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ANTIBACTERIAL ACTIVITY OF RED DRAGON FRUIT EXTRACT (*HYLOCEREUSPOLYRHIZUS*) ON *STREPTOCOCCUSMUTANS*

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ABSTRACT

Objective: The objective of the study was to evaluate the antibacterial activity of the red dragon fruit extract (*Hylocereuspolyrhizus*) to the growth of *Streptococcus mutans* (*S. mutans*).

Methods: Red dragon fruit peel and pulp extract were obtained by maceration using ethanol 96%. Pathological isolates *S. mutans* was obtained from Department of Microbiology, Faculty of Dentistry, University of Jember. The inhibition testing method has used well diffusion method. The treatment is started by giving red dragon fruit peel and pulp extract concentration of 100%, 50%, 0.2% chlorhexidine and *aquadest* sterile in each of the wells, then incubated for 24 h and was measured using a digital caliper. The antibacterial activities were assessed by the presence of inhibition zones after incubating the plates at 37 °C for 24 h.

Results: Based on the results of inhibition zone calculation, on average the biggest inhibition zone found in the positive control group(16.84 mm), then followed by peel100% (10.34 mm), peel50%(8.57 mm), pulp100% (6.79 mm), and pulp50%(5.93 mm). The higher concentration on the peel and pulp of the fruit shows the bigger inhibition zone diameter. The Kruskall-Wallis test showed a significant difference ($p<0.05$) and Mann Whitney test results showed a significant difference between the study group ($p<0.05$).

Conclusion: The red dragon fruit extract (*Hylocereuspolyrhizus*) showed significant antibacterial activity against *S. mutans*, and inhibition power ability of fruit peel extract is larger in inhibiting the *S. mutans* than its pulp ability.

Keywords: Red dragon fruit extract, Antibacterial activity, *Streptococcus mutans*

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INTRODUCTION

A current review of the available epidemiological data from many countries clearly indicates that there is a marked increase in the prevalence of dental caries. If remedies are not initiated, there could be a serious negative impact upon the future oral and systemic health of the global community, as well as a strain on the dental profession and a major increase in the cost of dental services [1]. The prevalence of dental caries in Indonesia for people over 12 y old reached 53.2% with details of active caries experience and have not done any treatment. Indonesia's DMF-T index at that same age is 4.6; this indicates there is 460 teeth decay per 100 peoples. This index will be increased along with the increased age and this number is considered high [2].

Streptococcus mutans (*S. mutans*) is a normal flora in the oral cavity that can transform into pathogens if there is an increase in a big number of colonies. This bacteria is the main cause of dental caries through carbohydrates fermentation on plaques on the surface of the tooth. Carbohydrates fermentation will produce a formation and hoarding of acid that resulted in the destruction and decalcification of tooth enamel, thus forms caries. Epidemiological studies in different populations claim that in the case of dental caries *S. Mutans* was found in a number of 74-94%. So to reduce the occurrence of caries, bacteria's activity must be inhibited [3, 4].

Antibacterial material is a material containing compounds that can inhibit or kill microorganisms. Antibacterial compounds can be derived from natural or synthetic chemicals. Synthetic chemicals such as chlorhexidine is a powerful antiseptic agent but shows toxicity reaction and side-effects on the tissues of the oral cavity. Natural antibacterial material can be derived from plants, such as red dragon fruit (*Hylocereuspolyrhizus)* [5].

Dragon fruit cultivated in Indonesia starting in the year 2000. Red dragon fruit as natural antibacterial materials are cheaper, easier to obtain and has natural deposits that reduce the risk of side effects commonly caused by synthetic antibacterial materials. Dragon fruit is effective in maintaining heart health, eye health, lowers blood sugar levels and rich in antioxidants. According to nutritionists, red dragon fruits are sweeter and contains more nutrients and vitamins than the white one. The total phenolic content on a red dragon fruit is also greater than the white dragon fruits [6].

Red dragon fruit contains antibacterial content of betalain pigments which belongs tobetacyanin the alkaloids, the phenols such as flavonoids and vitamin C, while the peel of this fruit contains flavonoids, alkaloids and these terpenoids. The content of phenols, such as flavonoids are naturally found in plants and vegetables. Phenolic compounds may inhibit the growth of bacteria with the ability of damaging the cytoplasmic membrane and proteins as well as inactivated some bacterial enzymes [7]. Polyphenols are not only beneficial to health but they also have antimicrobial and antiviral properties. Tea polyphenols are also active in inhibiting the growth of streptococcus mutans and excellent in reducing the formation of S. mutans [8].

The usage of fruit is only as consumption of fruit pulp alone, about 30-35% of the fruit peel has no further use. In fact, the results of previous studies shows the total content of phenolic substances in the peel of red dragon fruit is larger than in the red dragon fruit pulp with 70% ethanol extract. Red dragon fruit peel extract is also proved to be able to inhibit the growth of *Salmonella pullorum* and *Staphylococcus aureus* [9]. Based on the explanation above, researchers are interested in examining the activity of antibacterial extracts of dragon fruit (*Hylocereuspolyrhizus*) peel and pulp through its inhibit power against the growth of *S. mutans.*

MATERIALS AND METHODS

Material

Preparation of red dragon fruit extract (*Hylocereuspolyrhizus*)

This type of research is laboratories experimental research using the post-test only control group design. The research held at the Laboratory of Microbiology, Laboratory of Bioscience Dental Hospital of Jember University, and Laboratory of Botany Faculty of

Mathematics and Natural Science of Jember University, Indonesia. This study passed ethical approval by Medical Research Ethical Committee of Faculty of Medicine, University of Jember No.1072/ H25.1.11/KE/2016. The making of the fruit extracts begins with washing clean the dragon fruit with running water, then separate the peel and pulp of the fruit. Then cut dragon fruit pulp into small pieces then dried or rinse in a shady place for 4 d. Furthermore, Dragon fruit peels roasted using the oven in 50 ℃ for 3 h. The dried peel of dragon fruit mashed using the blender, then sifted so that it becomes simplicial powder and after that weighed. Dry weight obtained by the red dragon fruit peel of 84.54 g. The next process is ethanol 96% maceration. Soaking is done by using a ratio of 7.5 times the weight of the powder so the amount of ethanol addition is 634 ml. Simplicial already formed is placed in a glass jar, then soak using ethanol solvent during±3 d with stirring twice daily in the morning and the afternoon. After the third day, filter it using filter paper. Maceration product then evaporated so it does not contain any ethanol using a rotary evaporator at 45-50 °C, so that the extracts obtained from the fruit peel is thick with the concentration of 100%. As much as 3.44 g 100% peel and pulp extract concentration obtained on the making of the red dragon fruit peel extract.

Preparation of *Streptococcus mutans* (*S. mutans*) suspension

Suspension of *S. mutans* is made by adding 1 ose isolates of *S. mutans* into test tubes containing liquid media BHI-B as much as 2 ml. Then cover the test tubes using cotton and then put in a desicator and incubated with temperature 37 °C for 24 h. Growth of *S. mutans* was marked by the presence of turbidity in the media. After 24 h, suspension of *S. mutans* in the test tube vibrated using a thermolyne and measured its absorbance with the standard McFarland 0.5 and 0.05 absorbance with the wavelength of 560 nm using the spectrophotometer [10].

The making of Brain Heart Infusion Agar media (BHI-A**)** is carried out by mixing 10.4 gBHI-A and 200 ml *aquadest* sterile into an erlenmeyer flask. The mixture is stirred and heated to above the stove until homogeneous. So the media is then covered using cotton and sterilized in an autoclave at 121 ℃ for 15 min. BHI-A media and then incubated for 24 h to test the sterilization. BHI-Amedia sterile will remain crystal clear. When the BHI-Amedia is already warm, then poured into 8 sterile petri dish each of as many as 25 ml with a thickness of 6 mm. Then 0.5 ml suspension of *S. mutans* is inoculated BHI-A media and leveled with gigaskrin in order for the suspension in the media spread evenly, then left until the material becomes solid, approximately in 15 min [11].

Antibacterial test

Well diffusion method is used for inhibition power testing method. On each a petri dish which has been inoculated bacteria, 6 well holes is made using sterile borer diameter 5 mm. All treatment is done in laminar flow to avoid contamination. After making 6 holes on 8 petri dish finished, then add red dragon fruit peel extract 100% and 50%, Dragon fruit pulp extract 100% and 50%, positive control, negative control and on each hole using micro pipet which is given a yellow tip. As many as 20 µl red dragon fruit peel extract and flesh on each hole for each treatment group. All the treatments is also done in laminar flow to avoid contamination. This tip is always substituted for each treatment group of samples.

Measurement of the diameter of inhibition zones is done after incubated for 24 h, using a digital caliper on the area around the holes. The diameter of the inhibition zone is measured from edge to edge (breakpoint) crossed through the center of the hole. There are no inhibition zone if the result of the measurement is said to be 0.00 mm. If there is an oval inhibition zone, then measurement may use long diameter (a mm) and short (b mm) then sum them up and divide in two. The measurement is carried out 3 times with different people and taken on average. Three observers were previously given an explanation about the measurements terms, to equate the perception in measuring the inhibition zone.

RESULTS AND DISCUSSION

In the well diffusion method, the zones of inhibition produced by the sensitive organisms selected for the study were measured using calipers and recorded after the incubation period. In the study, positive control (chlorhexidine) was found to produce zones of inhibition against *S. Mutans* but was insensitive to the negative control (*aquadest* sterile); so no zone of inhibition was noted for the negative control. The zones of inhibitions of red dragon fruit (*Hylocereuspolyrhizus*) peel and pulp extract against the growth of *S. Mutans* shown in table 1. The difference test results on the entire research groups are depicted in table 2.

Based on the results of inhibition zone calculation, on average the biggest drag zone found in the group of positive control (16.84 mm), then followed by Peel group 100% (10.34 mm), Peel group 50%(8.57 mm), group Pulp 100% (6.79 mm), and Pulp group 50% (5.93 mm). The higher concentration on the peel and pulp of the fruit shows the bigger inhibition zone diameter. In addition, the resulting of inhibition zones from red dragon fruit peel is larger than thepulpone.

Table 1: The average of inhibition zones of red dragon fruit peel and pulp extract against *Streptococcus mutans* growth

Extract/Concentration	Zone of inhibition (mm)
Negative control (*Aquadest* sterile)	0.00±0.00
Pulp 50%	5.93±0.28
Pulp 100%	6.79±0.20
Peel 50%	8.57±0.59
Peel 100%	10.34±1.05
Positive Control (Chlorhexidine)	16.84±1.05

Data represents mean±Standard error of mean (n = 8)

Table 2: Difference test results on the entire research groups with kruskal-wallis

Chi-Square	*Df*	Significance
45.767	5	0.000

Inhibition power ability of dragon fruit peel extract is larger in inhibiting the *S. mutans* than its palpability. This difference is suspected because of the total phenolic content of red dragon fruit peel is larger than in the pulp. Red dragon fruit peel contains flavonoids, alkaloids and terpenoids, while red dragon fruitpulp has the same content plus vitamin C but do not contain terpenoids [12].

The content of other chemical exists in red dragon fruit peel as well aspulp is the alkaloid. The mechanism of the alkaloid is that it interferes with peptidoglycan that enables the constitution of the components of the bacterial cell, so the cell-wall layers are not fully formed and cause the death of these cells. In alkaloids, there is also a group of nitrogen base that reacts with amino acid compounds that make up the cell walls of bacteria and bacterial DNA. This reaction results in changes in the structure and composition of amino acids, which leads to changes in the genetic balance in the DNA chain; thus, the cell is damaged and promotes bacterial cell lysis, causing the death of bacterial cells. [13]. Alkaloid exists in red dragon fruit peel as well

aspulp is betacyanin. Betacyanin is the main components of redpigment which include the rare alkaloid that is soluble in water (Betalain). Betacyanin has proven antibacterial power for Gram-positive bacteria *Staphylococcus aureus* [14]. On the red dragon fruit pulp also contains vitamin C which can inhibit the growth of bacteria. This content is proven to inhibit the adhesion of *Escherichia coli* in uroepitelial cells on previous research. Therefore this vitamin is potentially reducing the risk of other pathogens bacterial infection [15].

There are three mechanisms antibacterial compounds of flavonoids, namely by the way of inhibiting nucleic acid synthesis, inhibiting cytoplasmic membrane function, and inhibiting energy metabolism of bacteria. Flavonoids can inhibit the synthesis of nucleic acids of bacteria through the cluster B ring that suspected of a role in DNA intercalation process, or through hydrogen bonds with the nucleic acid basic arrangement of bacteria that will inhibit the synthesis of DNA or RNA. In addition, flavonoids also have the ability to interfere with the activity of the transpeptidase peptidoglycan so that the formation of the cell wall is disturbed. As a result, the bacterial cell cannot withstand the internal osmotic pressure between atmospheric 5-20, where the pressure is enough to break up the cells if the cell walls are destroyed [16].

Flavonoids possibly have the ability to form a complex extracellular and able to dissolve the layer of lipids and proteins on the cell membrane of bacteria. Damage to the cell membrane can cause changes in the permeability of the cell membrane, where the various essential components of intracellular, such as nucleic acids, nucleotide, and others can get out from a cell so it will inhibit the function of the cytoplasm cell membrane. By the inhibition of nucleic acids synthesis and cytoplasmic membrane functions of bacteria will result in impaired cell metabolism, so bacterial growth will be hampered or even experiencing death [17]. Flavonoids as antimicrobial create a bond with phospholipids in the cell membrane of bacteria by reducing the permeability of the membrane; hence the cells become lysis and cause denaturation of proteins, inhibiting the formation of cytoplasmic proteins, nucleic acids and bonding with ATP-ase in the cell. The damage from cell membrane results in leakage of critical components such as proteins, nucleic acids and nucleotides which are the result of cell permeability disturbance so the cells unable do the activities of life and stunted growth or even death [18].

The other compound that act as an antibacterial is alkaloids. Alkaloids have antibacterial activity as since the compound is known as DNA interkalator and DNA synthesis inhibitor. Alkaloids are heterocyclic nitrogen compounds which contains at least one nitrogen atom and include as an alkaline. This base cluster reacts with acidic compounds in the bacterial cell such as DNA which is the main constituent of the cell nucleus. By the disruption of DNA, then the synthesis of proteins and nucleic acids in the cell will be disturbed [19].

Beside flavonoids and alkaloids, the antibacterial activity of red dragon fruit peelis also thought to derive from terpenoids compounds. The ability of terpenoids as the antibacterial compound is associated to the mechanism of action of these compounds that can react with the transmembrane protein (porin) on the outer membrane of bacterial cell walls, forming a strong polymer bond that resulted in the destruction of porin. Destruction of the porin is the influx of nutrients and other compounds will reduce the permeability of the cell wall, so bacterial growth will be stunted or die [13, 20].

Red dragon fruit peel extract concentrations 100% have a greater capability in inhibiting the growth of *S. mutans* compared to the red dragon fruit peel extract concentrations of 0%, and the pulp of the fruit extract concentrations 100% had greater inhibition power than the 50% concentrations, but it has less ability than the positive control group (chlorhexidine 0.2%). Beside antibacterial agents, structure and composition of bacterial cells also have an important role in those antibacterial mechanisms. *S. mutans* is a Gram-positive bacteria that have the structure of the sheath cells, which is relatively simple, consisting of two to three layers, which are membranes, cytoplasm and a thick peptidoglycan layer. In contrast to Gram-negative bacteria that have numerous layered cell sheath structure and extremely complex, such as lipoprotein layer, lipopolysaccharide and peptidoglycan layer. This causes *S. mutans*, as one of the Gram-positive bacteria, is more susceptible to the antimicrobial compounds because the antimicrobial material is easy to get into cells and found the target to work [21].

Growth inhibitory mechanisms by antimicrobial material in the form of reaction to the membrane or cell wall of bacteria can cause disruption to the absorption and transport of bacteria nutrients as well as disorders of energy metabolism in bacteria, especially on Gram-positive bacteria that have a simple cell sheath structure [22]. The ability of antimicrobial material such as flavonoids, alkaloids, and terpenoids which were contained in red dragon fruit peel extract is expected to kill or suppress the growth or reproduction of *S. mutans* which is a bacterial cause of caries. So, in the end, it can lower the risk of the occurrence of caries.

CONCLUSION

Based on the results of this research, it can be concluded that the red dragon fruit extract (*Hylocereuspolyrhizus*) showed significant antibacterial activity against *Streptococcus mutans*, and inhibition power ability of fruit peel extract is larger in inhibiting the growth of *Streptococcus. mutans* than its pulp ability.

AUTHORS CONTRIBUTIONS

Declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yani Corvianindya Rahayu, DDS, M. KG collected the data, analyzed the data, all the laboratory work performed, wrote the introduction, the material and method, and also discussion part. Dr. Ardo Sabir, DDS, M. Kes and Dyah Setyorini, DDS, M. Kes designed the study and proof-read the whole manuscript.

CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Bagramian RA, Godoy FG, Volpe AR. The global increase in dental caries. A pending public health crisis. Am J Dent 2009;22:3-8.
2. Riset Kesehatan Dasar (Riskesdas). Kesehatan gigi dsn mulut. Badan Penelitian dan Pengembangan Kesehatan. Kementerian Kesehatan Republik Indonesia; 2013. p. 110-8.
3. Octiara E, Budiardjo SB. Streptococcus mutans: Faktor virulensi dan target spesifik vaksin. Maj Ked Gigi 2008;13:180-5.
4. Purwandari P, Dewi N, Lia Y Budiarti. The influence of peat water to the colony number of aerob bacteria in the mouth. J Dentomaxillofac Sci 2016;1:95-8.
5. Pratiwi R. Perbedaan daya hambat terhadap streptococcus mutans dari beberapa pasta gigi yang mengandung herbal. Maj Ked Gigi 2005;38:64-7.
6. Nurliyana R, Syed ZI, Mustapha SK, Aisyah MR, Kamarul RK. Antioxidant study of pulp and peel of dragon fruits: a comparative study. Int Food Res J 2010;17:367-75.
7. Amalia S, Wahdaningsih S, Untari EK. Antibacterial activity testing of n-hexane fraction of red dragon (hylocereuspolyrhizus britton and rose) fruit peel on staphylococcus aureus ATCC 25923. Trad Med J 2014;19:89-94.
8. Fajriani, Yusriyanti M. Comparison of efficacy rinsing with cocoa seed extract, green tea catechins extract and lime extract to salivary ph in children suffering ECC (Early Childhood Caries). J Dentomaxillofac Sci 2018;3:20-3.
9. Ichsan MI, Abdul HA, Fakhrurrazi, Dewi M, Jalaluddin M. Antibacterial test of red dragon fruit extract peel (hylocereus polyrhizus) against bacteria salmonella pullorum. J Med Vet 2018;12:9-14.
10. Nwadinigwe AO, Onyeidu EG. Bioremediation of crude oil-polluted soil using bacteria and poultry manure monitores through soybean productivity. Pol J Environ Stud 2012;21:171-6.
11. Elias, Anil J. General chemistry experiments. Universities Press: (India) Private Limited; 2002.

12. Choo WS, Yong WK. Antioxidant properties of two species of Hylocereus fruits. Adv Appl Sci Res 2011;2:418-25.
13. Dharmautama M, Tetelepta R, Ikbal M, Warti AEA. Effect of mangrove leaves extract (avicennia marina) concentration on the growth of streptococcus mutans and candida albicans. J Dentomaxillofac Sci 2017;2:155-9.
14. Ali S, Khan MR, Irfanullah, Sajid M, Zahra Z. Phytochemical investigation and antimicrobial appraisal of parrotiopsis jacquemontiana (Decne) rehder. BMC Complementary Altern Med 2018;18:43.
15. Hussein ZG, Ali N, Mohammed AK. The effect of pomegranate peel extract and vitamin c in comparison with gemifloxacin on inhibiting adhesion of escherichia coli to uroepithelial cells. Zanco J Med Sci 2013;17:429-34.
16. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Curr Med Chem 2015;22:132-49.
17. Chushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agric 2005;26:343-56.
18. Setyorini D, Rahayu YC, Sistyaningrum T. The effects of rinsing red beetroot (Beta vulgaris L.) juice on streptococcus sp. dental plaque. J Dentomaxillofac Sci 2017; 2:15-7.
19. Karou S, Canini Y, Montesano S, Colizzi Te. Antibacterial activity of alkaloids from sidaacuta. Afr J Biotechnol 2005;4:1452-7.
20. Cowan M. Plant products as antimicrobial agents. Clin Microbiol 1999;12:564-82.
21. Davidson PM, Sofos JN, Brenem AL. Antimicrobial in foods. 3rd ed. New York: Marcel Dekker Inc; 2005.
22. Malanovic N, Lohner K. Gram-positive bacterial cell envelopes: the impact on the activity of antimicrobial peptides. Biochimica et Biophysica Acta 2016:936-46.