

## Antibacterial Activity of Cherry Leaf (*Muntingia Calabura L.*) Extract on *Porphyromonas Gingivalis* Growth

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### Abstract

Pathological inflammatory condition that often complained and neglected in Indonesian is periodontal disease . The main ethiology of periodontitis is dental plaque. *Porphyromonas gingivalis* is one of anaerobic Gram-negative bacteria which contained in dental plaque. Increase in *P. gingivalis* colony will escalate damage in periodontal tissues. One of the alternative to control microorganism growth is by antibacterial agents in cherry leaf based material which contain flavonoids, saponins and tannins as active substance. Objective: the aim of this study was to determine the effect of cherry leaf extract to *P. gingivalis* growth and to determine the maximum concentration in inhibiting the growth of *P. gingivalis*. Methods: This research used experimental laboratories methods with the post test only control group design. This research used well diffusion with 8 samples of each group. BHI-A was filled into petridish and inoculated by *P. gingivalis*. The holes was made 5 mm diameter and was filled 20µl cherry leaf extract concentration 100%, 50%, 25%, 12,5%, 6,25%, aquadest steril (K-) and Chlorhexidine (K+). Petridish was placed in desicator and incubator at 37°C for 24 hours. Zone of inhibition were measured by using digital caliper. Result and Conclusion: Cherry leaf extract contain antibacterial effect which can inhibit *P. gingivalis* increase in number with maximum concentration of 100%..

### 1. INTRODUCTION

Oral health is an important part in general aspect of life, including good digestion,

articulate speaking, and good communication to socialize. Unfortunately, the oral health problems both in developed and developing

country still has less concern even often, being ignored (Kwan et al, 2005). For example, on 2011, *Survei Kesehatan Rumah Tangga* (Household health survey) Depkes RI stated that 60% Indonesia's citizen has suffered periodontal disease (Kementrian Kesehatan Republik Indonesia, 2011).

Periodontal disease is chronic inflammation disease in periodontium, which could affect gingiva and even underlying tissues such as alveolar bone (Newman et al, 2015). There are two classification of etiology of this disease, which are local factor (dental plaque) and sistemic factor that could related to each other. In mature dental plaque, the proportion of gram-negative bacteria such as *Porphyromonas gingivalis* (*P. gingivalis*) is higer and could induce the disease. *P. gingivalis* virulence factor such as fimbriae, protease, lipopolysaccharide and hemagglutinin could start the tissues destruction and interfere with host defense mechanisms (Andrian et al, 2006). The higher *P. gingivalis* colonization, the more the destruction will become.

Plaque control must be performed to inhibit the dental plaque maturation, which could be done mechanically or chemically. Tooth brushing is primary method, but in some people there is lack of motivation to continue this method so the chemical agents could be used to maximize the plaque control (Menon and Ramamurthy, 2014). Chlorhexidine mouthwash is proved effective to hold off both gram-positive and negative bacteria growth (Gupta, 2012). However, long term-used chlorhexidine may affect the tooth colour, mucosa desquamation, and interrupt the oral microorganism balance. Therefore, many natural products have been observed as an alternative agent, such as cherry tree (*Muntingia Calabura L*).

Cherry tree is a useful as a shade and often found on the edge of the sidewalk, parking lot, and on the banks of the river is not terrible or in places that usually dry prolonged Cherry leaf contains flavonoid, saponnin, tannin which are well-known for their antibacterial and

antiinflammation properties. Flavonoid release the transduction energy towards bacteria cytoplasm and inhibit bacteria mobility. Tannin could destruct the protein structure of bacteria. Recent study shows that the cherry leaf extract inhibit the glucosyltransferase activity of *Streptococcus mutans* (Isnarianti et al, 2013). However, this effect still has not yet observed towards *P. gingivalis*. Therefore, based on the content of cherry leaf and previous study, we want to observe the antibacterial activity of this leaf towards *P. gingivalis* growth.

## 2. MATERIALS AND METHODS

This experimental study was using *P. gingivalis* ATCC 33277 colonies which have already fulfilled the 0,5 McFarland standard with 0,05 absorbance at 560 nm wavelength. There were three groups; experimental (P), positive (K+) and negative (K-) control group which are consisted of eight samples in each group. The K+ group was tested with chlorhexidine, K- group was tested with sterile aquadest, and the P group was tested with cherry leaf extract in different concentrations (100%, 50%, 25%, 12,5%, 6,25%). Well-method was used to observe the antibacterial activity of each treatment.

Bacterial colony and plant have already been identified first, followed by the plant extraction process. We used the the 3rd, 4th, and 5th cherry leaves from the tip at 16.30 p.m. The leaves were washed by running water and dried with oven at 40-500C for 24 hours (Majidah, 2014). After being dried off, the leaves were blended and sieved (80 mesh) until the simplicia was formed. The simplicia was soaked into 96% ethanol for 72 hours followed by filtering process. The results product was being concentrated with rotary evaporator which produced the viscous extract that could be diluted later.

25 ml BHI-A was poured into steril petri dish in  $\pm$  5 mm thickness. The *P. gingivalis* colony was inoculated by using cotton bud to strike the media surface. The wells were made

with 5 mm diameter sterile borer. 20  $\mu$ l of 100%, 50%, 25%, 12,5%, 6,25% cherry leaves extract was poured into the P group well (P100, P50, P25, P12,5 and P6,25 respectively) with yellow tip micropipette. In K(-) group, the well was poured with sterile aquadest while in K+ group, chlorhexidine was being used. After the treatment was performed, all petri dishes were collected into the desicator and incubated on 37°C for 24 hours. The inhibition zone which formed around the well was measured with digital calipers. The data was collected and analyzed by using SPSS software

### 3. RESULTS

Figure 1 shows the antibacterial activity of cherry leaf extract (*Muntingia calabura* L.) towards *P. gingivalis* growth. Figure 2 shows the histogram of *P. gingivalis* inhibition zone in each groups

Table 1 describes the result of statistical analysis of Mann Whitney test which shows, there are significance difference ( $p < 0,05$ ) but not in all groups.

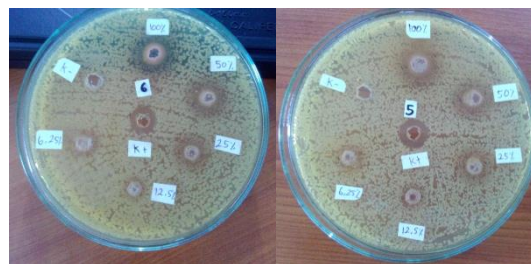
**Table 1.** Mann Whitney analysis of cherry leaf extract inhibition zone toward *P. Gingivalis*

Groups	K+	K-	P 100	P 50	P 25	P 12,5	P 6,25
K+	-	0,000*	0,002*	0,505	0,234	0,000*	0,000*
K-	0,000*	-	0,000*	0,000*	0,000*	0,000*	0,000*
P 100	0,000*	0,000*	-	0,000*	0,000*	0,000*	0,000*
P 50	0,505	0,000*	0,000*	-	0,001*	0,000*	0,000*
P 25	0,234	0,000*	0,000*	0,001*	-	0,000*	0,000*
P 12,5	0,000*	0,000*	0,000*	0,000*	0,000*	-	0,007*
P 6,25	0,000*	0,000*	0,000*	0,000*	0,000*	0,007*	-

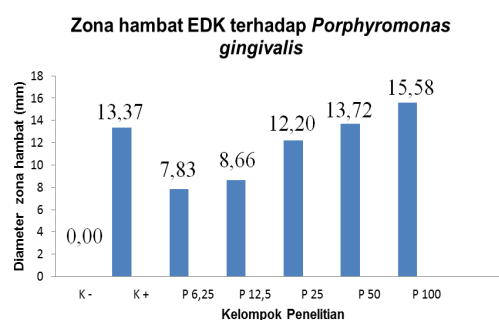
(\*) shows the significant difference ( $p < 0,05$ )

### 4. DISCUSSIONS

Based on the results, cherry leaf extract (*Muntingia calabura* L) showed antibacterial activity towards *P. gingivalis* growth which is in correspondence to Mintowati et al. (2013) who stated cherry leaf extract contains flavonoid, triterpene, steroid, saponnin, and tannin. These active compounds are potent as antibacterial, antioxidant and antiinflammation agent and their properties will be highly



**Figure 1.** Inhibition zone of cherry leaf extract in different concentrations 100%, 50%, 25%, 12,5%, 6,25%, sterile aquadest and chlorhexidine



**Figure 2.** Histogram of mean inhibition zone in each groups toward *P.gingivalis*.

optimized after being extracted (Mintowati et al. 2013)

In this study we used maceration method and ethanol that serve as solvent to pull out the polar, non-polar, or semipolar active compound (Poeloengan, 2007). This method showed good outcome because the active compounds in cherry leaf extract were being completely extracted with the result of the formation of inhibited zone in all experiment groups (Susanti, 2009).

Nazri et al. (2011) stated that diameter of inhibition zone is in correspondence with its antibacterial strength. There are three categories which are 15-20 mm (strong) ,10-14 mm (medium), and 0-9 mm (weak). Figure 2. shows that antibacterial strength of P 12,5 and P 6,25 group were considered weak; P 50 and P 25 group were medium; while P 100 group were considered strong.

Cherry leaf extract exclude antibacterial activity from its flavonoid compound by inhibiting the DNA and RNA formation through A and B ring in hydrogen-bond. This could increase the accumulation of nucleic acid and base which could destroy the cell wall permeability, lysosome and microsomes (Cushnie and Lamb, 2005). Saponin compound can disrupt the *P. gingivalis* membrane permeability, so the important structures such as protein and nucleotide might be leaked out (Agung et al, 2013). Tannin can stop the bacterial adhesion and transportation of enzyme and protein in cell membrane (Cushnie and Lamb, 2005).

In K+ group, chlorhexidine gluconate 0,2% was used because it is often being used in dentistry as a cleansing or therapeutic agent (Lindskog et al, 1998). Antibacterial mechanism of chlorhexidine is through the disruption of cell membrane and stimulate the interaction between positive and negative ion of phosphate in bacterial cell. This interaction may open the pathway so the chlorhexidine might penetrate the cell and toxified it (Filho et al, 2008). Meanwhile, there is no inhibition zone in K- group because the sterile aquadest does not possess antibacterial properties.

Based on statistical analysis, there are significance differences almost in all groups, however there is none between the K+ group and P50 group also in K+ and P25 group. Compare to other concentration, 100% group was showing the largest inhibition zone (15,58 mm). Therefore, 100% cherry leaf extract is the maximum concentration that shows the strongest inhibition towards *P. gingivalis*

growth and could be developed as an alternative agents to chlorhexidine gluconate.

## 5. CONCLUSIONS

Cherry leaf extract (*Muntingia calabura L.*) has antibacterial activity towards *P. gingivalis* growth. The maximum concentration was 100%. We suggest to perform toxicity test of cherry leaf extract towards the cell, understand the relationship between cherry leaf extract towards other oral pathogens, isolate the active compound of cherry leaf extract in order to reveal cherry leaf extract positive effect toward oral health.

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