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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 debridement 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).

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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% CO_2).

Rejuvenation *Streptococcus mutans*

A swab of bacterial cultures were taken with a sterile wire loop (oese) 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% CO_2), 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).

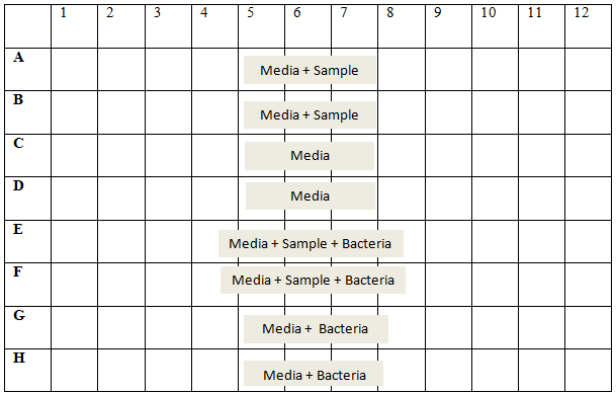

Figure 1. Minimal inhibitory concentration (MIC) of papain with dilution method on 96 well microplate

MH liquid media pipette into *a microwell plate* 150µL. Then, pipette 150µL sample (papain), put in *a microwell plat e* 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% CO_2). 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 e 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).

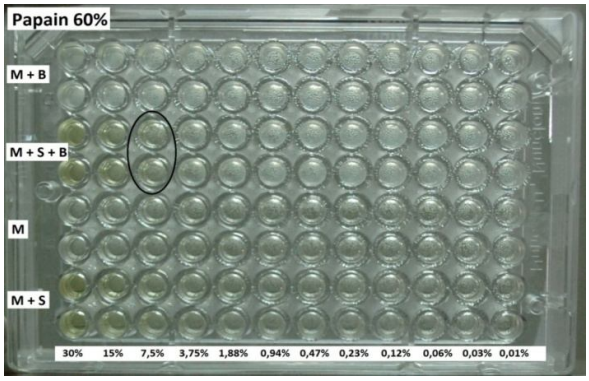

Figure 2. The MIC of papain against *Streptococcus mutans* in 96 *well microplate.*

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

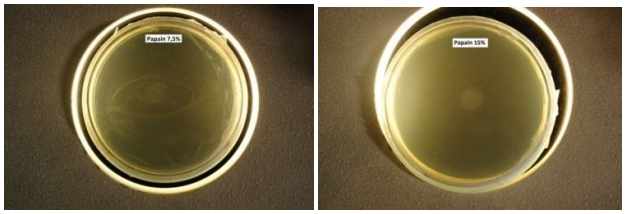

Figure 3. The MBC of papain against *Streptococcus mutans* on Muller Hinton media

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	0,059	0,053	0,052	0,048	0,046	0,048	0,047	0,047	0,047	0,05	0,048	0,046
B	0,063	0,049	0,047	0,049	0,046	0,046	0,045	0,045	0,045	0,045	0,046	0,045
C	0,045	0,044	0,041	0,043	0,043	0,041	0,043	0,043	0,041	0,042	0,043	0,047
D	0,045	0,042	0,044	0,04	0,041	0,042	0,045	0,042	0,043	0,042	0,042	0,047
E	0,061	0,056	0,052	0,073	0,093	0,132	0,156	0,173	0,156	0,147	0,14	0,143
F	0,061	0,055	0,051	0,073	0,097	0,116	0,138	0,125	0,15	0,15	0,141	0,132
G	0,132	0,132	0,132	0,128	0,117	0,116	0,117	0,129	0,115	0,108	0,114	0,13
H	0,123	0,13	0,131	0,129	0,134	0,13	0,113	0,127	0,123	0,109	0,116	0,121
%conc.	30	15	7,5	3,75	1,875	0,9375	0,4688	0,2344	0,1172	0,0586	0,0293	0,0146

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* Bharwajd *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 inhibiton zonw 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 degradated. The breakdown of peptide bonds involving Cysteine-25 and deprotonation by histidine-159. Aspargin-175 helps orientation of the imidazole ring of histidine-159 resulting in deprotonation (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 anti-trypsin. When alpha 1 anti-trypsin does not exist, papain will break down collagen molecules (Bharwadj *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 lipoteikhoik. 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 fimbria is mainly composed of proteins, polysaccharides and teichoic acid. Open space between the fimbria 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[5] 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.

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