Chapter

Curcuma Xanthorrhiza Roxb. An Indonesia Native Medicinal Plant with Potential Antioral Biofilm Effect

Dewi F. Suniarti, Ria Puspitawati, Rezon Yanuar and Ranny R. Herdiantoputri

Abstract

Most common oral diseases are directly related to oral biofilm, a complex community of microorganisms inhibiting the oral cavity. Recent studies provide deeper knowledge on how free-floating bacteria form a structurally organized micro-ecosystem and on its pathogenicity and its self-defense mechanisms; thus, creating an understanding of the challenges in eliminating oral biofilm and maintaining the balance of oral ecosystem. Chlorhexidine has been the standard oral antimicrobial agent for decades. However, studies showed that it is less effective against bacteria in the form of biofilm that leads to an ongoing search of another method to fight against biofilm, including the use of plant-derived compounds. Medicinal plants are known to contain secondary metabolites, which are not only important in protecting the plant from any harmful environment but also potential as antimicroorganism and antioral biofilm for humans. *Curcuma xanthorrhiza* Roxb., containing xanthorrhizol (XNT), an essential bioactive compound, is an Indonesian native medicinal plant proven to have antibacterial and antibiofilm activities by several in vitro studies. The understanding of biofilm formation, its resistance to common drugs, and the potential role of *C. xanthorrhiza*-derived compounds as antibacterial and antibiofilm may contribute to developing *C. xanthorrhiza* into the alternative weapon against oral biofilm-related diseases.

Keywords: *Curcuma xanthorrhiza* Roxb., xanthorrhizol, oral biofilm, antibacterial

1. Introduction

Oral biofilm or dental plaque is the complex community of microorganisms that can be found on the surfaces of various orodental tissues, especially on tooth surfaces. It had become a common knowledge that oral biofilm directly causes several oral diseases such as dental caries, periodontal disease, i.e., gingivitis and periodontitis, and many other oral diseases [1]. Compared with the planktonic microorganism, oral biofilm is masses of bacteria that form structure known as extracellular matrix

(ECM), that allows microorganism to persist under environmental conditions, and able to resist antimicrobial drugs [2]. In biofilm, there is a unique cell-to-cell communication system, namely quorum sensing (QS) that allows bacteria to detect and respond to cell population density mediating gene expression [3, 4]. It has been reported that QS is also responsible in antimicrobial resistance through regulating bacteria multidrug resistance (MDR) efflux pumps, regulating biofilm formation, and regulating bacterial secretion systems [5–9].

For many decades, antimicrobial agents, i.e., chlorhexidine (CHX) have become the best weapon against bacteria in oral cavity. However, CHX is less effective against biofilm bacteria because of the drug resistance properties of biofilm [10, 11]. This condition led researchers to develop another method to fight against biofilm, including use of alternative drugs, such as plant-derived compounds or essential oils. On the other hand, medicinal plants or herbs have been proved empirically and scientifically to have some important biological activities. As antibacterial and antibiofilm, medicinal plant-derived compounds and essential oils could inhibit biofilm formation by inhibiting peptidoglycan synthesis, modulating QS, and damaging bacteria membrane structures [12, 13]. Nowadays the use of natural products and their derivatives in dentistry, especially to prevent dental caries, is receiving large attention [14]. Moreover, many studies have reported the effect of various medicinal plant extracts on inhibiting biofilm formation and inhibiting bacterial adhesion. These suggest that medicinal plant-derived compounds might become promising alternative therapy in dental care.

Curcuma xanthorrhiza Roxb., known as Javanese turmeric or "temulawak," is a native Indonesian medicinal plant, which has been utilized traditionally as an ingredient of *jamu* (Indonesia herbal supplement and medicine) [15]. Most people use the rhizome as they believe it has medicinal effect for stomach illness, liver ailments, constipation, bloody diarrhea, dysentery, arthritis, children's fevers, hypotriglyceridemic, hemorrhoids, vaginal discharge, rheumatism, and skin eruptions empirically [16, 17]. Furthermore, the beneficial medicinal effect of *C. xanthorrhiza* has been proven in scientific studies. *C. xanthorrhiza* has been confirmed to have pharmacology effects such as anti-inflammatory, antibacterial, antioxidative, neuroprotective, nephroprotective, antitumor, and hepatoprotective activities [18–22]. Recently, in dentistry scope, the development of *C. xanthorrhiza*-derived compound as antibacterial drug has been extensively studied especially in East Asia and Southeast Asia countries. Xanthorrhizol (XNT) is the one of main active compound isolated from the essential oil of the rhizomes of *C. xanthorrhizza*, has a variety of pharmacological activities, one of that is antibacterial effects [23]. The bactericidal and bacteriostatic activity of xanthorrhizol against several oral bacteria has been reported using planktonic or biofilm models and showed promising result.

Thus, the use of *C. xanthorrhiza*-derived compounds as antibacterial and antibiofilm agent could be advantageous because natural-based medicines have fewer side effects. In this chapter, we will outline and summarize about inhibition of biofilm formation, mechanism action, and potential roles of *C. xanthorrhiza*-derived compounds as antioralbacterial and antioralbiofilm.

2. Oral biofilm and the most common oral infectious disease

The human oral cavity is a dynamic environment, which houses the most diverse microbiota, inhabited by more than 700 species of bacteria that colonize in the

surfaces of both hard and soft tissues [24]. Inside the oral cavity there are two types of bacteria: a single free-living cell known as planktonic bacteria mostly found in saliva, and multicellular-living, where the cells are sessile and live in biofilm. Oral biofilm is a complex community of microorganisms, which are attached on the oral surface and embedded in an extracellular matrix. Thus, the biofilm-associated bacteria differ compared with the planktonic bacteria in many ways, for example, growth rate, gene expression, transcription, and translation because bacteria biofilm lives in different complex microenvironments due to higher cell density of heterogeneous bacteria community [25]. The formation of the three-dimensional structure of biofilm causes the bacteria to be protected from the various environmental stresses, such as antimicrobial drugs.

The development of oral biofilm is a multistep process. The initial stage is pellicle formation on tissue surface, which is composed of a variety of host-derived molecules and source of receptors such as mucins, agglutinins, proline-rich proteins, phosphate-rich proteins, and enzymes such as α-amylase that could be recognized by early colonizer. These receptors allow various planktonic bacteria, which have been classified as early colonizer, such as *Streptococci* species that constitute around 60–90% of the bacteria that first colonize the teeth, and other bacteria include *Actinomyces* sp., *Capnocytophaga* sp., *Eikenella* sp., *Haemophilus* sp., *Prevotella* sp., *Propionibacterium* sp., and *Veillonella* sp. [26]. However, at this stage, the bacteria are still susceptible against antimicrobial drugs, because the biofilm matrix structure is not completely formed.

The interaction between the early-colonizing bacteria has been shown to regulate many gene expression in response to the environment and provide specific direct binding sites (not through salivary glycoprotein for various other bacteria to colonize) and promote the development of biofilm. The bacteria that bind to this initial layer of biofilm are known as known as late colonizers such as *Fusobacterium nucleatum*, *Treponema* sp., *Tannerella forsythensis*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, etc. They recognize polysaccharide or protein receptors on the pioneer bacteria cell surface and then attach on them [26]. The presence of late colonizer bacteria causes the change of environment and proportional shift, for example, relative amount of *Streptococci* sp. and *Neisseria* sp. is decreased, while the amount of *Actinomyces* sp., *Corynebacterium* sp., *Fusobacterium* sp., and *Veillonella* sp. increases [27]. The proportional shift occurs due to the interaction between bacteria in the community and the change of environment in biofilm. The competitive and cooperative interaction in biofilm may be essential to develop a successful mixed-species colonization.

During biofilm formation, there's cell-to-cell communication in the biofilm called QS. This phenomenon is mediated through production and release of chemical signals by bacteria termed autoinducer (AI), as response to changes in bacterial density and environment in biofilm. This mechanism initiates modification in gene expression to regulate cell or group behavior. During the maturation biofilm phase, QS also plays an essential role in extracellular matrix (ECM) production [28]. The ECM is a mixture of secreted high-molecular-weight polymers produced by bacteria, consisting of three major components: extracellular polysaccharides (EPS), proteins, and extracellular DNA, which form a cross-linked meshwork that serves as a shield [29]. At this stage, the biofilms show maximum resistance to antimicrobial drugs. The presence of biofilm ECM represents a strong barrier. The molecules of antimicrobial drugs must diffuse through the biofilm matrix to inactivate the bacterial cells. The biofilm ECM contains numerous anionic and cationic molecules that can bind charged molecules

of antimicrobial drugs [30]. The resistance provided by ECM may be discouraged by longer exposure and higher concentration of antimicrobial drugs; however, the toxicity for oral application should be the main consideration.

The drug resistance of oral biofilm against antimicrobial drugs becomes the main problem in eliminating oral biofilm. Other mechanisms that have been proposed to explain how bacteria protect itself from the effects of antimicrobials such the ability to adapt to various stress responses; the decrease of growth rate and metabolism; efflux pump mechanism; and QS [7, 10, 31].

Dental caries is the most common oral infectious disease characterized by acidic damage on the tooth surface due to a localized structural demineralization that leads to cavitation [32]. The bacteria that are responsible for the initiation of such cavitation process are the acidogenic, Gram-positive, facultative anaerobic bacteria, *Streptococcus mutans*. *S. mutans* along with other species from the same genus, *S. mitis,* are some of the early colonizers of oral biofilm that provide adherence for other microorganisms promoting the growth and maturation of the biofilm. Recent study by Dongyeop Kim et al. [33] showed that the rotund-shaped biofilm with corona-like cell segregation where *S. mutans* located at the very core created a highly acidic region at the interface between the biofilm and enamel, resulting in the characteristics of localized demineralized surface as commonly seen in clinical setting [33]. Therefore, not only dental caries is a diet-dependent disease but also a biofilm-dependent disease [32]. As the understanding of the nature of dental caries grows, the approach of caries management has been shifted from the previously popular approach that focused more on the symptomatic treatment and removal of carious tissue to be replaced by artificial structure, to the current approach that emphasizes the preventive measures: restriction of dietary sugar consumption, removal of bulk bacterial mass through brushing, and reduction of cariogenic bacteria in dental biofilm through chemotherapeutic methods [34].

While dental caries is a result of a chronic destruction of the tooth hard tissue itself, periodontal disease on the other hand is an inflammatory disease of the surrounding tissue of tooth, which may result in loss of attachment, and induced and maintain by the resident of oral biofilm, especially the biofilm located in the gingival crevices that stay in contact with the gingival epithelium [35, 36]. Different from the microbes of the dental caries-related biofilm located on the tooth surface whose ability is to transform carbohydrate into damaging acidic substrates, the microbes of the biofilm in the gingival crevices gain their source of nutrient mainly from the protein-rich gingival cervicular fluid (GCF) accommodating the growth of Gram-negative bacteria, some of which are responsible for the progression of periodontal diseases [35]. Gram-negative, anaerobic, proteolytic bacteria, namely *P. gingivalis*, *Prevotella intermedia*, and *A. actinomycetemcomitans,* are mostly found in the periodontal biofilm and linked to periodontal diseases due to their ability to release toxins that induce host proinflammatory response, which in turn creates an ecological shift to a dysbiosis and causes damage to the periodontal structure [37, 38].

2.1 Current treatment and challenges using CHX and other antibacterial agents/mouth rinse

The general treatments of periodontal disease are mechanical debridement and ensuring that the proper oral hygiene is maintained by the patient. The use of antibiotics for periodontal disease other than aggressive periodontitis is still controversial to date [36]. Concern has been raised toward drug tolerance and resistance of periodontal bacteria. A study done in Colombia showed that bacterial isolates from subgingival

biofilm of patient with aggressive periodontitis (*A. actinomycetemcomitans, P. gingivalis,* and *Tannerella forsythia)* were resistant to amoxicillin, azithromycin, and metronidazole [39]. Considering the nature of periodontal biofilm, mechanical disruption of the biofilm's integrity and reduction of the biofilm mass prior to the administration of antibiotics are considered essential [40].

Although CHX is considered as the gold standard antimicrobial agent in the oral cavity, there are some drawbacks of its usage: the risk of extrinsic staining on tooth surface, alteration in taste perception, and increase in calculus formation [41, 42]. Moreover, the effectiveness of CHX for biofilm eradication is also questioned. Due to the fact that *S. mutans* is the early colonizer of dental biofilm and that it inhibits the lowest strata, administration of CHX results in a concentration gradient from the outermost surface of the biofilm toward its innermost area that in turn exposes the *S. mutans* to only subinhibitory concentration of CHX [33, 43]. This suggestion is supported by another research conducted by spatially mapping the architecture of dental biofilm, which found that the intact corona structure of biofilm that conceals *S. mutans* cells in the core beneath layers of other microbes provides enhanced antimicrobial tolerance against CHX [33]. On the other hand, increasing the concentration of CHX in the aim to eliminate the dental caries-related biofilm is not recommended because the wide spectrum nature of CHX will disturb the balance of the oral environment by perturbing the commensal microbiome. As a prevention of periodontal diseases, several studies found its benefit to prevent bacterial surface adhesion, thus preventing the biofilm formation [44]. However, when the biofilm has formed, Gram-negative bacteria such as *P. gingivalis* are able to secrete outer membrane vesicles to bind CHX and provide protection to the bacteria in the biofilm community [43].

To avoid the aforementioned side effects and concerns, treatment and prevention alternatives from many natural products, herbs, and medicinal plants, in the form of extracts and essential oils, have been developed. Medicinal plant's extract from *Acacia arabica*, *Tamarix aphylla* L., and *Melia azadirachta* L. showed evidence of reducing oral biofilm formation and cleaning the well-developed oral biofilm [45]. Medicinal plant from South East Asia, *C. xanthorrhiza* Roxb., has also been proven through several studies to have eradication and inhibition effects against oral bacteria and candida biofilm [23, 46–53].

3. *Curcuma xanthorrhiza* Roxb

Curcuma xanthorrhiza Roxb., known as Java turmeric or "temulawak," is a native Indonesian medicinal plant that is mainly cultivated in Southeast Asian countries such as Indonesia, Malaysia, Thailand, Vietnam, and Philippines. For a long time, it has been used to enhance the flavor and color of food. Moreover, this plant has been believed and utilized as medication and supplement [15, 17]. In a few decades, turmeric plants including *C. xanthorrhiza* became the main subject of interest in research because many of its biological activities have been confirmed by experimental scientific studies. In addition, *C. xanthorrhiza may* be used as a treatment for COVID-19 because of its ability to inhibit proinflammatory cytokines [54]. However, it's still requiring more evaluation, especially in the clinical trial setting. Thus, recently market demand for *C. xanthorrhiza* rhizome has increased globally.

C. xanthorrhiza is a low-growing plant (2–2.5 m) with a root known as rhizome that looks like ginger. This plant can grow in the lowlands to an altitude of 1500 meters above sea level and has a habitat in tropical forests. The main part of

C. xanthorrhiza that has been proved to have beneficial medicinal activity is rhizome [15]. The rhizome of *C. xanthorrhiza* contains terpenoid and curcuminoid compounds, which reportedly have beneficial properties such as antioxidant, anti-inflammatory, antitumor, and anticancer effects [18, 20–22, 55]. The shape of the rhizome of *C. xanthorrhiza* is oval round shape, 3–4 branched, and reddish brown, dark yellow, or dark green in skin color (**Figure 1**). The rhizome flesh is dark, orange or brown in color, has a sharp pungent aroma and tastes bitter.

3.1 Phytochemical properties of *C. xanthorrhiza* Roxb

The rhizome of *C. xanthorrhiza* contains curcuminoids (1–2%), essential oil (3–12%), xanthorrhizol (44.5%), and camphor (1.39%). Moreover, xanthorrhizol (XNT), a bisabolene-type sesquiterpenoid compound isolated from essential oil of rhizome's *C. xanthorrhiza*, had been well established to possess various medicinal effects XNT is one of the most explored and studied phytochemicals, especially its antibacterial, antifungal, and antibiofilm activity. The major group of secondary metabolites has been identified in the rhizome of *C. xanthorrhiza* and can be seen in **Figure 2** [17]. However, the variation of active metabolite of *C. xanthorrhiza* might be influenced by several external factors, such as climate, sun intensity, altitude, and temperature of cultivation. For example, the high percentage of starch is influenced by the altitude of cultivation. The bioactive compound XNT and curcuminoid also reported higher in low altitude, high temperature, and low rainfall [56]. Thus, these are the challenges for development standardization phytomedicine, because of the vast variation of external factor and the different method of cultivation in each site.

3.1.1 C. xanthorrhiza Roxb. *Extraction preparation*

The *C. xanthorrhiza*-derived products, such as extract or as pure compounds, have provided unlimited opportunities for new drug discovery. However, to take advantage of the beneficial effect of the medicinal plant, an extraction process is carried out to obtain the active secondary metabolite. The extraction solvent selection is very essential because it affects the stability and metabolite profiles that implicate the efficacy of medicinal plant extract. Several commonly used solvents are ethanol, methanol, dichloromethane, acetone, and water [57, 58]. Proper actions must be taken to assure that potential compound is not lost or destroyed during the extraction process.

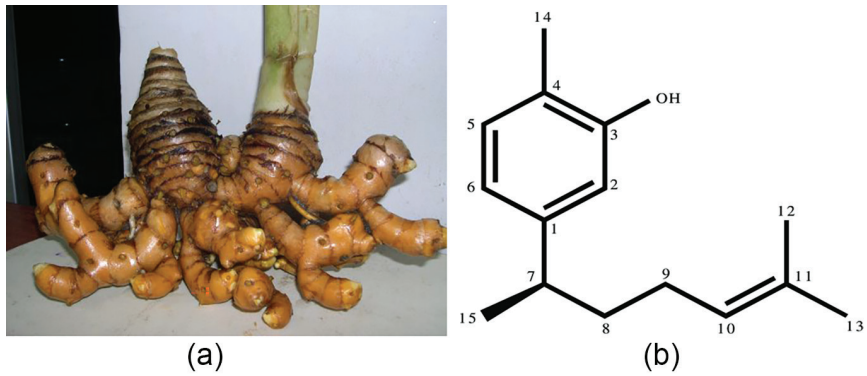


Figure 1.
(a) Rhizome of C. xanthorrhiza *Roxb. (b) Chemical structure of xanthorrhizol.*

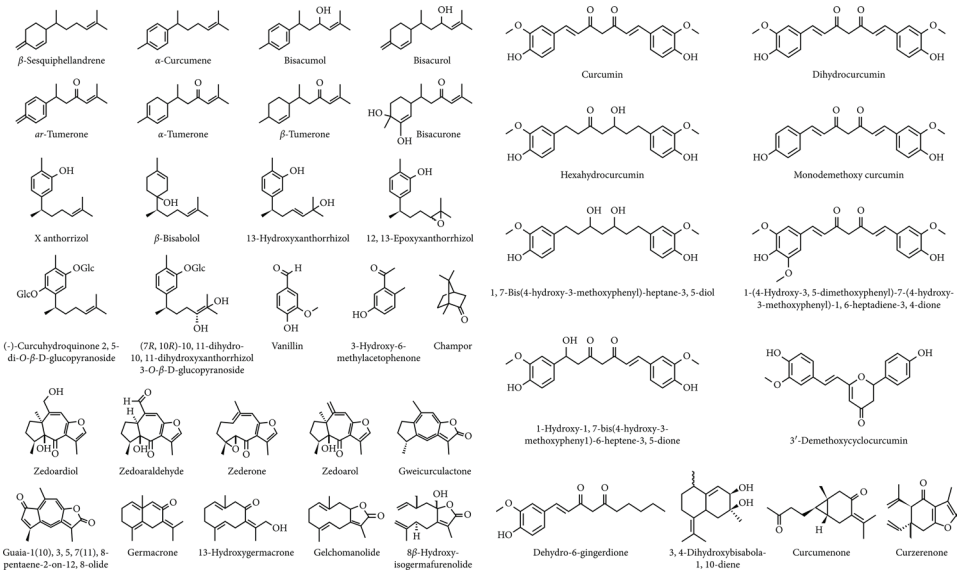


Figure 2.
Secondary metabolite compound of rhizome C. xanthorrhiza *Roxb.*

3.1.2 Xanthorrhizol isolate

XNT is an essential bioactive compound isolated from essential oil of rhizome *C. xanthorrhiza*. There are several methods used to extract the essential oil and XNT, i.e., supercritical fluid carbon dioxide extraction (SCFE-CO_2), Soxhlet extraction, and percolation process [59]. According to Salea et.al (2014), extraction using SCFE-CO_2 method will result in higher XNT compared with Soxhlet or percolation extraction method. Besides that, the conventional method to isolate XNT, which costs less, is still applicable and more efficient, while SCFE-CO_2 method is more applicable in large-scale production in the industry [59].

The interest in XNT as an antibacterial has attracted some researchers to develop as plant-derived drugs. The molecular weight and solubility of XNT are 218.33 g/mol and 28.90 μg/ml, respectively. This makes XNT have lower molecular weight and higher solubility compared with bioactive compound curcumin [60, 61]. Thus, it was expected that XNT might easily penetrate the surface of biofilm. According to the chemical structure, XNT and curcuminoid contain phenolic compounds and hydrocarbons.

4. Antibacterial and antibiofilm activity

4.1 Antibacterial

The antibacterial activities of *C. xanthorrhiza* have been studied using various preparations such as extract or fraction preparation and XNT isolation. *C. xanthorrhiza* extract and XNT have been reported to be effective against a variety of oral bacteria. They have been evaluated by standard in vitro susceptibility tests such as minimum inhibitory concentration (MIC) and minimum bactericidal concentration

(MBC). Our studies have shown that the effectiveness of *C. xanthorrhiza* ethanol extract against Gram-positive bacteria was superior compared with its effect against Gram-negative bacteria. In addition, the efficacy of *C. xanthorrhiza* extract and XNT against Gram-positive bacteria is comparable to CHX [48, 49, 52].

The antibacterial activity of C. *xanthorrhiza* is believed to emerge from XNT and curcuminoid compounds. The mechanism of action of phenol compounds, through interaction between the hydroxyl group (-OH) of the phenol compound with bacterial cells wall to facilitate hydrogen bonds subsequently causes alteration of bacterial membrane permeability. The high concentration of phenol can penetrate into cells subsequently leading to protein coagulation on the cell membrane and cell lysis [62, 63].

The Gram-negative bacteria are more resistant to phenol due to the complexity of their cell wall. Gram-positive bacteria possess thick cell walls containing many layers of peptidoglycan and teichoic acids. In contrast, Gram-negative bacteria possess thinner cell walls, but consist of a few layers of peptidoglycan surrounded by lipid membrane (lipopolysaccharides and lipoprotein). The complex cell wall of Gram-negative bacteria has been predicted to slow down the passage of chemicals. This was supported by a previous study by Inouye et al. [64], which concluded that the antibacterial effect of polyphenols was generally more effective against Gram-positive bacteria than Gram-negative [64].

XNT isolate is more effective against bacteria compared with the extract form. Since the crude extract contains various types of bioactive compounds or phytochemicals, usually unnecessary components are still carried away during the extraction process, for example, starch found in *C. xanthorrhiza* extract. Moreover, that unnecessary component has been suggested can affect the bioactive compounds activity. The XNT has been reported effective against several Gram-negative bacteria such as *Fusobacterium nucleatum* and *Enterococcus faecalis* [65, 66].

In addition, a clinical study evaluated the effectiveness of XNT, neem, cetylpyridinium chloride, and 0.2% CHX to decontaminate 60 children's toothbrushes after being used. Their result showed that the antimicrobial effect of XNT on *S. mutans* (78% reduction in *S. mutans*) was higher compared with CHX, but lesser than neem and cetylpyridinium chloride [67].

4.2 Antibiofilm

The *C. xanthorrhiza* extract and XNT also have been reported to have activity as antibiofilm against several oral bacteria in single species biofilm models. The antibiofilm activity of *C. xanthorrhiza* has been reported in various phases of biofilm formation. Rukayadi study reported that the activity of XNT as an antibiofilm was dependent on the concentration, exposure time, and the phase growth of biofilm. XNT is more effective in the early phase of biofilm formation [68]. Consistent with that, our study also demonstrated that the antibiofilm activity of *C. xanthorrhiza* ethanol extract is more effective in the early phase of biofilm formation. These indicate that the EPS matrix of mature biofilm implicates the resistance [46, 50, 51]. Although high concentration of XNT (1000 μg/mL) reportedly completely killed the biofilm, toxicity should be a major concern.

C. xanthorrhiza extract and XNT have been reported to inhibit several single species biofilm formations in in vitro study. Although not completely eliminated, bacteria were removed in the adhesion phase and early accumulation phase of biofilm development. The mechanism of inhibition biofilm formation is still not clear

yet. However, it has been reported that *C. xanthorrhiza* extract has shown to inhibit acid production of *S. mutans* biofilm [53]. Moreover, *C. xanthorrhiza* extract is also reported to have anti-QS or quorum quenching activity [69]. The high level of tannin, phenol, phenolic compound in *C. xanthorrhiza* is suggested to precipitate the proteins that are vital for *rhl* system in *Pseudomonas aeruginosa.* By inhibiting the *rhl* system, the swarming activity of *P. aeruginosa* is inhibited, thus the QS will not take place [69, 70]. Besides that, killing the cells by cell lysis will also degrade and detach the biofilm.

Besides inhibiting the biofilm formation, *C. xanthorrhiza* extract and XNT also reportedly can eradicate the mature biofilm. The in vitro study against single species 72-hour *S. mutans* biofilm model, treated with *C. xanthorrhiza* methanol extract, showed significant fewer colony forming unit (CFU). The TEM and SEM observation showed changes of peptidoglycan layer of *S.mutans* and fewer intact bacteria after treatment [53].

Because the biofilm matrix can limit the penetration of antimicrobial agents, Cho et al. [71] explored the nanoemulsion form of *C. xanthorrhiza* oil in order to facilitate the ease of penetration. The single species *S. mutans* biofilm model, which was treated with nanoemulsion of *C. xanthorrhiza* oil, showed higher dead cells compared with the live cells. Furthermore, quantitative analysis of live/dead biomass and biofilm thickness based on the CLSM images showed that the live/dead ratio with nano-emulsion treatment was 50% less compared with control. It was also reported that nanoemulsions, which were prepared using sonication, are more suitable to be used as antibiofilm materials than emulsions without sonication [71]. These results indicate that *C. xanthorrhiza* extract can penetrate the *S. mutans* biofilm and kill that cell.

Another in vitro study against root canal biofilm *F. nucleatum* presented that XNT at concentrations 1.25% and 1.5% reported similar eradication activity compared with 2.5% NaOCl [72].

The antibiofilm activity of *C. xanthorrhiza* extract and XNT has also been demonstrated in multispecies biofilm models. CLSM analysis demonstrated that biofilm treated with XNT at 2 and 10 μg/ml for 30 min results in reduced bacterial viability in a dose-dependent manner against saliva and multispecies oral biofilm. Moreover, when exposed to 1000 μg/mL XNT, all biofilm cells were completely killed. These results indicate that XNT provides antibiofilm properties by eradicating bacteria viability [73].

Generally, multispecies biofilms were considered to be more resistant to anti-biofilm agent compared with single species biofilms. To evaluate this notion, we tested dual species biofilm models (combination Gram-positive and Gram-negative bacteria) treated with *C. xanthorrhiza* ethanol extract, then measured the minimum of biofilm eradication (MBEC) using MTT-assay to assess the viability cell (**Table 1**). Our study demonstrated that *C. xanthorrhiza* ethanol extract was better eradicating dual-species biofilm (for example, *S. sanguinis* with *Porphyromonas gingivalis*; or *S. mutans* with *A. actinomycetemcomitans*), whereas not effective against single-species *P. gingivalis* biofilm nor single-species *A. actinomycetemcomitans* [46, 51]. This result may be possible due to the antagonist interaction between *S. sanguinis* and *P. gingivalis* that causes an incomplete formation of the EPS matrix surrounding the biofilm. It is supported by a clinical study by Stingu et al. [74], who reported that the presence of *S. sanguinis* has an influence on the presence of *P. gingivalis*, where *S. sanguinis* was found more in healthy gingival sulcus [74], while *P. gingivalis* vice versa. *S. sanguinis* also can produce bacteriocin called streptomycin and hydrogen peroxide, which can inhibit the growth of *P. gingivalis* [75].

No	Tested biofilm species	Effect	Reference
1	4–24 hr. *S. sanguinis* ATCC 10556	In the early phase of biofilm formation (4 hr), at concentration 15% shows eradicate biofilm equivalent to CHX. While in 12 hr. and 24 hr. biofilm formation, the $MBEC_{50}$ is 0.5% and 20%, respectively. However, the result at maximum concentration was smaller compared to CHX.	[46]
2	4–24 hr. *S. mutans* ATCC 25175	In the early phase of biofilm formation (4 hr) and 12 hr., at concentration 15–20% shows eradicate biofilm equivalent to CHX. While in 24 hr. biofilm formation, at concentration 20–25%, showed equivalent to CHX	[50]
3	4–24 hr. *Porphyromonas gingivalis* ATCC 33277	In 12 hr. biofilm formation, the $MBEC_{50}$ is 0.5%. However Not effective against 24 hr. biofilm formation. Only reduced <40% bacteria viability	[46]
4	4–24 hr. *Aggregatibacter actinomycetemcomitans* NCTC 9710	In 12 hr. biofilm formation, at concentration 20% the viability still 50%. However Not effective against 24 hr. biofilm formation. Only reduced <30% bacteria viability	[51]
5	4–24 hr. *S. sanguinis–P. gingivalis*	In the early phase of biofilm formation (4 hr), at concentration 15% shows eradicate biofilm equivalent to CHX. While in 12 hr. and 24 hr. biofilm formation, the $MBEC_{50}$ is 0.5%. However, the result at maximum concentration was smaller compared to CHX.	[46]
6	4–24 hr. *S. mutans–P. gingivalis*	In the early phase of biofilm formation (4 hr) and (12 hr), shows can eradicate biofilm. But in the mature phase (24 hr), it is not effective. Maximum concentration only eradicates 50% bacteria viability.	[50]
7	4–24 hr. *S. mutans–A. actinomycetemcomitans*	Only effective in the early phase of biofilm formation (4 hr), at maximum concentration reduce 90% the bacteria viability. While in 12 hr. and 24 hr. only reduce 50% and 20% bacteria viability, respectively	[51]

Table 1.
Effect of C. xanthorrhiza ethanol extract *against single and dual species biofilm.*

5. Conclusion

A fight against oral infectious disease is a fight against an adaptive, highly advanced, multispecies, pathogenic oral microbial community comprising oral biofilm. Inhibition and elimination of oral biofilm by means of preventing and treating oral diseases require pharmacological developments in finding alternative therapies that are able to dodge the defensive nature of oral biofilm and avoid cytotoxicity to the host while maintaining the homeostasis of the oral environment. *Curcuma xanthorrhiza* Roxb.–derived compounds such as XNT have been repeatedly proven to be a promising alternative therapy in dental care for its antimicrobial and antibiofilm activity. The phenolic compound of XNT has been proven to alter the permeability of the bacterial cell wall that leads to cell lysis. It is also proposed to prevent QS by inhibiting the swarming activity of bacteria. Further research to obtain the most effective form of compound and research in clinical settings are still needed to fully harness its potential.

Acknowledgements

The authors would like to thank the Universitas Indonesia for providing grants to our studies.

Author details

Dewi F. Suniarti[1]*, Ria Puspitawati[1], Rezon Yanuar[2] and Ranny R. Herdiantoputri[3]

1 Faculty of Dentistry, Department of Oral Biology, Universitas Indonesia, Jakarta, Indonesia

2 Faculty of Dentistry, Department of Pharmacology, Health Sciences University of Hokkaido, Tōbetsu, Japan

3 Department of Oral Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

*Address all correspondence to: dewisuniarti17@gmail.com

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