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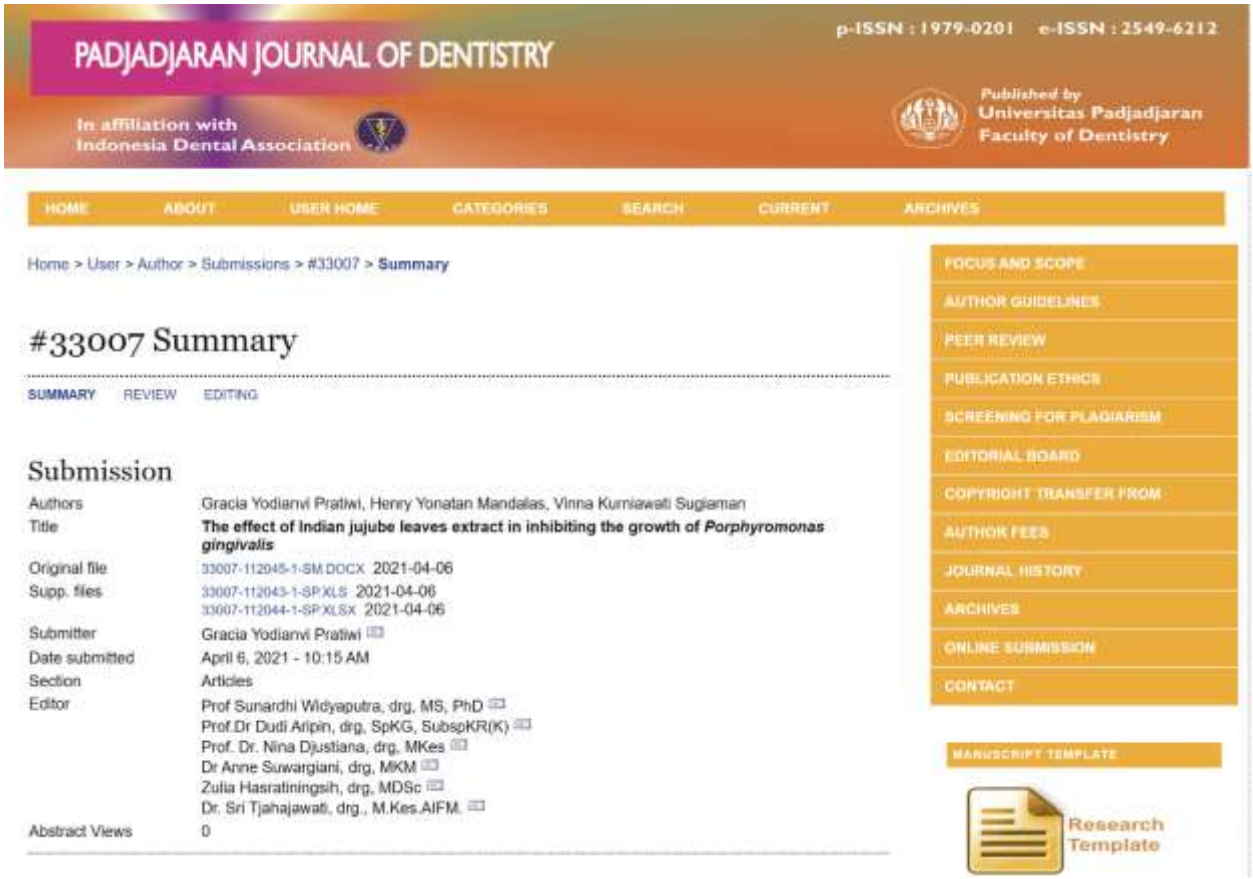

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#33007 Summary

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The effect of indian jujube (Ziziphus mauritiana Lam.) leaves extract in inhibiting the growth of Porphyromonas gingivalis

Gracia Yodianvi Pratiwi*, Henry Yonatan Mandalas**, Vinna Kurniawati Sugiaman***

*Faculty of Dentistry, Universitas Kristen Maranatha, Bandung, 40164, Indonesia

**Departement of Periodontology, Faculty of Dentistry Universitas Kristen Maranatha, Bandung, 40164, Indonesia

***Departement of Oral Biology, Faculty of Dentistry Universitas Kristen Maranatha, Bandung, 40164, Indonesia

ABSTRACT

Introduction: Periodontitis is an inflammatory disease that destroying tooth-supporting tissues and is associated with increased risk factors of systemic diseases. The main pathogen of periodontitis is the bacterium *Porhyromonas gingivalis*, which is a gram negative, anaerob, pleomorphic, coccobacillus, non motile, and assacharolytic. Antibiotics such as clindamycin, doxycycline, amoxicillin, and metronidazole are recommended for the treatment of periodontitis. However, improper administration of antibiotics can lead to antibiotic drug resistance. Each part of the plant produces various kinds of bioactive compounds, one of them is the leaves of Indian jujube (*Ziziphus mauritiana Lam)* which have the main bioactive compounds such as saponins, tannins, and flavonoids which have antimicrobial activities against some pathogenic microorganism. **Methods:** The method used in this study was the disc diffusion test based on the Clinical and Laboratory Standard Institute. The *Porphyromonas gingivalis* bacteria was collected from the Faculty of Dentistry, Padjajaran University, Bandung and the Indian jujube (*Ziziphus mauritiana Lam*) fresh leaves collected from one of the plantations in Probolinggo, East Java. In this study, tests used various concentrations of Indian jujube leaves extract, namely 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. **Results:** Then the results were classified according to David and Stout's inhibition zone classification. The results of the One-Way ANOVA statistical test showed a p-value of 0.0002. **Conclusion:** The Indian jujube leaves extract has effect in inhibiting the growth of *Porphyromonas gingivalis*.

Keywords: Indian Jujube (*Ziziphus mauritiana Lam*) leaves extract, ability of inhibition, Porphyromonas gingivalis, periodontitis, disc diffusion test.

INTRODUCTION

Chronic periodontitis is an asymptomatic and slowly progressing disease. However, the presence of several modifying factors such as systemic and environmental factors such as diabetes mellitus,

plaque accumulation, smoking, or stress can cause disease progression to become more aggressive. Chronic periodontitis most often occurs in adulthood although it can occur in children and adolescents due to the accumulation of plaque and calculus.[1] The bacteria most often found in chronic periodontitis are *Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia, Campylobacter rectus*, and *Fusobacterium nucleatum*.[2] It is known that the high prevalence of chronic periodontitis in adolescents, adults, and the elderly makes chronic periodontitis a serious problem for the oral health of the community.[3]

The prevalence of periodontitis in Indonesia is high. Data from the RISKESDAS 2018 shows that the percentage of chronic periodontitis in Indonesia is 74.1%.[4] According to WHO, the prevalence of periodontitis in the world is at a high level of around 10-15% of the world's population.[5] The highest incidence of periodontitis occurred in men, which was about 57% compared to women, which was around 39%. The high prevalence of periodontitis in men was related to the factors mentioned in the previous paragraph whereby about 18.8% of periodontitis in men was due to smoking and 9.8% was due to diabetes.[6]

Porphyromonas gingivalis is a gram-negative, anaerobic, asakarolytic, and black pigmented bacterium that is associated with the development of periodontal and systemic disease.[7] *Porphyromonas gingivalis* appears as the primary etiological agent in the pathogenesis and inflammatory processes of periodontal disease.[8] Virulence factors such as lipopolysaccharide (LPS), fimbriae, protease, capsule, adhesive domain, and outer membrane vesicle owned by *Porphyromonas gingivalis* can invade local periodontal tissues, take nutrients for growth, and defend themselves from the body's defense mechanisms.[8,9] These pathogenic bacteria appear as much as 85.75% in the sample subgingival plaque in patients with chronic periodontitis and about 40% -100% of adults with chronic periodontitis are infected by these opportunistic bacteria.[9]

Several classes of antibiotics are recommended for use as a treatment for chronic periodontitis, such as clindamycin, doxycycline, amoxicillin, and metronidazole.[10] Improper administration of antibiotics can lead to antibiotic resistance, therefore microbiologists in the world are trying to find new antimicrobial agents using natural plant products.[11] Indonesia is the second-largest center of biodiversity after Brazil in the world, which consists of tropical plants of which 7,000 have medicinal properties.[12] Based on data from Farmakope Indonesia, many plants can be used as substitutes for chemical antimicrobial agents such as fennel fruit, African leaves, bidara leaves, binahong leaves, ceremai leaves, moringa leaves, and many more.[13]

Ziziphus mauritiana Lam or Indian Jujube is a tree that grows in tropical and subtropical areas in various regions of the world. The Indian Jujube leaves is believed to be able to treat asthma, fever, accelerate wound healing, and overcome infection.[14] Therefore, several researchers conducted phytochemical tests of the extract of Indian Jujube leaves and the results showed that this Indian Jujube leaves extract contains major bioactive components which can inhibit the growth of microorganisms.[15] The results of phytochemical analysis of the Indian Jujube leaves extract are known to contain flavonoids, tannins, saponins, resins, quinones, glucose, terpenoids, and polyphenols.[14,15]

According to previous research, the bioactive compounds in Indian Jujube leaves extract is very effective in overcoming bacterial infections and can be used as an antifungal, antioxidant, and as a cancer treatment.[16]

Based on fundamentals, researchers were interested in studying the effect of Indian Jujube (*Ziziphus mauritiana Lam.*) leaves extract in inhibiting the growth of *Porphyromonas gingivalis*. The present study was aimed to provide scientific knowledge about the effect of Indian Jujube leaves extract in inhibiting the growth of the bacterium Porphyromonas gingivalis.

METHODS

The sample used in this study was the bacterium Porphyromonas gingivalis ATCC 33277 obtained from the Microbiology Laboratory of the Faculty of Dentistry, Universitas Padjajaran, Bandung. The leaves of the Indian Jujube used are taken from the plantations in Probolinggo, East Java. This plant has been identified by the staff of the Biology Research Center-LIPI Cibinong, Bogor.

Tools and Materials

Tools: Evaporator, Grinder, Analytical Balance (AXIS, AGN220C), Autoclave (HiClave, H-50), Microwave (SHIVAKI, SMW 103), Biosafety Cabinet (BSC) level II (Telstar Bio II Advance Plus), Whatman Filter Paper no. 3 (GE Healthcare, 1003-090), Sterile cotton swab (OneMed, 67723), Caliper (MODERN, SIGMA 6 ”), Disposable Petri dish (ThermoFisher Scientific, 101VR20), Incubator (Binder, CB53), Micropipette (20- 200 µl, 100-1000 µl) (Eppendorf), Tip (20-200 µl, 100-1000 µl) (Borosil), 1.5 ml Effendorf Tube (SPL, 60015-1), Vortex (WiseMix, VM-10), Falcon tube 15 ml (SPL, 50015), Falcon tube 50 ml (SPL, 50050), Serological Pipet (SPL, 91005).

Materials: Porphyromonas gingivalis ATCC 3277, Indian Jujube leaves extract (*Ziziphus mauritiana Lam*), 0.9% NaCl solution, Mueller Hinton Agar (MHA) (Himedia, M173), Mueller Hinton Broth (MHB) (Himedia, M391), 100% DMSO (Merck, 1.02952. 1000), Chlorhexidine 0.2% (Minosep, 0101220-C065), ddH2O.

Extract of Indian Jujube Leaves

The extract method used was maceration with 70% ethanol as the solvent. The Indian Jujube leaves are separated from the whole plant. The Indian Jujube leaves are dried. Dried Indian Jujube leaves are weighed as much as 2,500 grams or 2.5 kilograms. Simplicia was put into a maceration vessel and 3 liters of 70% ethanol for soaking was added. Soaking is carried out for 24 hours, stirring occasionally. Maceration is repeated several times. Furthermore, the simplicia that has been soaked is filtered with filter paper to separate the pulp and filtrate, the obtained filtrate is collected and put into a glass bottle. The filtered simplicia is then concentrated in a vacuum rotary evaporator with a temperature of 50^0C and a pressure of 40rpm, then evaporated using a water bath to obtain a thick ethanol extract of Indian Jujube leaves. The Indian Jujube leaves extract was then carried out

by phytochemical tests and the dilution process using DMSO 10% to obtain concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%.

Preparation of Suspension and Culture of Porphyromonas Gingivalis Bacteria

Following are the steps for making a test bacterial suspension based on the following research, as much as 10.5 grams of MHB medium was dissolved in 500 mL ddH2O, the *Phorphyromonas gingivalis* ATCC 33227 colony that had been cultured on Mueller Hinton Agar (MHA) medium was inoculated into Mueller Hinton Broth (MHB) medium. The suspension in the test tube was homogenized using a vortex mixer. The turbidity of the solution was then adjusted to the turbidity of the McFarland 0.5 standard solution so that an inoculum was obtained with the number of bacteria around 1-2 × 108 CFU / mL. The standard density accuracy of bacterial suspension with Mc Farland 0.5 can also use a spectrophotometer by inserting 1 ml of suspension into 6 well plates, with a light length of 1 cm. The Mc Farland standard of 0.5 has an absorbance reading of 0.08 to 0.1 at a wavelength of 620-625 nm.

The steps to culturing the *Porphyromonas gingivalis* bacterial suspension into Mueller Hinton Agar (MHA) are first to take 19 grams of MHA medium dissolved in 500 mL ddH2O. The medium is heated using a microwave until boiling and homogeneous. The medium was sterilized using an autoclave at a temperature of 121^0C, for 20 minutes. The MHA medium was poured on a petri dish to make an agar plate. A sterile cotton swab is dipped in a bacterial suspension whose turbidity has been pre-adjusted with the standard solution of McFarland 0.5. Cotton swab is rubbed onto the surface of the MHA evenly and left at room temperature for 3 to 5 minutes until the suspension is absorbed into the agar.

Disc Diffusion Test Procedure

Paper discs with a diameter of 6 mm were immersed in 1 mL of 10% DMSO solution as a negative control, Indian Jujube leaves extract with various concentrations and chlorhexidine 0.2% as a positive control for 5 minutes. In each medium that has been cultured with bacteria, a paper disc is placed. Each medium was incubated for 24 hours at 37^0C. The inhibition zone for the growth of the *Porphyromonas gingivalis* bacteria that formed around the paper disc was measured using a caliper with units of mm as research data. Then do the repetition of the same procedure as with the sample above three times based on Federer's Formula.

RESULTS

Based on the results of qualitative phytochemical tests, the Indian Jujube leaves extract used in this study contained the following bioactive compounds:

Table 1. Qualitative Phytochemical Screening Results of Indian Jujube Leaves Extract

Bioactive Compounds	Results
Alkaloids	+
Saponin	+
Tannin	+
Phenolic	+
Flavonoids	+
Triterpenoid	+
Steroid	+
Glycoside	+

The research was conducted in December 2020 at Microbiology Laboratory of Aretha Medika Utama, Bandung with three repetitions according to the calculation of Federer's formula. In this study, twelve treatments were carried out for each repetition, namely disc paper soaked with 0.2% chlorhexidine as a positive control, 10% DMSO as a negative control, and Indian Jujube leaves extract which had been diluted into ten concentrations, namely 10%, 20%, 30%. , 40%, 50%, 60%, 70%, 80%, 90%, and 100%. Each repetition used two Petri dishes containing agar planting media and cultured bacteria. Each petri dish in one repetition received six different treatments. Then the incubation was carried out for 24 hours in a CO^2 incubator at a temperature of 37^0C. Based on the data obtained, then the measurement of the inhibition zone produced by each treatment is continued by using a caliper. The results of the measurement of the inhibition zone of Indian Jujube leaves extract will be grouped into the inhibition category according to Davis and Stout.

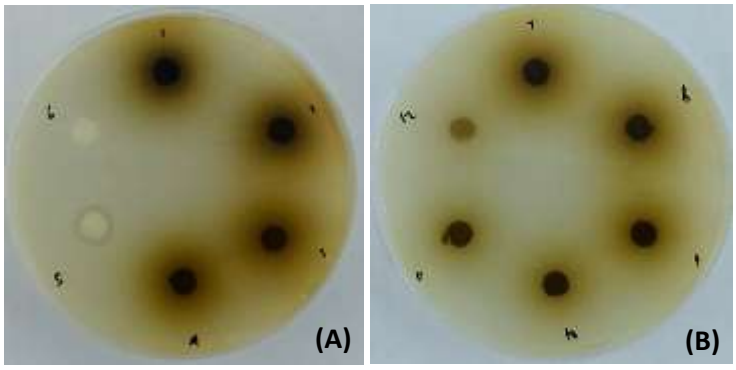

Figure 1. Inhibition Zone Observation of Indian Jujube Leaves Extract on Porphyromonas gingivalis

In Figure 1 (A) it can be seen that label number 1 shows the inhibition zone of the 100% extract concentration, label number 2 shows the inhibition zone of the extract concentration of 90%, label number 3 shows the inhibition zone of the extract concentration of 80%, label number 4 shows the zone inhibition of the extract concentration of 70%. Label number 5 indicates an inhibition zone of 0.2% chlorhexidine. Label number 6 shows the zone of inhibition of the 10% DMSO negative control.

In Figure 1 (B) label number 7 shows the inhibition zone of the 60% concentration, label number 8 shows the inhibition zone of the extract concentration of 50%, label number 9 shows the inhibition zone of the extract concentration of 40%, label number 10 shows the inhibition zone of the concentration extract 30%, label number 11 shows the inhibition zone of the extract concentration of 20%, and label number 12 shows the inhibition zone of the extract concentration of 10%.

Table 2. Inhibition Zone Measurement Results of Indian Jujube Leaves Extract

Treatments	Inhibition Zone (mm)			Mean	Deviation Standard
	1	2	3		
Positive Control (CHX 0.2%)	10.90	11.33	10.96	11.06	0.23
Negative Control (DMSO 10%)	0.00	0.00	0.00	0.00	0.00
Indian Jujube Leaves Extract 10%	0.00	0.00	0.00	0.00	0.00
Indian Jujube Leaves Extract 20%	0.00	0.00	0.00	0.00	0.00
Indian Jujube Leaves Extract 30%	0.00	0.00	0.00	0.00	0.00
Indian Jujube Leaves Extract 40%	9.38	8.38	8.35	8.70	0.59
Indian Jujube Leaves Extract 50%	10.86	8.73	8.57	9.39	1.28
Indian Jujube Leaves Extract 60%	10.80	9.58	9.20	9.86	0.84
Indian Jujube Leaves Extract 70%	11.04	10.90	11.09	11.01	0.10
Indian Jujube Leaves Extract 80%	10.46	11.09	11.08	10.88	0.36
Indian Jujube Leaves Extract 90%	11.14	11.64	11.14	11.31	0.29

Indian Jujube Leaves Extract 100%	11.80	12.60	11.41	11.94	0.61

David and Stout categorized the zone of inhibition into four categories, namely if the resulting zone of inhibition was less than 5 mm (<5 mm) the antimicrobial agent was categorized as weak, 5 mm - 10 mm was categorized as moderate, 10 mm - 19 mm was categorized as strong, and more than 20 mm (> 20 mm) are categorized as very strong.[17] Based on these categories, the measurement results of the inhibition zone of Indian Jujube leaves extract can be categorized as follows:

Table 3. Inhibition Zone Classification of Indian Jujube Leaves Extract

Indian Jujube Leaves Extract	Mean	Classification
Indian Jujube Leaves Extract 10%	0.00	Weak
Indian Jujube Leaves Extract 20%	0.00	Weak
Indian Jujube Leaves Extract 30%	0.00	Weak
Indian Jujube Leaves Extract 40%	8.70	Moderate
Indian Jujube Leaves Extract 50%	9.39	Moderate
Indian Jujube Leaves Extract 60%	9.86	Moderate
Indian Jujube Leaves Extract 70%	11.01	Strong
Indian Jujube Leaves Extract 80%	10.88	Strong
Indian Jujube Leaves Extract 90%	11.31	Strong
Indian Jujube Leaves Extract 100%	11.94	Strong

The data were tested for data normality with the Lilliefors normality test which is a non-parametric normality test and it was found that the data were normally distributed. Therefore, the calculation of statistical analysis can use the One Way ANOVA analysis test. Before that, the data homogeneity test was carried out first using the Barttlet homogeneity test and the results were that the data was not homogeneous. In this study, the data entered into the analysis test was only the inhibition zone produced by the positive control and the concentration 40% - 100% of Indian Jujube leaves extract because the negative control and the concentration 10% - 30% of Indian Jujube leaves extract did not produce an inhibition zone. The following are the results of the One Way ANOVA analysis test in the eight groups:

Table 4. One Way ANOVA Test Results

Mean	*n*	*Std. Dev*	*Treatment*
11.06	3	0.23	K+
8.70	3	0.59	40%
9.39	3	1.28	50%
9.86	3	0.84	60%

11.01	3	0.10	70%
10.88	3	0.36	80%
11.31	3	0.29	90%
11.94	3	0.61	100%
10.52	24	1.17	Total

Source	*SS*	*df*	*MS*	*F*	*p-value*
Treatment	24.93	7	3.56	8.57	.0002
Error	6.64	16	0.42		
Total	31.57	23			

Based on the results of the One Way ANOVA test, it can be seen that the p-value is 0.0002, because the significant value is lower than 0.05, meaning that there is a significant difference in the inhibition zone of the eight treatments seen from the average inhibition zone. Because the data was not homogeneous, a further One Way ANOVA test was carried out using the t-test to see which groups had significant differences, a further test would be carried out using the post hoc analysis t-test. The results of the post hoc analysis test can be stated that the use of Indian Jujube leaves extract starting from a concentration of 70% can inhibit the growth of *Porphyromonas gingivalis* bacteria with the same ability as a positive control of chlorhexidine 0.2%.

DISCUSSION

The main bioactive compounds contained in Indian Jujube leaves extract that has antibacterial functions are flavonoids, saponins, and tannins. These saponins and tannins have been tested in previous studies by being injected into the blood vessels and proven to have antibacterial properties.[18,19] The tannins contained in the Indian Jujube leaves extract to have a way of working by the properties of the tannins themselves, namely they can bind macromolecules such as proteins so that tannins can inhibit the formation of cell walls or outer membrane vesicles in Porphyromonas gingivalis.[19] Then the tannins will penetrate the bacterial internal membrane and bind to bacterial DNA, resulting in disruption of the bacterial metabolic process and absorption of nutrients. This causes the bacteria to experience cell death.[20]

Saponins are molecules that can attract and bind water and destroy fats or lipids, thereby lowering the surface tension of cells where bacteria cannot survive.[21] This decrease in cell surface tension is due to saponins having membrane lytic activity which affects the ion transport of bacterial cells.[22] Also, saponins also can inhibit the process of hemolysis by causing a decrease in erythrocyte

permeability, but at high concentrations, it will cause damage to erythrocytes.[23] Hemolysis is carried out by Porphyromonas gingivalis bacteria to obtain nutrients by destroying erythrocytes and then binding heme, when this process is inhibited, bacterial metabolic disorders occur and absorption of nutrients which causes a decrease in the functional ability of bacteria and the death of bacteria.[8,22] Apart from saponins and tannins, other active compounds such as flavonoids and alkaloids also have antibacterial properties.[14]

Flavonoids have been tested in-vitro to show the resulting antibacterial activity.[24] several types of flavonoids have antibacterial activity, including apigenin, galanin, naringenin, epigallocatechin, errors, and their derivatives of flavones and isoflavones.[25] The mechanism of action of flavonoids is to inhibit the secretion of bacterial enzymes such as the protease enzyme or gingipain produced by Porphyromonas gingivalis.[26] Gingipain functions to degrade fibrinogen and heme host proteins which contribute to inhibition of blood clotting so that bleeding gets worse and the amount of heme needed by bacteria increases.[9] When the protease or gingipain enzymes are inhibited, the bacteria lose their source nutrition and decreased function such as difficulty adhering to target cells and cell death.[27,28]

Flavonoids can also increase the secretion of inflammatory cytokines such as interleukin 1ß, interleukin 6, interleukin 8, and tumor necrosis factor or TNFα.[24,25] These inflammatory cytokines are glycoproteins that play an important role in the body's response to infection.[29] Meanwhile, the alkaloid mechanism as an antibacterial is by interfering with the composition of the peptidoglycan component so that the bacterial cell wall is not formed completely.[30] After the bacterial cell wall is damaged, alkaloids damage the lipopolysaccharide composition which causes depolarization and cytoplasmic leakage. So that it disrupts homeostasis or the balance of bacterial cells and causes bacterial cell death.[31]

The inhibition zone that is formed can be caused by the presence of antibacterial activity produced by the active compound derived from the extract of the Indian Jujube leaves extract. Table 2 states the results of the research on the measurement of the inhibition zone produced by administering Indian Jujube leaves extract, positive control, and negative control measured using a caliper after being incubated for 24 hours with a temperature of 37^0C. The results showed that the inhibition zone formed starting with the concentration of 40% - 100% Indian Jujube leaves extract and 0.2% chlorhexidine positive control. While negative control DMSO 10% and Indian Jujube leaves extract concentrations of 10% - 30% did not form an inhibition zone.

The absence of the formation of an inhibition zone in the concentration of 10% - 30% Indian Jujube leaves extract can be caused because the amount of bioactive compound content from the extract is not sufficient to inhibit bacterial growth.[32] Besides, this can also be caused by the structure of the bacterial cell wall.[33] Cell wall Gram-negative bacteria have three layers, namely the outer membrane of lipoproteins, phospholipids, and lipopolysaccharides. The lipid content in the cell wall of gram-negative bacteria ranges from 11% - 22%.[34] In gram-positive bacteria, the structure of the cell wall is simpler so that its growth is easier to inhibit. Therefore, the amount of bioactive compound content with the structure of the bacterial cell wall is related, where the bioactive compounds contained in

the extract are more difficult to damage the cell walls of gram-negative bacteria.[35] This causes bacteria to still be able to multiply cells and not have their growth stunted.[32,35]

Based on the results of the research, the resulting large inhibition zone is bigger when the concentration used is also getting bigger. This can be due to the dilution carried out in the extract of Indian Jujube leaves, where the smaller the concentration, the ratio of the extract is less than the solvent. When the amount of solvent is more than the extract, the number of bioactive compounds is less so that the antibacterial activity is not as good as the concentration of a larger extract. So that the greater the concentration of the inhibitory power, the stronger it will be, which is then supported by table 3 where there is an increase in the strength of the inhibitory power. Strong inhibition starting from the extract concentration of 70%, supported by the results of the one-way ANOVA statistic which states that its inhibiting ability is equivalent to 0.2% chlorhexidine in inhibiting the growth of *Porphyromonas gingivalis* bacteria.

However, the size of the inhibition zone is not only influenced by bioactive compounds but several other things that can affect the formation of the inhibition zone. Things that can affect the formation of the inhibition zone other than bioactive compounds are the time of absorption of bacteria into the agar.[36] Other factors such as turbidity of the suspension, incubation temperature, incubation time, and thickness of the media for growth can also affect.[37] Suspension turbidity using the Mc Farland 0.5 or equivalent to a colony of 1.5 x 10^8 and equivalent to the results of the examination using a spectrophotometer, namely 0.08 to 0.1 with a wavelength of 625 nm.[38] The incubation temperature used was around 35^0C - 37^0C. The thickness of the media must also be considered, which is about 4 mm. [36.37]

When the turbidity of the bacterial suspension is less than the Mc Farland standard, the resulting diameter will be larger and vice versa. Then when the temperature used is below 35^0C the resulting diameter will be smaller and when the temperature used is more than 37^0C the diameter will be bigger because the extract diffusion is not good. When the thickness of the agar medium is less than 4 mm the extract diffusion is faster so that the diameter is larger and vice versa. These factors as a whole can be controlled during the testing procedure. This inhibition zone testing uses the disc diffusion method according to the standards based on the Clinical and Laboratory Standard Institute.

CONCLUSION

Based on the results of the study, it can be concluded that there is an effect of the Indian Jujube (*Ziziphus mauritiana Lam.)* leaves extract in inhibiting the growth of *Porphyromonas gingivalis* bacteria.

REFERENCES

1. Carranza FA, Hogan EL. Clinical periodontology. 11th ed. Philadelphia: WB Saunders Co. 2012.

2. Belibasakis, Georgios N, Bostanci, Nagihan. Pathogenesis of Periodontal Disease Biological Concepts for Clinicians. Switzerland AG: Springer. 2018.

3. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. IJHS. 2017;1(2):72-80.

4. Badan Penelitian dan Pengembangan Kesehatan RI. Laporan Nasional Riset Kesehatan Dasar 2018. Indonesia: Kementerian Kesehatan RI. 2018.

5. Al-Saidy AH, Karim SA, Rashed HJH. Prevalence of chronic periodontitis among patients attended the department of periodontology, school of dentistry, university sulaimani. IOSR Journal of Dental and Medical Sciences. 2015;14(9):114-120.

6. Ioannidou E. The sex and gender intersection in chronic periodontitis. Frontiers in Public Health. 2017;5(189):1-8.

7. Klein AB, Tenorio LE, Lazinski DW, Camili A, Duncan MJ, Hu LT. Identification of essential genes of the periodontal pathogen Porphyromonas gingivalis. BMC Genomics. 2012;13(578):1-17.

8. Nakayama M, Ohara N. Molecular mechanisms of Porphyromonas gingivalis host cell interaction on periodontal diseases. Japanese Dental Science Review. 2017;53(4):134-140.

9. How KY, Song KP, Chan KG. Porphyromonas gingivalis: An Overview of Periodontopathic Pathogen below the Gum Line. Front Microbiol. 2016;7(53):1-14

10. Rams TE, Degener JE, Winkelhoff AJ. Antibiotic Resistance in Human Chronic Periodontitis Microbiota. J Periodontal. 2014;85(1):160-169.

11. Gerits E, Verstraeten N, Michiels J. New approaches to combat Porphyromonas gingivalis Biofilms. Journal of Oral Microbiology. 2017;9(1):1-15.

12. Lestari P. Studi Tanaman Khas Sumatera Utara Yang Berkhasiat Obat. Jurnal Farmanesia. 2016;3(1):11-21.

13. Kementerian Kesehatan Republik Indonesia. Farmakope Herbal Indonesia. Edisi 3. Kementrian Kesehatan Republik Indonesia. 2017.

14. Ghasam Al A, Muzaini Al M, Qureshi AK, Elhassan, Khan AR, Farhana AS, et al. Phytochemical Screening, Antioxidant, and Antimicrobial Activities of Methanolic Extract of Ziziphus Mauritiana Lam. Leaves Collected from Unaizah, Saudi Arabica. International Journal of Pharmaceutical Research & Allied Sciences. 2017;6(3):33-46.

15. Abalaka EM, Daniyan YS, Mann A. Evaluation of the antimicrobial activities of two Ziziphus species (Ziziphus mauritiana Lam and Ziziphus spinachristi L.) on some microbial pathogens. African Journal of Pharmacy and Pharmacology. 2010;4(4):135-139.

16. Priyanka P, Shashank B, Suresh D, Aanchal J. Phytochemical Studies Of The Secondary Metabolites Of Ziziphus Mauritiana Lam Leaves. International Journal of Current Pharmaceutical Research. 2012;4(3):153-155.

17. Ouchari L, Boukeskasse A, Bouizgarne B, Ouhdouch Y. Antimicrobial Potential of Actinomycetes Isolated from Unexplored Hot Merzouga Dessert and Their Taxonomic Diversity. Moroccan Coordinated Collections of Microorganism. 2018:1(1):1-24.

18. Dangoggo SM, Hassan LG, Sadiq IS. Phytochemical Analysis and Antibacterial Screening of Leaves of Diospyros Mespiliformis and Ziziphus Spina-Christi. 2012;1(1):31-35.

19. Salazar MC, Hernandez SR, Perez JO, Guillen RJ, Lagunas BC, Camacho LM, et al. Antibacterial Activities of Tannic Acid Against Isolated Ruminal Bacteriafrom Sheep. Elsevier. 2018;117(1):255-258

20. Yazaki K, Arimura G, Ohnishi T. Hidden Terpenoids in Plants: Their Biosynthesis, Localization, and Ecological Roles. Plant & Cell Physiology. 2017:58(10):1615-1621.

21. Ji YS, Lestari ND, Rinanda T. Uji Aktifitas Antibakteri Ekstrak Etanol Kelopak Bunga Rosella (*Hibiscus sabdariffa L.*) Terhadap Streptococcus pyogenes Secara In Vitro. Jurnal Kedokteran Syah Kuala. 2012:12(1):31-36.

22. Jurtshurk P. Medical Microbiology. 4th ed. Galveston: Baron. 2000.

23. Permatasari GA, Besung IN, Mahatmi H. Daya Hambat Perasaan Daun Sirsak Terhadap Pertumbuhan Bakteri Escherichia coli. Indonesia Medicus Veterinus. 2013:2(2):162-169.
24. Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. Antibacterial Activity of Flavanoids and Their Structure-Activity Relationship: An update review. Wiley. 2018:1(1):1-28.

25. Alfaridz F, Amalia R. Klasifikasi dan Aktivitas Farmakologi Dari Senyawa Aktif Flavanoid. Farmaka. 2016:16(3):1-9.

26. Jain C, Khatana S, Vijayvergia R. Bioactivity of Secondary Metabolites of Various Plants. International Journal of Pharmaceutical Sciences and Research. 2019:10(2):494-504.

27. Arifin B, Ibrahim S. Struktur, Bioaktivitas dan Antioksidan Flavonoid. Jurnal Zarah. 2018:6(1):21-29.

28. Kaczmarek B. Tannic Acid with Antiviral and Antibacterial Activity as A Promising Component of Biomaterials. MDPI. 2020:1(1):1-13.

29. Sapara TU, Waworuntu O, Juliatri. Efektivitas Antibakteri Ekstrak Daun Pacar Air (Impatiens balsamina L.) Terhadap Pertumbuhan Porphyromonas gingivalis. Pharmacon. 2016:5(4):10-18.

30. Irawati L, Acang L, Irawati N. Ekspresi Tumor Necrosis Factor Alfa Dan Interleukin-10 (IL10) Pada Infeksi Malaria Falciparum. 2008:1(32):16-28.

31. Johnson AM. Saponins as Agents Preventing Infection Caused By Common Waterborne Pathogens. The University of Texas. 2013:1(1):1-126.

32. Khusuma A, Safitri Y, Yuniarni A, Rizki K. Uji Teknik Difusi Menggunakan Kertas Saring Media Tampung Antibiotik dengan Escherichia Coli sebagai Bakteri Uji. Jurnal Kesehatan Prima. 2019:13(2):151-155.

33. Kaseng ES, Muhlishah N, Irawan S. Uji Daya Hambat Terhadap Pertumbuhan Bakteri Uji Staphyloccocus aureus dan Escherichia coli Ekstrak Etanol Daun Mangroove Rhizophora mucronata dan Efek Antidiabetiknya Pada Mencit yang Diinduksi Aloksan. Jurnal Bionature. 2016:17(1):1-6.

34. Oroh SB, Kandou FEF, Pelealu J, Pandiangan D. Uji Daya Hambat Ekstrak Metanol Selaginella delicatula dan Diplazium dilatatum Terhadap BAkteri Staphyloccocus aureus dan Escherichia coli. Jurnal Ilmiah Sains. 2015:15(1):52-58

35. Retnaningsih A, Primadiamanti A, Marisa I. Uji Daya Hambat EKstrak Etanol Biji Pepaya Terhadap Bakteri Escherichia coli dan Shigella dysentriae Dengan Metode Difusi Sumuran. Jurnal Analisis Farmasi. 2019:4(2):122-129.

36. Chairunisa I, Indradi RB. Aktivitas Antibakteri dan Kandungan Fitokimia Ekstrak Etanol Alga Merah (Eucheuma cottonii). Farmaka. 2020:17(1):105-110.

37. Dwiyanti RD, Nurlailah, Widiningsi IK. Efektivitas Air Rebusan Daun Binahong (Anredera cordifolia) Terhadap Pertumbuhan Salmonella typhi. Medical Laboratory Technology Journal. 2015:1(1):1-6.

38. Zeniusa P, Ramadhan MR, Nasution SH, Karima N. Uji Daya Hambat Ekstrak Etanol Teh Hijau Terhadap Escherichia coli Secara In Vitro. Majority. 2019:8(2):136-143.

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Bukti konfirmasi submit revisi pertama yang telah direvisi (16 september 2021)

The effect of indian jujube (Ziziphus mauritiana Lam.) leaves extract in inhibiting the growth of Porphyromonas gingivalis

Gracia Yodianvi Pratiwi*, Henry Yonathan Mandalas**, Vinna Kurniawati Sugiaman***

*Faculty of Dentistry, Universitas Kristen Maranatha, Bandung, 40164, Indonesia

**Departement of Periodontology, Faculty of Dentistry Universitas Kristen Maranatha, Bandung, 40164, Indonesia

***Departement of Oral Biology, Faculty of Dentistry Universitas Kristen Maranatha, Bandung, 40164, Indonesia

ABSTRACT

Intorduction: Periodontitis is an inflammatory diseases that causing destruction of tooth supporting tissues and is associated with increased risk factors of systemic diseases. The main pathogen of periodontitis is the bacterium *Porhyromonas gingivalis*, which is a gram negative, anaerob, pleomorphic, coccobacillus, non motile, and assacharolytic. Antibiotics such as clindamycin, doxycycline, amoxicillin, and metronidazole are recommended for the treatment of periodontitis. However, improper administration of antibiotics can lead to antibiotic drug resistance. Each part of the plant produces various kinds of bioactive compounds, one of them is leaves of Indian jujube (*Ziziphus mauritiana Lam)* which have the main of bioactive compounds such as saponins, tannins, and flavonoids which have antimicrobial activities against some pathogenic microorganism. **Methods:** The method used in this study was the disc diffusion test based on the Clinical and Laboratory Standard Institute. The *Porphyromonas gingivalis* bacteria was collected from the Faculty of Dentistry, Padjajaran University, Bandung and the Indian jujube (*Ziziphus mauritiana Lam*) fresh leaves collected from one of the plantations in Probolinggo, East Java. In this study, tests used various concentrations of Indian jujube leaves extract, namely 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. **Results:** Then the results were classified according to David and Stout's inhibition zone classification. The results of the One-Way ANOVA statistical test showed a p-value of 0.0002. **Conclusion:** The Indian jujube leaves extract has a significant effect in inhibiting the growth of *Porphyromonas gingivalis*.

Keywords: Indian Jujube (*Ziziphus mauritiana Lam*) leaves extract, ability of inhibition, Porphyromonas gingivalis, periodontitis, disc diffusion test.

INTRODUCTION

Chronic periodontitis is an asymptomatic and slow progressing disease. However, the presence of several modifying factors such as systemic and environmental factors such as diabetes mellitus, plaque accumulation, smoking, or stress can cause disease progression become more aggressive. Chronic periodontitis most often occurs in adulthood although it can occur in children and adolescents due to the accumulation of plaque and calculus.[1] The bacteria most often found in chronic periodontitis are *Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia, Campylobacter rectus*, and *Fusobacterium nucleatum*.[2] It is known that the high prevalence of chronic periodontitis in adolescents, adults and the elderly makes chronic periodontitis a serious problem for the oral health of the community.[3]

The prevalence of periodontitis in Indonesia is high. Data from the RISKESDAS 2018 shows that the percentage of chronic periodontitis in Indonesia is 74.1%.[4] According to WHO, the prevalence of periodontitis in the world is at a high level of around 10-15% of the world's population.[5] The highest incidence of periodontitis occurred in men, which was about 57% compared to women, which was around 39%. The high prevalence of periodontitis in men was related to the factors mentioned in the

previous paragraph whereby about 18.8% of periodontitis in men was due to smoking and 9.8% was due to diabetes.[6]

Porphyromonas gingivalis is a gram-negative, anaerobic, asakarolytic, and black pigmented bacterium that is associated with the development of periodontal and systemic disease.[7] *Porphyromonas gingivalis* appears as the primary etiological agent in the pathogenesis and inflammatory processes of periodontal disease.[8] Virulence factors such as lipopolysaccharidae (LPS), fimbriae, protease, capsule, adhesive domain and outer membrane vesicle owned by *Porphyromonas gingivalis* can invade local periodontal tissues, take nutrients for growth, and defend themselves from the body's defense mechanisms.[8,9] These pathogenic bacteria appear as much as 85.75% in the sample subgingival plaque in patients with chronic periodontitis and about 40% -100% of adults with chronic periodontitis are infected by these opportunistic bacteria.[9]

Several classes of antibiotics are recommended for use as a treatment for chronic periodontitis, such as clindamycin, doxycycline, amoxicillin, and metronidazole.[10] Improper administration of antibiotics can lead to antibiotic resistance, therefore microbiologists in the world are trying to find new antimicrobial agents using natural plant products.[11] Indonesia is the second largest center of biodiversity after Brazil in the world, which consists of tropical plants of which 7,000 have medicinal properties.[12] Based on data from the Farmakope Indonesia, there are many plants that can be used as substitutes for chemical antimicrobial agents such as fennel fruit, African leaves, bidara leaves, binahong leaves, ceremai leaves, moringa leaves, and many more.[13]

Ziziphus mauritiana Lam or Indian Jujube is a tree that grows in tropical and subtropical areas in various regions of the world. The Indian Jujube leaves is believed to be able to treat asthma, fever, accelerate wound healing, and overcome infection.[14] Therefore, several researchers conducted phytochemical tests of the extract of Indian Jujube leaves and the results showed that this Indian Jujube leaves extract contains major bioactive components which can inhibit the growth of microorganisms.[15] The results of phytochemical analysis of the Indian Jujube leaves extract are known contains flavonoids, tannins, saponins, resins, quinones, glucose, terpenoids, and polyphenols.[14,15] According to previous research, the bioactive compounds in Indian Jujube leaves extract is very effective in overcoming bacterial infections and can be used as an antifungal, antioxidant, and as a cancer treatment.[16]

Based on fundamentals, researchers were interested in studying the effect of Indian Jujube (*Ziziphus mauritiana Lam.*) leaves extract in inhibiting the growth of *Porphyromonas gingivalis*. The present study was aimed to provide scientific knowledge about the effect of Indian Jujube leaves extract in inhibiting the growth of the bacterium Porphyromonas gingivalis.

METHODS

The sample used in this study was the bacterium Porphyromonas gingivalis ATCC 33277 obtained from the Microbiology Laboratory of the Faculty of Dentistry, Universitas Padjajaran, Bandung. The

leaves of the Indian Jujube used are taken from the plantations in Probolinggo, East Java. This plant has been identified by the staff of the Biology Research Center-LIPI Cibinong, Bogor.

Tools and Materials

Tools : Evaporator, Grinder, Analytical Balance (AXIS, AGN220C), Autoclave (HiClave, H-50), Microwave (SHIVAKI, SMW 103), Biosafety Cabinet (BSC) level II (Telstar Bio II Advance Plus), Whatman Filter Paper no. 3 (GE Healthcare, 1003-090), Sterile cotton swab (OneMed, 67723), Caliper (MODERN, SIGMA 6 "), Disposable Petri dish (ThermoFisher Scientific, 101VR20), Incubator (Binder, CB53), Micropipette (20- 200 µl, 100-1000 µl) (Eppendorf), Tip (20-200 µl, 100-1000 µl) (Borosil), 1.5 ml Effendorf Tube (SPL, 60015-1), Vortex (WiseMix, VM-10), Falcon tube 15 ml (SPL, 50015), Falcon tube 50 ml (SPL, 50050), Serological Pipet (SPL, 91005).

Materials : Porphyromonas gingivalis ATCC 3277, Indian Jujube leaves extract (*Ziziphus mauritiana Lam*), 0.9% NaCl solution, Mueller Hinton Agar (MHA) (Himedia, M173), Mueller Hinton Broth (MHB) (Himedia, M391), 100% DMSO (Merck, 1.02952. 1000), Chlorhexidine 0.2% (Minosep, 0101220-C065), ddH2O.

Extract of Indian Jujube Leaves

The extract method used was maceration with 70% ethanol as the solvent. The Indian Jujube leaves are separated from the whole plant. The Indian Jujube leaves are dried. Dried Indian Jujube leaves are weighed as much as 2,500 grams or 2.5 kilograms. Simplicia was put into a maceration vessel and 3 liters of 70% ethanol for soaking was added. Soaking is carried out for 24 hours, stirring occasionally. Maceration is repeated several times. Furthermore, the simplicia that has been soaked is filtered with filter paper to separate the pulp and filtrate, the obtained filtrate is collected and put into a glass bottle. The filtered simplicia is then concentrated in a vacuum rotary evaporator with a temperature of 50^0C and a pressure of 40rpm, then evaporated using a water bath to obtain a thick ethanol extract of Indian Jujube leaves. The Indian Jujube leaves extract was then carried out by phytochemical tests and the dilution process using DMSO 10% to obtain concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%.

Preparation of Suspension and Culture of Porphyromonas Gingivalis Bacteria

Following are the steps for making a test bacterial suspension based on the following research, as much as 10.5 grams of MHB medium was dissolved in 500 mL ddH2O, the *Phorphyromonas gingivalis* ATCC 33227 colony that had been cultured on Mueller Hinton Agar (MHA) medium was inoculated into Mueller Hinton Broth (MHB) medium. The suspension in the test tube was homogenized using a vortex mixer. The turbidity of the solution was then adjusted to the turbidity of the McFarland 0.5 standard solution so that an inoculum was obtained with the number of bacteria around 1-2 × 108 CFU / mL. The standard density accuracy of bacterial suspension with Mc Farland 0.5 can also use a spectrophotometer by inserting 1 ml of suspension into 6 well plates, with a light length of 1 cm. The Mc Farland standard of 0.5 has an absorbance reading of 0.08 to 0.1 at a wavelength of 620-625 nm.

The steps to culturing the *Porphyromonas gingivalis* bacterial suspension into Mueller Hinton Agar (MHA) are first take 19 grams of MHA medium dissolved in 500 mL ddH2O. The medium is heated using a microwave until boiling and homogeneous. The medium was sterilized using an autoclave at a temperature of 121^{0}C, for 20 minutes. The MHA medium was poured on a petri dish to make an agar plate. A sterile cotton swab is dipped in a bacterial suspension whose turbidity has been pre-adjusted with the standard solution of McFarland 0.5. Cotton swab is rubbed onto the surface of the MHA evenly and left at room temperature for 3 to 5 minutes until the suspension is absorbed into the agar.

Disc Diffusion Test Procedure

Paper discs with a diameter of 6 mm were immersed in 1 mL of 10% DMSO solution as a negative control, Indian Jujube leaves extract with various concentrations, and chlorhexidine 0.2% as a positive control for 5 minutes. In each medium that has been cultured with bacteria, a paper disc is placed. Each medium were incubated for 24 hours at 37^{0}C. The inhibition zone for the growth of the *Porphyromonas gingivalis* bacteria that formed around the paper disc was measured using a caliper with units of mm as research data. Then do the repetition of the same procedure as with the sample above three times based on Frederer's Formula.

RESULTS

Based on the results of qualitative phytochemical tests, the Indian Jujube leaves extract used in this study contained the following bioactive compounds:

Table 1. Qualitative Phytochemical Screening Results of Indian Jujube Leaves Extract

Bioactive Compounds	Results
Alkaloids	+
Saponin	+
Tannin	+
Phenolic	+
Flavonoids	+
Triterpenoid	+
Steroid	+
Glycoside	+

The research was conducted in December 2020 at Microbiology Laboratory of Aretha Medika Utama, Bandung with three repetitions according to the calculation of Frederer's formula. In this study, twelve treatments were carried out for each repetition, namely disc paper soaked with 0.2% chlorhexidine as a positive control, 10% DMSO as a negative control, and Indian Jujube leaves extract which had been diluted into ten concentrations, namely 10%, 20%, 30%. , 40%, 50%, 60%, 70%, 80%, 90%, and 100%. Each repetition used two petri dishes containing agar planting media and cultured bacteria. Each petri dish in one repetition received six different treatments. Then the incubation was carried out for 24 hours in a CO^2 incubator at a temperature of 37^0C. Based on the data obtained, then the measurement of the inhibition zone produced by each treatment is continued by using a caliper. The results of the measurement of the inhibition zone of Indian Jujube leaves extract will be grouped into the inhibition category according to Davis and Stout.

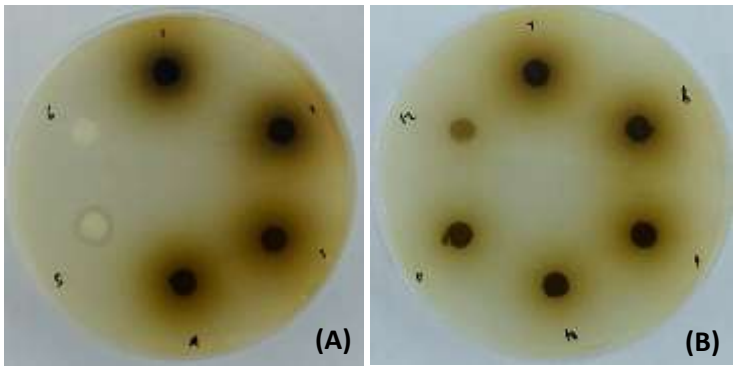


Figure 1. Inhibition Zone Observation of Indian Jujube Leaves Extract on Porphyromonas gingivalis

In Figure 1 (A) it can be seen that label number 1 shows the inhibition zone of the 100% extract concentration, label number 2 shows the inhibition zone of the extract concentration of 90%, label number 3 shows the inhibition zone of the extract concentration of 80%, label number 4 shows the zone inhibition of the extract concentration of 70%. Label number 5 indicates an inhibition zone of

0.2% chlorhexidine. Label number 6 shows the zone of inhibition of the 10% DMSO negative control.

In Figure 1 (B) label number 7 shows the inhibition zone of the 60% concentration, label number 8 shows the inhibition zone of the extract concentration of 50%, label number 9 shows the inhibition zone of the extract concentration of 40%, label number 10 shows the inhibition zone of the concentration extract 30%, label number 11 shows the inhibition zone of the extract concentration of 20%, and label number 12 shows the inhibition zone of the extract concentration of 10%.

Table 2. Inhibition Zone Measurement Results of Indian Jujube Leaves Extract

Treatments	Inhibition Zone (mm)			Mean	Deviation Standard
	1	2	3		
Positive Control (CHX 0.2%)	10.90	11.33	10.96	11.06	0.23
Negative Control (DMSO 10%)	0.00	0.00	0.00	0.00	0.00
Indian Jujube Leaves Extract 10%	0.00	0.00	0.00	0.00	0.00
Indian Jujube Leaves Extract 20%	0.00	0.00	0.00	0.00	0.00
Indian Jujube Leaves Extract 30%	0.00	0.00	0.00	0.00	0.00
Indian Jujube Leaves Extract 40%	9.38	8.38	8.35	8.70	0.59
Indian Jujube Leaves Extract 50%	10.86	8.73	8.57	9.39	1.28
Indian Jujube Leaves Extract 60%	10.80	9.58	9.20	9.86	0.84
Indian Jujube Leaves Extract 70%	11.04	10.90	11.09	11.01	0.10
Indian Jujube Leaves Extract 80%	10.46	11.09	11.08	10.88	0.36
Indian Jujube Leaves Extract 90%	11.14	11.64	11.14	11.31	0.29
Indian Jujube Leaves Extract 100%	11.80	12.60	11.41	11.94	0.61

David and Stout categorized the zone of inhibition into four categories, namely if the resulting zone of inhibition was less than 5 mm (<5 mm) the antimicrobial agent was categorized as weak, 5 mm - 10 mm was categorized as moderate, 10 mm - 19 mm was categorized as strong, and more than 20 mm (> 20 mm) are categorized as very strong.[17] Based on these categories, the measurement results of the inhibition zone of Indian Jujube leaves extract can be categorized as follows:

Table 3. Inhibition Zone Classification of Indian Jujube Leaves Extract

Indian Jujube Leaves Extract	Mean	Classification
Indian Jujube Leaves Extract 10%	0.00	Weak
Indian Jujube Leaves Extract 20%	0.00	Weak
Indian Jujube Leaves Extract 30%	0.00	Weak
Indian Jujube Leaves Extract 40%	8.70	Moderate
Indian Jujube Leaves Extract 50%	9.39	Moderate
Indian Jujube Leaves Extract 60%	9.86	Moderate
Indian Jujube Leaves Extract 70%	11.01	Strong
Indian Jujube Leaves Extract 80%	10.88	Strong
Indian Jujube Leaves Extract 90%	11.31	Strong
Indian Jujube Leaves Extract 100%	11.94	Strong

The data was tested for data normality with the Lilliefors normality test which is a non-parametric normality test and it was found that the data were normally distributed. Therefore, the calculation of statistical analysis can use the One Way ANOVA analysis test. Before that, the data homogeneity test was carried out first using the Barttlet homogeneity test and the results were that the data was not homogeneous. In this study, the data entered into the analysis test was only the inhibition zone produced by the positive control and the concentration 40% - 100% of Indian Jujube leaves extract because the negative control and the concentration 10% - 30% of Indian Jujube leaves extract did not produce an inhibition zone. The following are the results of the One Way ANOVA analysis test in the eight groups:

Table 4. One Way ANOVA Test Results

Mean	*n*	*Std. Dev*	*Treatment*
11.06	3	0.23	K+
8.70	3	0.59	40%
9.39	3	1.28	50%
9.86	3	0.84	60%
11.01	3	0.10	70%
10.88	3	0.36	80%
11.31	3	0.29	90%
11.94	3	0.61	100%
10.52	24	1.17	Total

Source	*SS*	*df*	*MS*	*F*	*p-value*
Treatment	24.93	7	3.56	8.57	.0002
Error	6.64	16	0.42		

Total	31.57	23

Based on the results of the One Way ANOVA test, it can be seen that the p-value is 0.0002, because the significant value is lower than 0.05, meaning that there is a significant difference in the inhibition zone of the eight treatments seen from the average inhibition zone. Because the data was not homogeneous, a further One Way ANOVA test was carried out using the t-test to see which groups had significant differences, a further test would be carried out using the post hoc analysis t-test. The results of the post hoc analysis test can be stated that the use of Indian Jujube leaves extract starting from a concentration of 70% can inhibit the growth of *Porphyromonas gingivalis* bacteria with the same ability as a positive control of chlorhexidine 0.2%.

DISCUSSION

The main bioactive compounds contained in Indian Jujube leaves extract that have antibacterial functions are flavonoids, saponins, and tannins. These saponins and tannins have been tested in previous studies by being injected into the blood vessels and proven to have antibacterial properties.[18,19] The tannins contained in the Indian Jujube leaves extract have a way of working in accordance with the properties of the tannins themselves, namely they can bind macromolecules such as proteins so that tannins can inhibit the formation of cell walls or outer membrane vesicles in Porphyromonas gingivalis.[19] Then the tannins will penetrate the bacterial internal membrane and bind to bacterial DNA, resulting in disruption of the bacterial metabolic process and absorption of nutrients. This causes the bacteria to experience cell death.[20]

Saponins are molecules that can attract and bind water and destroy fats or lipids, thereby lowering the surface tension of cells where bacteria cannot survive.[21] This decrease in cell surface tension is due to saponins having membrane lytic activity which affects the ion transport of bacterial cells.[22] In addition, saponins also has the ability to inhibit the process of hemolysis by causing a decrease in erythrocyte permeability, but at high concentrations it will cause damage to erythrocytes.[23] Hemolysis is carried out by Porphyromonas gingivalis bacteria to obtain nutrients by destroying erythrocytes and then binding heme, when this process is inhibited, bacterial metabolic disorders occur and absorption of nutrients which causes a decrease in the functional ability of bacteria and the death of bacteria.[8,22] Apart from saponins and tannins, other active compounds such as flavonoids and alkaloids also have antibacterial properties.[14]

Flavonoids have been tested in-vitro to show the resulting antibacterial activity.[24] There are several types of flavonoids that have antibacterial activity, including apigenin, galangin, naringenin, epigalocatekin, errors and their derivatives of flavones and isoflavones.[25] The mechanism of action of flavonoids is to inhibit the secretion of bacterial enzymes such as the protease enzyme or gingipain produced by Porphyromonas gingivalis.[26] Gingipain functions to degrade fibrinogen and heme host proteins which contribute to inhibition of blood clotting so that bleeding gets worse and the amount

of heme needed by bacteria increases.[9] When the protease or gingipain enzymes are inhibited, the bacteria lose their source nutrition and decreased function such as difficulty adhering to target cells and cell death.[27,28]

Flavonoids can also increase the secretion of inflammatory cytokines such as interleukin 1ß, interleukin 6, interleukin 8, and tumor necrosis factor or TNFα.[24,25] These inflammatory cytokines are glycoproteins that play an important role in the body's response to infection.[29] Meanwhile, the alkaloid mechanism as antibacterial is by interfering with the composition of the peptidoglycan component so that the bacterial cell wall is not formed completely.[30] After the bacterial cell wall is damaged, alkaloids damage the lipopolysaccharide composition which causes depolarization and cytoplasmic leakage. So that it disrupts homeostasis or the balance of bacterial cells and causes bacterial cell death.[31]

The inhibition zone that is formed can be caused by the presence of antibacterial activity produced by the active compound derived from the extract of the Indian Jujube leaves extract. Table 2 states the results of the research on the measurement of the inhibition zone produced by administering Indian Jujube leaves extract, positive control, and negative control measured using a caliper after being incubated for 24 hours with a temperature of 37^0C. The results showed that the inhibition zone formed starting with the concentration of 40% - 100% Indian Jujube leaves extract and 0.2% chlorhexidine positive control. While negative control DMSO 10% and Indian Jujube leaves extract concentrations of 10% - 30% did not form an inhibition zone.

The absence of the formation of an inhibition zone in the concentration of 10% - 30% Indian Jujube leaves extract can be caused because the amount of bioactive compound content from the extract is not sufficient to inhibit bacterial growth.[32] In addition, this can also be caused by the structure of the bacterial cell wall.[33] Cell wall Gram-negative bacteria have three layers, namely the outer membrane of lipoproteins, phospholipids, and lipopolysaccharides. The lipid content in the cell wall of gram-negative bacteria ranges from 11% - 22%.[34] In gram-positive bacteria, the structure of the cell wall is simpler so that its growth is easier to inhibit. Therefore, the amount of bioactive compound content with the structure of the bacterial cell wall is related, where the bioactive compounds contained in the extract are more difficult to damage the cell walls of gram-negative bacteria.[35] This causes bacteria to still be able to multiply cells and not have their growth stunted.[32,35]

Based on the results of the research, the resulting large inhibition zone is bigger when the concentration used is also getting bigger. This can be due to the dilution carried out in the extract of Indian Jujube leaves, where the smaller the concentration, the ratio of the extract is less than the solvent. When the amount of solvent is more than the extract, the number of bioactive compounds is less so that the antibacterial activity is not as good as the concentration of a larger extract. So that the greater the concentration of the inhibitory power, the stronger it will be, which is then supported by table 3 where there is an increase in the strength of the inhibitory power. Strong inhibition starting from the extract concentration of 70%, supported by the results of the one way ANOVA statistic which states that its inhibiting ability is equivalent to 0.2% chlorhexidine in inhibiting the growth of *Porphyromonas gingivalis* bacteria.

However, the size of the inhibition zone is not only influenced by bioactive compounds, but there are several other things that can affect the formation of the inhibition zone. Things that can affect the formation of the inhibition zone other than bioactive compounds are the time of absorption of bacteria into agar.[36] Other factors such as turbidity of the suspension, incubation temperature, incubation time, and thickness of the media for growth can also have an effect.[37] Suspension turbidity using the Mc Farland 0.5 or equivalent to a colony of 1.5 x 10^8 and equivalent to the results of the examination using a spectophotometer, namely 0.08 to 0.1 with a wavelength of 625 nm.[38] The incubation temperature used was around 35^0C - 37^0C. The thickness of the media must also be considered, which is about 4 mm. [36.37]

When the turbidity of the bacterial suspension is less than the Mc Farland standard, the resulting diameter will be larger and vice versa. Then when the temperature used is below 35^0C the resulting diameter will be smaller and when the temperature used is more than 37^0C the diameter will be bigger because the extract diffusion is not good. When the thickness of the agar medium is less than 4 mm the extract diffusion is faster so that the diameter is larger and vice versa. These factors as a whole can be controlled during the testing procedure. This inhibition zone testing uses the disc diffusion method according to the standards based on the Clinical and Laboratory Standard Institute.

CONCLUSION

Based on the results of the study, it can be concluded that there is an effect of the Indian Jujube (*Ziziphus mauritiana Lam.)* leaves extract in inhibiting the growth of *Porphyromonas gingivalis* bacteria.

REFERENCES

39. Carranza FA, Hogan EL. Clinical periodontology. 11th ed. Philadelphia: WB Saunders Co. 2012.

40. Belibasakis, Georgios N, Bostanci, Nagihan. Pathogenesis of Periodontal Disease Biological Concepts for Clinicians. Switzerland AG: Springer. 2018.

41. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. IJHS. 2017;1(2):72-80.

42. Badan Penelitian dan Pengembangan Kesehatan RI. Laporan Nasional Riset Kesehatan Dasar 2018. Indonesia: Kementerian Kesehatan RI. 2018.

43. Al-Saidy AH, Karim SA, Rashed HJH. Prevalence of chronic periodontitis among patients attended department of periodontology, school of dentistry, university sulaimani. IOSR Journal of Dental and Medical Sciences. 2015;14(9):114-120.

44. Ioannidou E. The sex and gender intersection in chronic periodontitis. Frontiers in Public Health. 2017;5(189):1-8.

45. Klein AB, Tenorio LE, Lazinski DW, Camili A, Duncan MJ, Hu LT. Identification of essential genes of the periodontal pathogen Porphyromonas gingivalis. BMC Genomics. 2012;13(578):1-17.

46. Nakayama M, Ohara N. Molecular mechanisms of Porphyromonas gingivalis host cell interaction on periodontal diseases. Japanese Dental Science Review. 2017;53(4):134-140.

47. How KY, Song KP, Chan KG. Porphyromonas gingivalis: An Overview of Periodontopathic Pathogen below the Gum Line. Front Microbiol. 2016;7(53):1-14

48. Rams TE, Degener JE, Winkelhoff AJ. Antibiotic Resistance in Human Chronic Periodontitis Microbiota. J Periodontal. 2014;85(1):160-169.

49. Gerits E, Verstraeten N, Michiels J. New approaches to combat Porphyromonas gingivalis Biofilms. Journal of Oral Microbiology. 2017;9(1):1-15.

50. Lestari P. Studi Tanaman Khas Sumatera Utara Yang Berkhasiat Obat. Jurnal Farmanesia. 2016;3(1):11-21.

51. Kementerian Kesehatan Republik Indonesia. Farmakope Herbal Indonesia. Edisi 3. Kementrian Kesehatan Republik Indonesia. 2017.

52. Ghasam Al A, Muzaini Al M, Qureshi AK, Elhassan, Khan AR, Farhana AS, et al. Phytochemical Screening, Antioxidant, and Antimicrobial Activities of Methanolic Extract of Ziziphus Mauritiana Lam. Leaves Collected from Unaizah, Saudi Arabica. International Journal of Pharmaceutical Research & Allied Sciences. 2017;6(3):33-46.

53. Abalaka EM, Daniyan YS, Mann A. Evaluation of the antimicrobial activities of two Ziziphus species (Ziziphus mauritiana Lam and Ziziphus spinachristi L.) on some microbial pathogens. African Journal of Pharmacy and Pharmacology. 2010;4(4):135-139.

54. Priyanka P, Shashank B, Suresh D, Aanchal J. Phytochemical Studies Of The Secondary Metabolites Of Ziziphus Mauritiana Lam Leaves. International Journal of Current Pharmaceutical Research. 2012;4(3):153-155.

55. Ouchari L, Boukeskasse A, Bouizgarne B, Ouhdouch Y. Antimicrobial Potential of Actinomycetes Isolated from Unexplored Hot Merzouga Dessert and Their Taxonomic Diversity. Moroccan Coordinated Collections of Mivroorganism. 2018:1(1):1-24.

56. Dangoggo SM, Hassan LG, Sadiq IS. Phytochemical Analysis and Antibacterial Screening of Leaves of Diospyros Mespiliformis and Ziziphus Spina-Christi. 2012;1(1):31-35.

57. Salazar MC, Hernandez SR, Perez JO, Guillen RJ, Lagunas BC, Camacho LM, et al. Antibacterial Activities of Tannic Acid Against Isolated Ruminal Bacteriafrom Sheep. Elsevier. 2018;117(1):255-258

58. Yazaki K, Arimura G, Ohnishi T. Hidden Terpenoids in Plants: Their Biosynthesis, Localization and Ecological Roles. Plant & Cell Physiology. 2017:58(10):1615-1621.

59. Ji YS, Lestari ND, Rinanda T. Uji Aktifitas Antibakteri Ekstrak Etanol Kelopak Bunga Rosella (*Hibiscus sabdariffa L.*) Terhadap Streptococcus pyogenes Secara In Vitro. Jurnal Kedokteran Syah Kuala. 2012:12(1):31-36.

60. Jurtshurk P. Medical Microbiology. 4th ed. Galveston: Baron. 2000.

61. Permatasari GA, Besung IN, Mahatmi H. Daya Hambat Perasaan Daun Sirsak Terhadap Pertumbuhan Bakteri Escherichia coli. Indonesia Medicus Veterinus. 2013:2(2):162-169.
62. Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. Antibacterial Activity of Flavanoids and Their Structure Activity Relationship: An update review. Wiley. 2018:1(1):1-28.

63. Alfaridz F, Amalia R. Klasifikasi dan Aktivitas Farmakologi Dari Senyawa Aktif Flavanoid. Farmaka. 2016:16(3):1-9.

64. Jain C, Khatana S, Vijayvergia R. Bioactivity of Secondary Metabolites of Various Plants. International Journal of Pharmaceutical Sciences and Research. 2019:10(2):494-504.

65. Arifin B, Ibrahim S. Struktur, Bioaktivitas dan Antioksidan Flavonoid. Jurnal Zarah. 2018:6(1):21-29.

66. Kaczmarek B. Tannic Acid with Antiviral and Antibacterial Activity as A Promising Component of Biomaterials. MDPI. 2020:1(1):1-13.

67. Sapara TU, Waworuntu O, Juliatri. Efektivitas Antibakteri Ekstrak Daun Pacar Air (Impatiens balsamina L.) Terhadap Pertumbuhan Porphyromonas gingivalis. Pharmacon. 2016:5(4):10-18.

68. Irawati L, Acang L, Irawati N. Ekspresi Tumor Necrosis Factor Alfa Dan Interleukin-10 (IL10) Pada Infeksi Malaria Falciparum. 2008:1(32):16-28.

69. Johnson AM. Saponins as Agents Preventing Infection Caused By Common Waterborne Pathogens. University of Texas. 2013:1(1):1-126.

70. Khusuma A, Safitri Y, Yuniarni A, Rizki K. Uji Teknik Difusi Menggunakan Kertas Saring Media Tampung Antibiotik dengan Escherichia Coli sebagai Bakteri Uji. Jurnal Kesehatan Prima. 2019:13(2):151-155.

71. Kaseng ES, Muhlishah N, Irawan S. Uji Daya Hambat Terhadap Pertumbuhan Bakteri Uji Staphyloccocus aureus dan Escherichia coli Ekstrak Etanol Daun Mangroove Rhizophora mucronata dan Efek Antidiabetiknya Pada Mencit yang Diinduksi Aloksan. Jurnal Bionature. 2016:17(1):1-6.

72. Oroh SB, Kandou FEF, Pelealu J, Pandiangan D. Uji Daya Hambat Ekstrak Metanol Selaginella delicatula dan Diplazium dilatatum Terhadap BAkteri Staphyloccocus aureus dan Escherichia coli. Jurnal Ilmiah Sains. 2015:15(1):52-58

73. Retnaningsih A, Primadiamanti A, Marisa I. Uji Daya Hambat EKstrak Etanol Biji Pepaya Terhadap Bakteri Escherichia coli dan Shigella dysentriae Dengan Metode Difusi Sumuran. Jurnal Analisis Farmasi. 2019:4(2):122-129.

74. Chairunisa I, Indradi RB. Aktivitas Antibakteri dan Kandungan Fitokimia Ekstrak Etanol Alga Merah (Eucheuma cottonii). Farmaka. 2020:17(1):105-110.

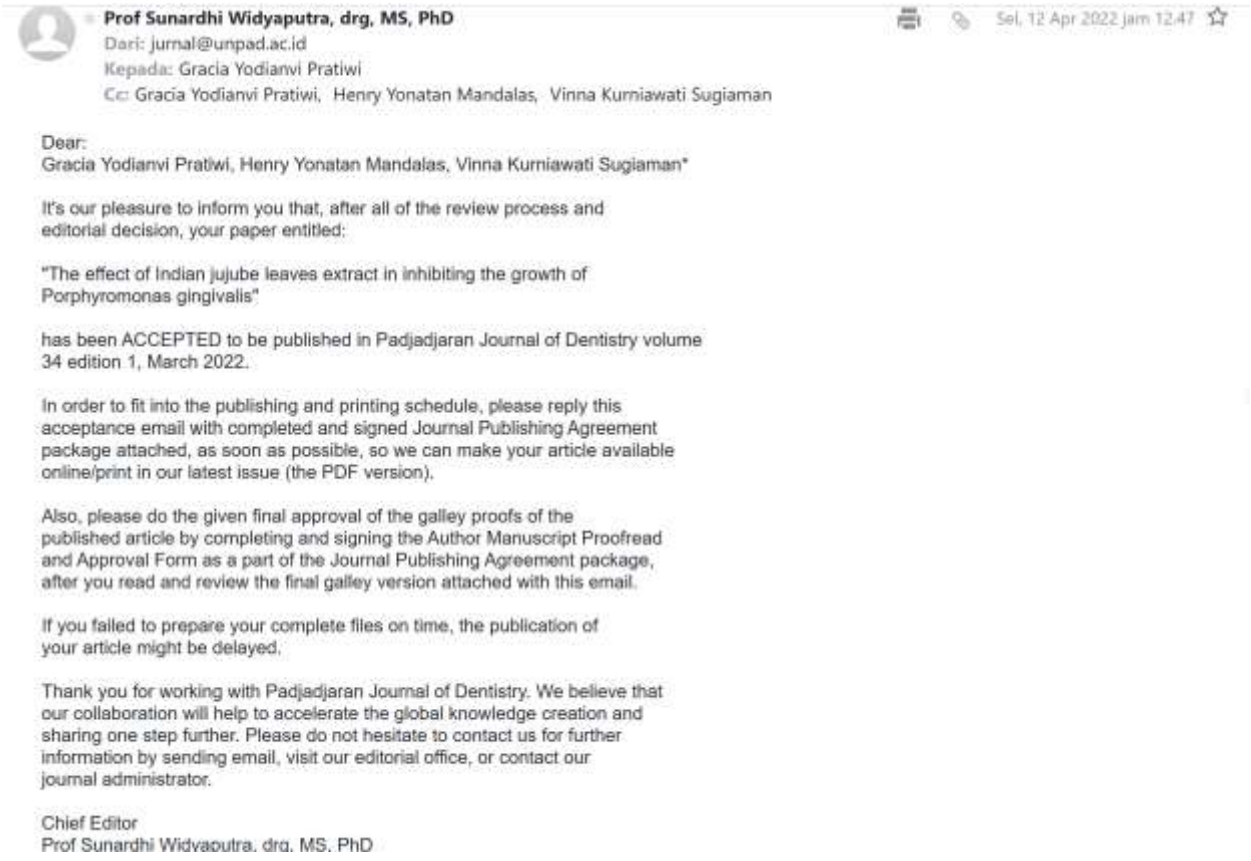
75. Dwiyanti RD, Nurlailah, Widiningsi IK. Efektivitas Air Rebusan Daun Binahong (Anredera cordifolia) Terhadap Pertumbuhan Salmonella typhi. Medical Laboratory Technology Journal. 2015:1(1):1-6.

76. Zeniusa P, Ramadhan MR, Nasution SH, Karima N. Uji Daya Hambat Ekstrak Etanol Teh Hijau Terhadap Escherichia coli Secara In Vitro. Majority. 2019:8(2):136-143.

Bukti melakukan review yang kedua

Bukti konfirmasi submit artikel yang telah revisi kedua

Bukti konfirmasi artikel diterima (12 April 2022)



Prof Sunardhi Widyaputra, drg, MS, PhD Sel, 12 Apr 2022 jam 12.47

Dari: jurnal@unpad.ac.id
Kepada: Gracia Yodianvi Pratiwi
Cc: Gracia Yodianvi Pratiwi, Henry Yonatan Mandalas, Vinna Kurniawati Sugiaman

Dear:
Gracia Yodianvi Pratiwi, Henry Yonatan Mandalas, Vinna Kurniawati Sugiaman*

It's our pleasure to inform you that, after all of the review process and editorial decision, your paper entitled:

"The effect of Indian jujube leaves extract in inhibiting the growth of Porphyromonas gingivalis"

has been ACCEPTED to be published in Padjadjaran Journal of Dentistry volume 34 edition 1, March 2022.

In order to fit into the publishing and printing schedule, please reply this acceptance email with completed and signed Journal Publishing Agreement package attached, as soon as possible, so we can make your article available online/print in our latest issue (the PDF version).

Also, please do the given final approval of the galley proofs of the published article by completing and signing the Author Manuscript Proofread and Approval Form as a part of the Journal Publishing Agreement package, after you read and review the final galley version attached with this email.

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Thank you for working with Padjadjaran Journal of Dentistry. We believe that our collaboration will help to accelerate the global knowledge creation and sharing one step further. Please do not hesitate to contact us for further information by sending email, visit our editorial office, or contact our journal administrator.

Chief Editor
Prof Sunardhi Widyaputra, drg, MS, PhD

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#33007 Summary

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Gracia Yodianvi Prathiwi, Henry Yonatan Mandalas, Vinna Kurniawati Sugiaman

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Authors

Gracia Yodianvi Prathiwi
Maranatha Christian University
Indonesia
Department of Periodontology, Faculty of Dentistry

Henry Yonatan Mandalas
https://orcid.org/0000-0002-9799-3824
Maranatha Christian University
Indonesia
Department of Periodontology, Faculty of Dentistry

Vinna Kurniawati Sugiaman
https://orcid.org/0000-0002-3688-6718
https://www.scopus.com/authid/detail.uri?authorId=57209256262
Maranatha Christian University
Indonesia
Department of Oral Biology, Faculty of Dentistry

Title and Abstract

The effect of Indian jujube leaves extract in inhibiting the growth of *Porphyromonas gingivalis*

Introduction: Periodontitis is an inflammatory disease that destroys tooth-supporting tissues and is associated with increased risk factors for systemic diseases. The main pathogen of periodontitis is the bacteria P. gingivalis, a Gram-negative, anaerobic, pleomorphic, coccobacillus, non-motile, and saccharolytic. The leaves of Indian jujube (Ziziphus mauritiana Lam.) have the main bioactive compounds such as saponins, tannins, and flavonoids which have antimicrobial activities against pathogenic microorganisms. This study aims to analyse various concentrations of the ethanol extract of Indian jujube leaves against P. gingivalis. **Methods:** The method used in this study was the disc diffusion test based on the Clinical and Laboratory Standard Institute. P. gingivalis preparation and the fresh leaves of Indian jujube collected from one of the plantations in Probolinggo, East Java. In this study, tests used various concentrations of Indian jujube leaves extract, namely 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%. Data antibacterial activity was classified according to David and Stout's inhibition zone classification. **Results:** Indian jujube leaves extract with a concentration between 10-30% had weak antibacterial activity, 40-60% had moderate antibacterial activity, and 70-100% had strong antibacterial activity. The largest inhibitory zone diameter against P. gingivalis was found at a concentration of 100%. **Conclusion:** Indian jujube leaves extract starting from a concentration of 70% can inhibit the growth of P. gingivalis with strong antibacterial activity.

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