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ANTIBACTERIAL ACTIVITY OF PAPAIN AGAINST STREPTOCOCCUS MUTANS ATCC 25175

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ABSTRACT

Papain is an enzyme extracted from papaya plants (Carica papaya L), including Caricaceae family. This enzyme shows a broad proteolytic activity against the protein, short chain peptides, amino acid ester and amid, including bacterial cell wall. The purpose of this study is to produce a proper papain concentration to inhibit the growth of or kill Streptococcus mutans. The type of research is an experimental laboratory by determining the minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) with a dilution method, and measured using a microplate reader. Papain’s minimum inhibitory concentration (MIC) papain against Streptococcus mutans was 7.5% and the minimum bactericidal concentration (MBC) was 15%. Papain has antibacterial activity to Streptococcus mutans.

INTRODUCTION

Papain is an enzyme derived from the papaya plant (Carica papaya L), including family Caricaceae (Ming et al., 2002 and Aravind et al., 2013). Indonesia is the 5th largest country in the world in papaya product after Brazil, Nigeria, Mexico and India. Utilization of papain in Indonesia is still very little (Muhidin, 1999). Papain has a molecular weight of 23.406 daltons, pH and temperature optimum between 3-9 and 65-80°C. Papain is a cysteine protease hydrolase enzyme is very stable and active, which consists of 212-218 amino acids and shows a strong degree of homologous (Ming et al., 2002 and Amri et al., 2012). Papain shows broad proteolytic activity against the protein, short chain peptides, amino acid ester and amid as well as widely used in the fields of food and medicine, also is biocompatible to the soft tissue (Sunarintyas, 2003). Papain can break peptide bonds involving amino acids, especially arginine, lysine, and phenylalanine residues that follow (Amri et al., 2012). Papain is a proteolytic enzyme, derived from papaya latex and most powerful enzyme produced from all parts of the papaya plant. Papain is bactericidal, bacteriostatic, anti-inflammatory and debride material and shows a broad proteolytic activity against the protein, short chain peptides, amino acid ester and amid (Osato et al., 1993 and Mahmood et al., 2005). The main bacteria that play role in the formation of caries is Streptococcus mutans, due to its ability to produce extracellular polysaccharides called glucans/fructans. High acidogenic Streptococcus mutans reach the terminal pH 3.4 within 18 hours of growth in broth medium. These bacteria have the ability to survive in an acidic environment (aciduric) and stay alive at low pH for a longer period than other oral Streptococcus species (Hamada and HD Slade, 1980 and www.mecriticalcare.net).

MATERIALS AND METHODS

The object of this study is papain powder (76220-25G) derived from Carica papaya (Sigma Co.) and stored at 2-8°C. The bacteria used were Streptococcus mutans ATCC 25175. Bacteria grown in Muller-Hinton medium (MH) plus sucrose at 37°C in an facultative anaerobic (5% CO₂).

Rejuvenation Streptococcus mutans

A swab of bacterial cultures were taken with a sterile wire loop (osee) and plated on Muller Hinton media. Then covered with parafilm and sterilized wire loop back. After it was
incubated at 37°C for 48 hours in an anaerobic (5% CO₂) it can be stored in the refrigerator for a certain period of time.

**Preparation of Streptococcus mutans Liquid Culture**

A small amount of a bacterial culture was taken with a sterile wire loop, incorporated into Muller Hinton liquid media, incubate at 37°C with 150 rpm for 24 hours.

**Determination of Optical Density (OD)**

Label liquid culture of *Streptococcus mutans* incorporated into the 1 ml cuvette as a blank. Calibration is done in a form, then set the OD at a wavelength of 600 nm with a UV-VIS spectrophotometer, absorbance recorded.

**Determination of Minimum Inhibitory Concentration (MIC)**

Liquid culture of *Streptococcus mutans* created with a turbidity of Mc Farland 0.5. Prepared microwell plate formats: media + samples, media, media + sample + bacteria, media + bacteria (made Duplo) (Figure 1) (Kaya et al., 2012).

![Figure 1. Minimal inhibitory concentration (MIC) of papain with dilution method on 96 well microplate](image)

MH liquid media pipette into a microwell plate 150μL. Then, pipette 150μL sample (papain), put in a microwell plate and performed a serial dilution of 12 times dilution. Liquid culture of *Streptococcus mutans* 10μL pipetted and put in a microwell plate. After it was incubated at 37°C for 24 hours in a state of facultative anaerobic (5% CO₂). Appointed and read the results with microplate reader at a wavelength of 630 nm, absorbance recorded.

**Determination of Minimal Bactericidal Concentration (MBC)**

MIC results from each well that there is no bacterial growth, as 100μL pipetted into petri dishes containing solid media (agar). Then spread evenly over the entire surface so the cup. After it was incubated for 24 hours at 37°C in an facultative anaerobic, if not clearly visible colonies grew, incubated for 2x24 hours (Kaya et al., 2012 and Hosgor et al., 2011).

**RESULTS**

The MIC of papain against *Streptococcus mutans* with initial concentration of 60% and a 24-hour incubation was 7.5% (Table 1 and Figure 2).

![Figure 2. The MIC of papain against Streptococcus mutans in 96 well microplate](image)

The MBC of papain against *Streptococcus mutans* with an incubation of 24 hours was 15% (Figure 3).

![Figure 3. The MBC of papain against Streptococcus mutans on Muller Hinton media](image)

**Table 1. The MIC of papain against Streptococcus mutans using a microplate reader**

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Inne S. Sasmita et al. Antibacterial activity of papain against Streptococcus mutans ATCC 25175
DISCUSSION
The minimum inhibitory concentration (MIC) papain against Streptococcus mutans was 7.5%, whilst the minimal bactericidal concentration (MBC) is 15%. Although papain is a proteolytic enzyme that has the characteristics of bactericidal, bacteriostatic and anti-inflammatory, requires a high concentration to inhibit the growth of and kill Streptococcus mutans Bharwaj et al. (2012) compared the antimicrobial activity of 2% chlorhexidine (100%), extract of Morinda citrifolia (86.02%), aloe vera gel (78.9%), papain gel (67.3%) and calcium hydroxide (64.3%) against Enterococcus faecalis. Phankhongsap et al. (2012) compared the effectiveness of the antimicrobial between papain with mangosteen pericarp extract and papain with propolis extract against mixture Streptococcus gordonii and Enterococcus faecalis with the inhibition zone size 11.25±0.66 and 10.42±0.72 mm, respectively. Minimum inhibitory concentration of the two materials were 25 mg/ml, while the MBC were 50 mg/ml.

The mechanism of papain through the cysteine-25 of the triad in the active site that can attack the carbonyl carbon in backbone of peptide chain so that frees the amino terminal. When this occurs in the peptide chain of the protein, the protein will be degraded. The breakdown of peptide bonds involving Cysteine-25 and deprotoation by histidine-159. Aspargin-175 helps orientation of the imidazole ring of histidine-159 resulting in deprotoation (Amri et al., 2012). All three of these amino acids work together in the active site so that the function of this enzyme is unique. In the active side of papain, Cysteine-25 and histidine-159 has activity as an active catalytic thiolate-imidazolium (Ming et al., 2002). Three-dimensional structure of papain consists of two structural domains with pocket of them. Pocket that contains the active site triad containing the same catalyst with chymotrypsin. Catalytic triad is composed of three amino acids; cysteine-25, histidine-159, and aspargin-175. Papain can catalyze peptide bond in proteins into simpler compounds such as amino acid and dipeptide (Ming et al., 2002). Papain works only in infected tissues due to deficiency of plasma anti-proteases called alpha 1 tripsyn. When alpha 1 tripsyn does not exist, papain will break down collagen molecules (Bharwaj et al., 2012; Phankhongsap et al., 2012 and Flindt, 1978).

Streptococcus mutans is the main bacteria causing caries with cell surface layer consists of 4 components: peptidoglycan, polysaccharide antigen, a protein (glycoprotein) and teichoic acids and glycerol from lipoteikhoit. Peptidoglycan cell wall of bacteria serves to protect the internal osmotic pressure, resulting molecules to the cytoplasm. Proteins and polysaccharides are synthesized in the membrane. Research ferritin labelled antigen showed that the fimbrin is mainly composed of proteins, polysaccharides and teichoic acid. Open space between the fimbrin and peptidoglycan cause bonding of bacteriophages. The relationship of these polymers in the walls will give the information about the breakdown of polysaccharides and proteins by proteolytic enzymes (Hamada and HD Slade, 1980). The use of papain in Dentistry is still rare, Sunarintyas used papain as an artificial teeth cleaning and has done biocompatibility test. Biocompatibility tests showed that exposure to papain 15.66 TU mg is not cytotoxic.

Skin tests and specific IgE examination in the blood serum showed that papain exposure does not cause hypersensitivity reactions in healthy people, except for allergy sufferers with a probability of 4.16%. Siregar et al. (2011) studied the difference effect between liquid of papaya extracts and papain enzyme to inhibit the growth of plaque and Streptococcus alpha in removable space maintainer.

REFERENCES
Phankhongsap A, Pattama C, Apa J, Jomjai P. Antimicrobial effectiveness of root canal irrigant from mangosteen pericarp extracts with papain and propolis extracts with papain on mixture of Streptococcus gordonii and Enterococcus faecalis. 1st Mae Fah Luang University International Conference. 2012.
Flindt, M. Allergy to alpha-amylose and papain. Lancet 1(8131): 1407. 1978