterial activity. A study reported that xanthorrhizol isolates from turmeric methanol extract contain an antibacterial effect on *S. sanguinis* and *Porphyromonas* [4]. A study by Reina (2015) reported that the MIC and MBC in turmeric

ethanol extract identified against *S. sanguinis* were 5% and 15%, respectively, whereas those identified against *P. gingivalis* could not be established.

A previous study has reported the sensitivity of *S. sanguinis* toward turmeric ethanol extract compared with *P. gingivalis* [7], which may be due to differences in physiology, bacterial morphology structure, and antibacterial action mechanisms of turmeric ethanol extract. Gram-positive bacteria cell wall has a simple structure compared with Gram-negative bacteria that has a more complex cell wall structure consisting of peptidoglycan, lipopolysaccharide, and lipoprotein [8]. It causes Gram-positive bacteria, such as *P. gingivalis,* to be more resistant compared with Gram-positive bacteria, such as *S. sanguinis*.

Turmeric ethanol extract against *Streptococcus sanguinis* biofilm

In this study, the efficacy from turmeric ethanol extract toward 4-h and 12-h *S. sanguinis* biofilms 4-h and 12-h is dose-dependent, whereas biofilm the effect of turmeric ethanol extract in the 24-h biofilm was not dose dependent. The efficacy of turmeric ethanol extract in eradicating 4-h *S. sanguinis* biofilms is equivalent to 0.2% chlorhexidine, whereas on 12-h and 24-h *S* biofilms, the efficacy of turmeric extract ethanol was smaller than 0.2% chlorhexidine.

The results showed that turmeric ethanol extract was identified to have greater eradication efficacy of biofilm on the initial phase of *S. sanguinis* biofilm formation. In the 4-h biofilm (adhesion phase), new *S. sanguinis* was suspected to attach to the well base and not yet produce EPS, which is one of the factors causing biofilm resistance. Thus, the turmeric ethanol extract molecules are identified to penetrate and eradicate biofilms. The 12-h biofilm or accumulation phase is a phase wherein bacterial attachment is still active. In this phase, bacterial colonization has been predicted to begin, and EPS formation begins [16]. The EPS matrix causes molecules from turmeric ethanol extract to be more difficult to penetrate and eradicate biofilms. In the 24-h biofilm (maturation phase), biofilms have been suspected to form an extracellular matrix. Biofilms that have formed EPS matrices are reported to produce peroxidase enzymes. Peroxidase enzymes can oxidize hydroxyl groups in xanthorrhizol to reduce antibacterial properties [16]. An interaction was found in *S. sanguinis*, implying the occurrence of resistance in biofilms that have matured [13].

Turmeric extract ethanol efficacy toward *Porphyromonas gingivalis* biofilm

In the 4-h, 12-h, and 24-h *P. gingivalis* biofilm, the effect of turmeric ethanol extract toward *P. gingivalis* biofilms is not dose dependent. In the 4-h biofilm model, *P. gingivalis* was predicted not to form a biofilm because *P. gingivalis* is some late colonizer bacteria; hence, biofilm formation is longer [17]. The test result with MTT showed no change from yellow to purple in the negative control group, indicating absence of live bacterial cell activity. The color change of the yellow MTT solution to purple indicates the presence of dehydrogenase enzyme activity in the living bacterial mitochondria [18]. In the 12-h and 24-h *P. gingivalis* biofilms, the efficacy of turmeric extract ethanol in eradicating the 12-h and 24-h *P. gingivalis* biofilms is lower than 0.2% chlorhexidine.

In this study, turmeric ethanol extract was shown to eradicate *P. gingivalis* biofilm in the early phase of biofilm formation. This result resembles the results of a study by Marina (2015) using the same turmeric ethanol extract on a *P. gingivalis* biofilm. In the 4-h biofilm (adhesion phase), suspected *P. gingivalis* has not yet formed a biofilm. Thus, in the 4-h *P. gingivalis* biofilm, the efficacy of turmeric extract ethanol was identified as incompatible with 0.2% chlorhexidine. Bacterial attachment is still active in the 12-h biofilm or accumulation phase. In this phase, *P. gingivalis* biofilms have been presumed to form on a well base. However, the *P. gingivalis* biofilm is not expected to form the EPS matrix; hence, the turmeric ethanol extract is still able to penetrate and eradicate the *P. gingivalis* biofilm. In the 24-h biofilm (phase maturation), biofilms have formed the EPS matrix. In addition, attachment and growth of biofilm metabolism become slower than in the accumulation phase. An interaction of *P. gingivalis* was observed in mature biofilms, implying the occurrence of biofilm resistance; hence, the turmeric ethanol extract can eradicate matured *P. gingivalis* biofilm less [13,16].

Turmeric extract ethanol efficacy identified toward combine biofilm

The effects of the identified turmeric ethanol extract were dose dependent in the 4-h and 12-h combination biofilm, whereas the effect of turmeric ethanol extract is not dose dependent in the 24-h biofilm combination.

The efficacy of turmeric extract ethanol is identified against a single 4-h biofilm of *S. sanguinis* and a 4-h combination biofilm equivalent to chlorhexidine. However, the 4-h *P. gingivalis* biofilm model cannot be compared because it is not yet forming a biofilm. Thus, to conclude against the 4-h biofilm, the efficacy of turmeric ethanol extracts in the eradication of single and combination *S. sanguinis* biofilms is the same. This is estimated because *S. sanguinis* bacteria are attached within only within 4 h to the well base because it is an early colonizer bacteria [16].

In the 12-h biofilm model, the efficacy of turmeric ethanol extract was identified in the eradication of single and combination *S. sanguinis* biofilms and unequal combinations with chlorhexidine. The study showed that turmeric ethanol extract was better in eradicating combination biofilm and single *S. sanguinis* compared with *P. gingivalis* biofilm. In the 24-h biofilm model, turmeric ethanol extract further erodes combined biofilms compared with single biofilms. An antagonist interaction between *S. sanguinis* and *P. gingivalis* bacteria was predicted to cause incomplete formation of the EPS matrix. Thus, the turmeric ethanol extract was able to eradicate the biofilms of *S. sanguinis* and *P. gingivalis* combinations compared with single biofilms of *P. gingivalis* and *S. sanguinis*. A study by Catalina *et al.* (2007) in clinical studies suggested that the presence of *S. chromosinis* has an influence on the presence of *P. gingivalis*. *S. sanguinis* was found more in healthy gingival sulcus compared with *P. gingivalis*. Otherwise, more *P. gingivalis* and less *S. sanguinis* were found in patients with periodontitis. *S. sanguinis* has been known to prevent periodontitis because of its ability to reduce the number of *P. gingivalis*. Another study by Zhang *et al.* (2000) showed that *S. sanguinis* reduced the number of *P. gingivalis* in their experiments in mice [19,20]. *S. sanguinis* can produce bacteriocin called sanguicin and hydrogen peroxide, which can inhibit the growth of *P. gingivalis* [12]. An *in vitro* study reported that proteins extracted from *S. sanguinis* bacteria had an antibacterial activity against *P. gingivalis* bacteria [21].

CONCLUSION

The results showed that identified Javanese turmeric ethanol extract (IJTEE) efficacy in eradicating biofilms is equivalent to chlorhexidine against the initial phase of biofilm formation. IJTEE is more effective against *S. sanguinis* biofilms than in *P. gingivalis* biofilms. Thus, turmeric Javanese ethanol extract can eradicate *S. sanguinis* and *P. gingivalis* biofilms.

CONFLICT OF INTEREST

The author reports no conflict of interest.

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