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THE INFLUENCE OF VARIOUS CONCENTRATED CHERRY *(MUNTINGIA CALABURA)* EXTRACT IN PREVENTING *LACTOBACILLUS ACIDOPHILUS IN VITRO*

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ABSTRACT

Background: Dental caries is a multifactorial illness caused by bacteria. *Lactobacillus acidophilus* is one of the bacteria causing caries, where it continues the caries process. That is why the antibacterial agent is needed. A cherry (*Muntingia calabura*) is fruit having many benefits for health, which one of them is as an antibacterial agent. It contains flavonoid, tannin, alkaloid, and terpenoid, which can function as the antibacterial agent. **Aim** :The study aimed to determine the influence of cherry (*Muntingia Calabura*) extract in preventing the growth of *L.acidophilus*. **Method**: *True experimental* laboratory with a *post-test only control group design* and 24 samples. Cherries were extracted by using maceration. Then, the phytochemical test was conducted to discover antibacterial substances. The antibacterial test was done by using the dilution method to know Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). **Results**: Minimum Inhibitory Concentration (MIC) could not be seen, and Minimum Bactericidal Concentration (MBC) showed that all concentration 6,25%, 12,5%, 25%, and 50% and positive control were able to kill a bacterium *L. acidophilus*. **Conclusions**: There is an influence of cherry extract on the grow
Keywords: cherry extract, antibacterial, and *Lactobacillus acidophilus*

INTRODUCTION

The dental and oral health are significant things to consider, because if the problems occur in that are, it will affect the other body organs. The general dental and oral problems which can be occur is caries. According to Riskesdas (2018), the prevalence of caries in Indonesia 88,8%, this number indicates that the caries problem in Indonesia is still in high prevalence.

The dental caries is defined as a damage on tooth tissue which is affected by biolfilm, since the carbohydrate fermentation which results to acid, as lactic acid which is produced by the bacteria in oral cavity. The bacteria which plays a role to form caries are S.mutans and Lactobacilli within plaque. While, Streptococcus mutans plays a role to initiate the formation of caries and Lactobacilli plays a role to continue the formation of caries.

Lactobacilli is a gram-positive bacteria which usually in non-motile or non-spore and result lactic acid as their main product from the result of fermentation metabolism. The lactobacilli is rarely found before the growth of caries lesion and on the beginning of caries formation. This bacteria is considered as microorganism initiator in the process of caries formation, especially in dentin. The role of Lactobacilli in this formation varies its species, one of them is Lactobacillus acidophilus. L. achidophilus is a type of bacteria which is identified on the saliva of caries people in the high volume.

The prevention on caries occurrence can be conducted in several ways including to the plaque control by brushing tooth and

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applying anti-bacterial agent. The use of anti-bacterial agent is still the first choice, because on the caries the bacteria plays the important role. On the other hand, the anti-bacterial agent is still not optimal, for instance the anti-bacterial agent is less effective because of bacterial immunity, as well as other side effects like dental staining. From those problems the researchers intend to look for the natural anti-bacterial agent which is expected to be effective and not cause to side effects.

Muntingia calabura is a type of plant from tropical America, which is familiarly known as Jamaican cherry, in Indonesia, this plant is known as *talok* or *kersen* (cherry). In a glance, this plant does not seem significant, but in fact, this plant has many advantages. Traditionally, the leaf extract and bark of cherry is benefitted as antiseptic in Peru. The cherry flower is benefitted to medicate headache and flu in Philippine. The cherry is also benefitted in food. But, in fact, the cherry itself contains many benefits for health as an anti-bacteria to heal uric acid, calm headache, overcame diabetes, anti-cancer, anti-inflammation, and heal digestive problems as diarrhea. Lately, a research has proven that the activity of anti-bacteria in the cherry extract is able to hamper several types of bacteria as *Escherichia coli*, *Alcaligenes faecalis*, *Bacillus megartarium*, *Bacillus subtilis*, *Klebseilla pneumoniae*, *Staphylococcus aureus* and *Salmonella typhmirium.* The previous research has also proven that if the cherry extract results anti-bacterial activity in order to obstruct the bacteria as *Pseudomonas aeruginosa* and *Streptococcus pyogenes.*

The cherry extract also contains active ingredients as tannin, alkaloid, terpenoid, flavonoid, and steroid. Those active ingredients are able to take role as anti-bacteria, which include to alkaloid, tannin, terpenoid, and flavonoid. Flavonoid has mechanism as the anti-bacteria which involves to hamper nucleic acid synthesis, energy metabolism, and cytoplasm membrane function. Terpenoid itself has unclear mechanism, but people have speculated the membrane obstruction is through lipophilic compound. Alkaloid has mechanism of obstruction in several ways as to obstruct nucleic acid synthesis and hamper Z ring. Tannin itself has mechanism to hamper bacteria by binding bacterial protein, obstruct enzyme, and interfere membrane.

Hence, this research is conducted by the researchers to identify the effectiveness of cherry extract (M.calabura) on the growth of L.acidophilus.

MATERIALS AND METHODS

This research was an experimental laboratorial research which exerted the post-test control group design. This research employed sample of cherry, particularly the species of Muntingia Caabura, next, it was extracted in maceration method. The total sample in this research were 24 fruits which would be divided into 6 groups. The treatment group was consisted of 4 levels of extract concentration: 25%,12,5%, 25%, and 50%. The control group was consisted of clorhexidine 0,2% as the positive control and sterile aquades as the negative control. The testing method of anti-bacteria was conducted in dilution and through two stages, first minimum inhibitory rate and second minimum kill rate. The minimum inhibitory rate was done in group of treatment which would be inserted into test tube and added 0,1 ml of L. achidophilus suspense in MRS-B, and homogenized by exerting vortex. Next, the reaction or test tube would be incubated in temperature of

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37°C for 24 hours. After that, it would be examined, compared to the KHM control which was determined by the tube which consisted of lowest drug concentration and was still able to obstruct the growth of germ. Further, for the minimum kill rate was conducted from each tube, it would be taken approximately 1 ose suspense of L.acidophilus and craved on MRS-A media. The next step, it was incubated in temperature 37°C for 48 hours, then, the lowest concentration would be examines in this step where the growth of germ colony was not existed, which was referred as KBM of cherry extract to L. acidophilus.

This research exerted descriptive analysis to analyze the research data. This research has acquired Ethical Clearance from the Ethical Commission of Health Research of Medicine Faculty of Diponegoro University and research permit in Microbiology Laboratory of Medicine Faculty of Diponegoro University, Semarang 112/EC/H/KEPK/FK-UNDIP/VIII/2019.

Findings

The product of cherry extract has been tested quantitatively and contained of flavonoid, terpenoid, alkaloid, and tannin as the table below:

Table 1. Phytochemical Test on Cherry Extract

Parameter	Value of Analysis Result
Flavonoid	493,821mg/100g
Terpenoid	0,325%
Alkaloid	0,229%
Tannin	54,434g/100g

Based on the KHM valuation, it showed no identification that all concentration of cherry extract and chlorhexidine 0,12 were able to obstruct the growth of L. acidophilus or not, because the media use was dark colored and the extract of cherry resulted to the dark color as well.

Table 2. KHM and KBM Test on Cherry Extract to L. acidophilus

Group	N	KHM Test (Clear)	KBM Test (Sterile)
Chlorhexidine 0,12%	4	-	4
Cherry extract with concentration level 6,25%	4	-	4
Cherry Extract with concentration level 12,5%	4	-	4
Cherry Extract with concentration level 25%	4	-	4
Cherry Extract with concentration level 50%	4	-	4

- = no identification

The KBM test or valuation showed that the cherry extract on all levels of concentration from the lowest to the highest level 6,25%, 12,5%, 25%, and 50% which have been inoculated by L. acidophilus bacteria was able to kill L. acidophilus bacteria and indicate that all sterile cups were same in the positive control of chlorhexidine 0,12% during 5 times of repetition.

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Table 3. KBM Test on Cherry Extract to L. acidophilus

Repetition	Concentration 6,25%	Concentration 12,5%	Concentration 25%	Concentration 50%
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+

+= ability to kill (no growth)
-= inability to kill

DISCUSSION

This research was in vitro experimental research which exerted L. acidophilus as the sample of gram-positive bacteria that has been tested through cherry extract in gradual concentration. The product of cherry extract has been made 100% in Drug and Herbal Laboratory of Medicine Faculty of Diponegoro University and tested quantitatively which contained of flavonoid, terpenoid, alkaloid, and tannin.

Based on the phytochemical test of flavonoid content on the cherry extract, it contained 493,821 mg/100g. Flavonoid was the main polyphenol class in structure C6C3-C6. The anti-bacterial activity from flavonoid could be given in there ways, as to kill the bacteria immediately, activate antibiotic, and weaken bacterial pathogenicity. According to a literature study which has stated that if the anti-bacterial mechanism of flavonoid covered to 3 aspects, as to ruin cytoplasm membrane which was caused by the perforation or decline of membrane fluidity, hamper the nucleic acid synthesis in form of topoisomerase obstruction, and obstruct energy metabolism through reductase inhibition of NADH- Cytochrome. Another research has also asserted that the anti-bacterial mechanism of flavonoid also consisted of inhibition of attachment and formation of bio-film, inhibition of porin on membrane cells, change from membrane permeability, and attenuation of pathogenicity.

Based on the phytochemical test of terpenoid content on cherry extract, it contained 0,325%. Terpenoid was the plant aroma which known as quinta essential or essential oil fraction. The mechanism from this compound was still controversial, but a speculation said that this terpenoid was able to hamper by interfering that membrane cell with lipophilic compound. The study from this literature has also demonstrated if the terpenoid was also able to against bacteria by interfering membrane cells, so it could affect bacteria permeability and osmoregulation ability or ability to remove toxic materials.

Based on the phytochemical test of alkaloid content on cherry extract, it contained 0,229%. Alkaloid was heterocyclic nitrogen compound which comprised of various groups of chemical nitrogen compound. Alkaloid has different anti-bacterial mechanism based on its class, which comprised of indolizidine, isoquinoline, quinolone, agelasine, and polyamine. This indolizidine worked by hampering nucleic acid synthesis, since it hampered the enzyme of dehydrofolate reductase in cell free test (assay free cell). Isoquinolone was able to obstruct by interfering Z ring and cell division. On the quinolone class, it has procedure to obstruct topoisomerase enzyme 2 and hamper breathing by reducing bacterial consumption

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of O2. Agelasine referred the anti-bacterial effect by hampering BCG 3185C enzyme. While, polyamine has procedure to hamper the bacteria by interfering outer membrane and integrity of cytoplasm membrane.

Based on the phytochemical test of tannin content on the cherry extract, it contained 54,434g/100g. Tannin was the common name to call a group of primary phenolic substance which could precipitate gelatin from the solution, which conceived astringent. Tannin itself has anti-bacterial mechanism as it has been explained in quinone, it might be related to their ability to deactivate microbe adhesion, enzyme, and cell envelope transport protein. They were complex to polysaccharides. The significance of anti-microbial mechanism from this activity has not explored specifically. The direct inactivation evidence of microorganism: the low concentration of tannin modified the tube morphology of Crinipellis perniciosa germ.

On the previous research which had conducted phytochemical test on the cherry extract but only qualitatively, it showed whether it has resulted to any content or not. This research was the first research which employed phytochemical test in quantitative.

The research on KHM test could not conclude on which concentration, the cherry extract was able to obstruct L. acidophilus bacteria. It was because the media use, MRS-A was in dark colored and the product of cherry extract was also in dark colored. Therefore, it was not indicated to the clear change and identified to which concentration the cherry extract could hamper L.acidophilus bacteria.

Another research on inhibitory power of cherry extract has been done by Novia, et al which referred that the cherry extract was able to inhibit the bacteria as S. pyogenes and P. aueroginosa. Besides, other research done by Singh R., et al also indicated that the ability to hamper bacteria as B. megarterium and B. subtilis through diffusion method. The diffusion method was only able to identify anti-bacterial activity, but was unable to identify whether it could inhibit or kill the bacteria or not.

The research on KBM concluded that all concentration of cherry extract were able to kill L. acidophilus bacteria. This statement was proven by no growth of L. acidophilus on MRS-A media that have been colonized by cherry extract on all levels of concentration for four times of repetition. The researcher employed extraction maceration method by exerting alcohol solvent to adapt with the previous researches. Until recently, there was no research which investigate the killing power of cherry extract to L. acidophilus, thus, it could not be compared to the other researches.

However, this research has weaknesses and restriction in the data collection method. This research exerted in vitro method, therefore, the research finding could not immediately applied to the human. The other weakness in this research was that the result of cherry extract in dark color, therefore, it could not identify the inhibition power of cherry extract to the bacteria. The probability of dark color which was resulted from the cherry extract because of various kinds of compound as oil or other compounds within the extract which have ability to inhibit, help or no ability at all, if the compound was able to inhibit or interfere, it would be usually done by compound separation or redistillation.

CONCLUSION

This research referred to the effect of cherry extract in several levels of concentration to the growth of Lactobacillus

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acidophilus. It was only able to be valued from the minimum killing rate of cherry extract, the cherry extract was able to kill L. acidophilus on all levels of concentration 6,25%, 12,5%, 25%, or even 50%. But, the minimum inhibition rate was not identified yet in this research, because the product was in dark colored.

REFERENCES

1. Badan Penelitian dan Pengembangan Kesehatan (Badan Litbangkes).Laporan Riskesdas 2017, Kemenkes RI. 2018;
2. Lehmann JH. Caries Process and Prevention Strategies: The Environment. JAMA J Am Med Assoc. 1970;214(12):2115–2115.
3. Chen F, Wang D. Novel technologies for the prevention and treatment of dental caries: a patent survey. Expert Opin Ther Pat. 2010;20(5):681–94.
4. Ahirwar SS, Gupta MK, Gupta G, Singh V. Screening, Isolation and Identification of Lactobacillus Species from Dental Caries of Children. Int J Curr Microbiol Appl Sci. 2017;6(1):497–503.
5. Badet C, Thebaud NB. Ecology of Lactobacilli in the Oral Cavity: A Review of Literature. Open Microbiol J . 2008;2(1):38–48. A
6. Kracher CM. Current Concepts in Preventive Dentistry.Dental Care.com. 2012;1–28.
7. Isnarianti R, Wahyudi IA, Puspita RM. *Muntingia calabura* L Leaves Extract Inhibits Glucosyltransferase Activity of *Streptococcus mutans*. J Dent Indones. 2013;20(3):59–63.
8. Pratama Y. Info Artikel. 2015;4(2252):3–6.
9. R. Singh, et al. Phytochemical Analysis of *Muntingia calabura* Extracts Possessing Anti-Microbial and Anti-Fouling Activities. Int J Pharmacogn Phytochem Res. 2017;9(6):826–32.
10. Anjani Novia dkk. Sabun Mandi Cair Antibakteri Dari Ekstrak Buah Kersen (*Muntingia Calabura L*). Indones J Chem Sci. 2016;5(3):225–8.
11. Pandey AK. Perspective on plant products as antimicrobials agents: A review. Pharmacologia. 2013. 4(7):469–80.
12. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. Curr Med Chem. 2015;22(1):132–49.
13. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564–82.
14. Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int J Antimicrob Agents. 2014;44(5):377–86.
15. Karpiński TM, Szkaradkiewicz AK. Microbiology of dental caries. Journal of Biology and Earth Sciences 3(1):21–4.
16. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet (London, England). 2007 Jan 6 [cited 2019 Apr 7];369(9555):51–9.
17. Dasar Dasar Karies - Edwina A.M. Kidd, Sally Joyston Bechal - Google Buku .[cited 2019 Apr 7].
18. Walley S. Essential microbiology for dentistry, 4th edition. China:Churchill Livingstone Elsevier. 2012. 350.
19. Roman-Mendez C, Badet C, Yanez A, Dominguez ML, Giono S, Richard B, et al. Identification of oral strains of Lactobacillus species isolated from

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Mexican and French children. J Dent Oral Hyg. 2009;1(1):009–16.
20. File:*Lactobacillus acidophilus* (259 08) *Lactobacillus acidophilus* (Döderlein bacillus).jpg - Wikimedia Commons. [cited 2019 Mar 24].
21. Mahmood ND, Nasir NLM, Rofiee MS, Tohid SFM, Ching SM, Teh LK, et al. *Muntingia calabura*: A review of its traditional uses, chemical properties, and pharmacological observations. Pharm Biol. 2014;52(12):1598–623.
22. Jasmine R, Selvakumar B, Daisy P. Investigating the mechanism of action of terpej. ,noids and the effect of interfering substances on an indian medicinal plant extract demonstrationg antibacterial activity. Int J Pharmaeutical Studies Research. 2011;2(2):19–24.
23. Gu Q. Mode of Action of the Compound chapter 5.2017
24. Elvika chandra pranata. Pengaruh Asap Cair berbagai konsentrasi dalam menghambat *Lactobacillus acidophilus* Skripsi. 2017.