ACTIVITY OF PERICARP EXTRACT OF MANGOSTEEN AGAINST ORAL *STREPTOCOCCI*

(*AKTIVITAS EKSTRAK PERICARP BUAH MANGGIS MELAWAN STREPTOCOCCI ORAL*)

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Abstract

The increasing prevalence of dental caries is still as a major world health problem. Caries is the direct result of acid production by cariogenic oral pathogens, especially *Streptococcus mutans*. New and better antimicrobial agents active against cariogenic bacteria with minimal side effects on the oral tissues are much needed, especially natural agents derived directly from plants. Phytochemical studies have shown that the extracts from various parts of mangosteen or *Garciniamangostana Linn* tree contain varieties of secondary metabolites such as prenylated and oxygenated xanthones, many of which have been found *in vitro* to have antimicrobial properties against oral pathogens. Several studies which examined the eficacy of herbal for human health have shown that xanthones from mangosteen have remarkable biological activities such as antioxidant, antimicrobial, anticancer etc, and had no cytotoxic effects on human gingival fibroblasts. Their results showed that among these xanthone derivatives obtain from pericarp extract of mangosteen, α-mangostin has the most potent antimicrobial activity against cariogenic *Streptococcus mutans*. It can be concluded that the strong antimicrobial activity of the pericarp extract of mangosteen is a good drug of choice that might be helpful in preventing the dental caries.

Keywords: *Garciniamangostana Linn*; oral pathogens; *Streptococcus mutans*; anti-caries agent.

Abstrak

Meningkatnya prevalensi karies gigi masih sebagai masalah kesehatan utama dunia. Karies adalah hasil produksi asam oleh patogen oral kariogenik, terutama *Streptococcus mutans*. Agen antimikroba yang baru dan lebih baik, yang aktif terhadap bakteri kariogenik dengan efek samping minimal pada jaringan mulut sangat dibutuhkan, terutama agen alami yang berasal langsung dari tanaman. Studi fitokimia telah menunjukkan bahwa ekstrak dari berbagai bagian pohon manggis atau *Garciniamangostana Linn* mengandung varietas metabolit sekunder seperti *xanthones* yang teroksigenasi dan *prenylated*, banyak di antaranya yang telah ditemukan secara *in vitro* memiliki sifat antimikroba terhadap patogen oral. Beberapa penelitian yang meneliti khasiat herbal untuk kesehatan manusia menunjukkan bahwa *xanthones* dari manggis memiliki aktivitas biologis yang luar biasa seperti antioksidan, antimikroba, antikanker dll, dan tidak memiliki efek sitotoksik pada fibroblast gingiva manusia. Hasil mereka menunjukkan bahwa di antara turunan *xanthone* ini diperoleh dari ekstrak *pericarp* manggis, α-mangostin memiliki aktivitas antimikroba yang paling kuat terhadap kariogen *Streptococcus mutans*. Dapat disimpulkan bahwa aktivitas antimikroba yang kuat dari ekstrak *pericarp* manggis adalah obat pilihan yang baik yang mungkin dapat membantu dalam mencegah karies gigi.

Kata Kunci: *Garciniamangostana Linn*; *oral pathogens; Streptococcus mutans; anti-caries agent.*

INTRODUCTION

Dental caries is one of the most prevalent and costly infectious diseases in the world. Because treatment is expensive, prevention is a major goal in caries-control programs.[1] *Streptococcus mutans* is the most common cariogenic bacteria that produces several virulence factors.[2,3] The caries process can present in smooth-surface as coronal caries and also as root caries.[3,4]

Microbial adhesion onto surfaces and the subsequent formation of biofilms are critical concerns for many biomedical and dental applications. The initial adhesion and the successful colonization of bacteria onto solid surfaces play a key role in biofilm formation and the pathogenesis of infections related to biomaterials.[5] Dental plaque plays a primary role in the etiology of dental caries, so their biological and cariogenic properties are basic to caries prevention.[6]

Accumulation of *Streptococci* in dental surface is considered a critical factor for the development of cariogenic biofilms, due to the production of acids and a consequent oral pH reduction, leading to a higher probability of dental tissues demineralization.[7] Eradication of such biofilms is extremely difficult[8] so inhibition or elimination of *S. mutans* is important for dental plaque control and caries prevention.[9]

An investigation has been done to suppress the caries-related microorganisms in dentine lesions using chlorhexidine and antibiotic.[10] Other studies used antimicrobial agents derived directly from plants. They found that the pericarp extract of *Garciniamangostana Linn*has agents active against cariogenic bacteria andhas no toxic effects on the oral tissues.[9,11,12] Although mangosteen extract has a broad-spectrum antibacterial activity, its benefits in dentistry especially ininhibition or eradication of carious bacterial have never been reported.

CARIES

Dental caries is one of the most prevalent and costly infectious diseasesin the world[1] and is among the most common chronic infectious diseases occurring in humans.[2] The caries process can presents in smooth-surfaces as coronal caries[3] and also as root caries.[4] Differences in the natural history and clinical manifestation of coronal and root dental caries imply that their respective microbial etiologies may differ as well.[4]

DENTAL PLAQUE BIOFILM

The oral cavity is a unique environment, as different types of surfaces (hard, soft, natural and artificial) share the same ecological niche. In order to survive within this ‘open growth system’ and to resist shear forces, bacteria need to adhere either to soft or hard tissues. Adhesion of oral bacteria to acquired enamel pellicle (AEP) leads to development of dental plaque biofilm.[5]

Dental plaque is the tooth-associated biofilm consisting of a microbial community and a matrix of polymer of bacterial and host origin, which is also found on the various restorative materials introduced by dental treatment.[6]This biofilm is colonized by a complex, relatively specific and highly interrelated range of microorganisms. The presence of pathogenic microorganisms within biofilms can lead to pathological processes such as caries and periodontal disease.[7]

BACTERIA

The most common cariogenic bacteria associated with human dental caries are *Streptococcus mutans* and *Streptococcus sobrinus*[3,8] and implicated as the primary etiologic agents of coronal and root caries.[4]Kato et al (2004)[6] also wrote that *Streptococci* including both, *S. mutans* and *S. sobrinus*, have been well known as the group of oral microorganisms which have virulence factors related to cariogenicity. Studies showed that *S. mutans* accounts for 74 – 94 %is overwhelmingly associated with coronal caries development in diverse population. *S. sobrinus* is less prevalent and has been correlated with smooth-surface caries. These two organisms display differenced in initial colonization and virulence mechanisms.[3]

Early studies on root caries have indicated that some specific caries such as *Actinomy-cesviscosus* might be involved. Other studies have reported that these lesions are associated with a microbial comparable to that of coronal caries. However, in more recent studies, no species or group of microorganisms was uniquely associated with the initiation and progression of root lesions. And no clear consensusamong researchers concerning the nature of the microbiota associated with root caries; moreover, the information that is available is in some instances superficial.[4]

On the other hand, Nascimento et al (2004)[4] wrote that no genotype of *S.mutans* is uniquely associated with the initiation and progression of caries, and that root and coronal caries can emerge in the presence of a broad spectrum of *S. mutans* clones. Also *S.mutans* showed a significantly greater frequency of isolation (59,2 %) than the other mutans *Streptococci*. Each of the subjects harboured two to ten genotypes of *S. mutans*, randomly distributed in different sites.

PATHOGENESIS OF CARIES

Streptococcus mutans and *S. sobrinus*have been well known which have virulence factors related to cariogenecity.[6] Two major virulence attributes of these organisms are their capabilities for sucrose-dependent adhesion and for glycolytic acid production at low pH values, which leads to enamel demineralization.[1]

*Streptococcus mutans*can change dietary sugars to water-insoluble glucans, which promote bacterial adhesion and plaque development. It is also capable of fermenting dietary sugars into organic acids, resulting in tooth demineralization and caries formation.[9]*Streptococcus mutans* and *S. sobrinus* drop the plaque pH to low levels by producing acids from carbohydrates and survive in this acidic environment. They also produce extracellular polysaccharides which may promote the dissolution of the tooth surfaces by increasing the porosity of plaque matrix and permitting deeper penetration of sugar.[6]

This cariogenic biofilm is considered a critical factor, due to the production of acids and a consequent oral pH reduction, leading to a higher probability of dental tissues demineralisation.[7] Shemes et al (2010)[5] also wrote that this biofilm causing dissolution of enamel by acid end-products resulting from carbohydrate metabolism.

THE INHIBITION EFFORTS OF CARIES PROCESS

Stepwise excavation procedures are suggested for the management deep dentinal lesions not only in the first but also in the second dentition in order to induce self-repair and arrest of the carious process and thus to maintain pulpal vitality. This preservation technique implies the combination of four well-proven hypothesis:(1) removal of the soft central biomass in a first step reduces the degree of infection in the cavity, (2) application of an antibacterial material (for instance calcium hydroxide) aiming of the induction of tertiary dentine apposition, (3) remineralization of partially demineralised dentine, and (4) prevention of reinfection of the underlying dentine with microorganisms from the oral cavity.[10]

In operative dentistry the common concept of infection control is primarily to remove carious tissue mechanically during the excavation procedure. On the other hands, antibacterial treatment of the dentine possibly suppresses the growth of bacteria under existing restorations and thus minimizes the risk of recurrent caries and damage to the pulp. Different approaches have been described, among them the addition of chlorhexidine to glass ionomers or the incorporation of antibiotics in dental cements.[10]

Wicht et al (2004)[10] studied the short-term effect of a 1 % chlorhexidine and 1 % thymol-containing varnish (Cervitec) and a demeclocycline/triamci nolone-containing ointment (Ledermix) on the microflora of carious dentine during a stepwise excavation procedure. They found that application of ledermix paste additionally diminishes the total anaerobic and facultative anaerobic counts.

Kunze et al (2010)[8] studied the use of carolacton for the prevention of dental caries. Carolacton is a secondary metabolite from the mycobacterium *Sorangiumcellulosum* which has high antibacterial activity against biofilm of *S. mutans*. Their results indicated that carolacton only weakly inhibited growth under both aerobic and anaerobic conditions. Planktonic growth of bacteria was only slightly impaired. They also reported that bacteria embedded in biofilms are more than 1000-fold less susceptible to the effects of commonly used antimicrobial compounds than are their planktonic counter-parts.

Pereira et al (2011)[7] studied the effects of photodynamic inactivation (PDI) using methylene blue as photosensitizer and low-power laser irradiation on the viability of biofilm formed by *S. mutans*andbiofilms formed by *Staphylococcus aureus* and *Candida albicans*, both isolated and associated. They concluded that biofilms formed *in vitro* by *S. mutans* were sensitive to PDI mediated by methylene blue dye. So, it might be a useful approach for the control of oral biofilms.

Alagan et al (2017)[13] found that organic compounds in seaweeds possess antimicrobial activities. They met with success that crude methanol extracts obtained from *Ulvalactuca Linn* effective against variety of pathogenic bacteria, includes *Staphylococcus aureus*. They used the disc diffusion method.

PLANTS EXTRACT FOR CARIES INHIBITION

Essential oils, green tea polyphenols, have been shown to exhibit antibacterial activity against *S. mutans*.[9] Plants are an important possible source of new bioactive compounds to combat dental caries because they produce a wide variety of secondary metabolites, many of which have been found *in vitro* to have antimicrobial properties against oral pathogens.[1] Extract from the pericarp of *Garciniamangostana Linn* fruit has been demonstrated the antimicrobial activity against a wide variety of microorganisms including several Gram-positive and Gram-negative bacteria especially those associated with skin infection, diarrhea, tuberculosis, or acne.[11]

The active chemical components that are present in medicinal plants like *Garciniamangostana* were responsible for its antimicrobial activity. Xanthones are a secondary metabolite found in some higher plant that involves mangosteen. Xanthones could be isolated from peel, whole fruit, bark, and leaves of mangosteen.[11] Among xanthone derivatives from mangosteen extract, α-mangostin has been known to exert the most potent antimicrobial activity.[7,11] On the other hand, Jung et al (2006)[12] found that two of active isolates obtained from the pericarp of *Garciniamangostana*, α-mangostinand δ-mangostin, were found to be major components of the CH_2Cl_2-soluble extract of it. Therefore, these two compounds may be used as marker components for quality control of this botanical dietary supplements. Torrungruang et al (2007)[9] showed the antibacterial activity of mangosteen pericarp extract against cariogenic *Streptococcus mutans*.

Torrungruang et al (2007)[9] studied the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. mutans* ATCC 25175 and $KPSK_2$*in vitro*. They compared the effect of α-mangostin to chlorhexidine, an antiseptic commonly used and is considered a gold standard for an anti-plaque agent in plaque control, against both strains of *S.mutans*. The MIC of α-mangostinwere 0.625 ug/ml for both strains, and of chlorhexidine were 0.312 and 0.625 ug/ml for ATCC 25175 and $KPSK_2$. It means that its antibacterial activity as inhibition was stronger than or at least equivalent to those commercially available antibacterial substances. The MBC of this extract were 0.625 and 1.25 ug/ml for ATCC 25175 and $KPSK_2$ compared to of chlorhexidine was 0.625 for both strains. These results suggesting that it acted bactericidally against *S. mutans*.

The bactericidal effect of mangosteen extract at two-times MBC (2.5 ug/ml) concentration in their results required an exposure time of at least 90 minutes. When the concentration was increased to 256 times MBC (160 ug/ml) it still required more than 5 minutes to completely kill the bacteria.[9]

DISCUSSION

The increasing prevalence of dental caries is still as a major world health problem.[1] There has many efforts to inhibit or arrest the caries process, from an easy method for example using the chlorhexidine gargle, chlorhexidine incorporating with filling material[10], until some mechanical and chemical methods for example excavation the carious biomasstissue and applied the antiseptic varnish or antibiotic paste. Also,effort to use a metabolite, extracted from other bacteria, named carolacton[8], and a photodynamic inactivation using methylene blue as photosensitizer and laser irradiation.[7,9]There were evidence that chlorhexidine can cause undesirable side effects.[9] Up to now those methods still in trial process and costly expensive. New and better anti-microbial agents active against cariogenic bacteria with minimal side effects on the oral tissues are very needed, especially natural agents derived directly from plants.

Mustaqimah and Auerkari (2017)[14] wrote about antioxidants of plants could be used as future choice to prevent periodontal diseases and caries occurrence. They wrote that total antioxidantcapacity (TAOC) levels in the serum of healthy periodontal persons is higher than in the periodontitis patients. Antioxidants may protect cells from the damage caused and to stabilize free radicals. These free radicals damage may lead to cancer and some diseases, which could be involved in neuro-degenerative diseases. Those free radicals or reactive oxygen species (ROS) are involved in the pathogenesis of periodontitis. It has also been discussed that α-mangostin as antioxidant of *G. Mangostana L* possessed strong antibacterial activity against *S. mutans*. On the other hand, phytochemical studies have shown that the pericarp extract of *Garciniamangostana Linn* contain varieties of secondary metabolites such as prenylated and oxygenated xanthones, which have antimicrobial properties against oral pathogens, Gram-positive and Gram-negative. Several studies showed that these xanthones have remarkable biological activities such as antioxidant, anti-inflammatory, anticancer, anti-allergy, antifungal, and antiviral.[1,11] One of these xanthones, α-mangostin, has the most potent antimicrobial activity against *S. mutans*.[9,11]

Nguyen and Marquis (2011)[1] found that antimicrobial actions of α-mangostin against oral streptococci was due to some points of view. They studied in inhibition of glycolysis, inhibition of membrane-bound enzymes, inhibition of glycolytic enzymes, inhibition of alkali production, inhibition of respiration, and killing of *S. mutans* by α-mangostin. It is found that α-mangostin is a potent inhibitor of glycolysis with an IC_{50} in the micromoles per litre range. Its capacity for reducing acid production from glycolysis was enhanced at acid pH values. Also, biofilm cells had greater resistance to α-mangostin than cells in suspensions.

The ATPase, a membrane-bound enzyme of *S. mutans* cell membrane is highly sensitive to inhibition effort of α-mangostin, especially in F_0 component. F-ATPase is composed of a major completes: F_1, the cytoplasmic, catalytic domain; and F_0, the proton-conducting membrane domain.[1]

Acid production by oral S*treptococci* is the direct result of glycolytic sugar catabolism. The inhibition of acid production suggested that α-mangostin could possibly affect the activity of glycolytic enzymes. The glycolytic enzymes of permeabilities cells found to be most sensitive to α-mangostin were lactic dehydrogenase, aldolase, and glyceraldehydes-3 phosphate dehydrogenase.Alkali production by S. mutans UA 159 was markedly inhibited by α-mangostin, with an IC_{50} of less than 30 umol. L^{-1} for intact cells. Thus, α-mangostin inhibition of alkali production likely involves inhibition of both the membrane permease catalysing L-malate uptake and MLE.[1]

*S. mutans*is capable of high rates of oxygen respiration, mainly involving the cytoplasmic enzyme NADH oxidase, and aerobic growth of the organism appears to be associated with oxidative stress. The result showed that respiration of intact cells of *S. mutans* was repressed by α-mangostin at concentration higher than 30 umol. L^{-1} with an IC_{50} close to 100 umol. L^{-1}.[1]

α-mangostin was found bactericidal for suspension cells at an initial concentration of 24 umol. L^{-1} with a D value time (for 90 % killing of the population) of approximately 5.8 min. This relatively rapid killing by α-mangostin suggests that killing could be due to irreversible inactivation of glycolysis.[1]

It also reported that pericarp extract of *Garciniamangostana Linn* had no cytotoxic effects on human gingival fibroblasts and in other human tissue cells for example heart tissues.[1] From all above data reports we can concludethat this plant extract could be used for preventing and arrest the caries process. As the bactericidal effect of *G.mangostana Linn* extract at lower concentration, two times of MBC or 2.5 ug/ml, required an exposure time of at least 90 minutes; and higher concentration, 256 times of MBC or 160 ug/ml, required more than 5 minutes to completely kill the *S.mutans*[9], it is needed to get a vehicle system to deliver the antibacterial agent to the oral cavity, since brushing or rinsing usually takes only a few minutes.

As the conclusions, the pericarp extract of mangosteen or *Garciniamangostana Linn* has strong and most potent antimicrobial activity against *Streptococcus mutans*.Xanthones from these extracts might be a good drug of choice and might be helpful in preventing and arrest the dental caries process, both in smooth surface or in dental niches. It is suggested to use xanthone in some experimental studies to reduce or arrest the dental caries process in community to get good achievement.

REFFERENCES

1. Nguyen PTM, Marquis RE. Antimicrobial actions of α-mangostin against oral streptococci. Can J Microbiol2011;57:217-25.
2. Wang B-Y, Deutch A, Hong J, Kuramitsu HK. Proteases of an Early Colonizer Can Hinder *Streptococcus mutans* Colonization *in vitro*. J Dent Res2011;90(4):501-5.
3. Li Y, Caufield PW, RedmoEmanuelsson I, Thorngvist E. Differentiation of *Streptococcus mutans* and *Streptococcus sobrinus* via genotypic and phenotypic profiles from three different populations. Oral MicrobiolImmunol 2001;16:16-23.
4. Nascimento MM, Hofling JF, Goncalves RB *Streptococcus mutans* Genotypes Isolated from Root and Coronal Caries. Caries Res2004;38:454-63.
5. Shemesh M, Tam A, Aharoni R, Steinberg D. Genetic adaptation of *Streptococcus mutans* during biofilm formation on different types of surfaces. BMC Microbiol 2010;10:51-60.
6. Kato K, Sato T, Takahashi N, Fukui K, Yamamoto K, Nakagaki H. A Method for Mapping the Distribution

Pattern of Cariogenic Streptococci within Dental Plaque in vivo. Caries Res 2004;38:448-53.
7. Pereira CA, Romeiro RL, Costa ACBP, Machado AKS, Junqueira JC, Jorge AOC. Susceptibility of *Candida albicans*, *Staphylococcus aureus*, and *Streptococcus mutans* biofilms to photodynamic inactivation: an in vitro study. Lasers Med Sci 2011;26:341-8.
8. Kunze B, Reck M, Dotsch A, Lemme A, Schummer D, Irschik H, et al. Damage of *Streptococcusmutans*biofilms by carolacton, a secondary metabolite from the myxobacterium*Sorangiumcellulosum*. BMC Microbiol 2010;10:199-11.
9. Torrungruang K, Vichienroj P, Chutimaworapan S. Antibacterial activity of mangosteen pericarp extract against cariogenic *Streptococcus mutans*. C U Dent J 2007;30:1-10.
10. Wicht MJ, Haak R, Schutt-Gerowitt H, Kneist S, Noack MJ. Suppression of Caries-Related Microorganisms in Dentine Lesions after Short-Term Chlorhexidine or Antibiotic Treatment. Caries Res 2004;38:436-41.
11. Vishnu PriyaV, Jainu M, Mohan SK, Saraswathi P, Gopan CSVS. Antimicrobial activity of pericarp extract of *Garciniamangostana Linn*. Int J Pharm Sci Res2010;1(8):278-81.
12. Jung H-A, Su B-N, Keller WJ, Mehta RG, Kinghorn AD. Antioxidant Xanthones from the Pericarp of *Garciniamangostana* (Mangosteen). J Agric Food Chem 2006;54:2077-82.
13. Alagan VT, Valsala RN, Rajesh KD. Bioactive chemical constituent analysis, *in vitro* antioxidant and antimicrobial activity of whole plant methanol extracts of Ulvalactuca Linn. B J Pharm Res 2017;15(1):1-14.
14. Mustaqimah DN, Auerkari EI. Antioxidants of plants as future choice to prevent periodontal diseases and caries occurence. Seminar in 2nd molecular and celullar life sciences. Surabaya, 2017