World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Inhibitory activity of jackfruit (*Atrocarpus heterophyllus Lam.*) leaf extract on the growth of *Porphyromonas gingivalis* bacteria

Eka Fitria Augustina [1,*], Lambang Bargowo [1], Alfarabi Anhar [2] and Dewi Nur Aini [2]

[1] *Department of Periodontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[2] *Dental Medicine Education Study Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.*

World Journal of Advanced Research and Reviews, 2023, 17(01), 126–132

Publication history: Received on 24 November 2022; revised on 04 January 2023; accepted on 06 January 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.17.1.0006

Abstract

Background: Periodontitis is an inflammation that affects the periodontal tissue, causing pocket formation, loss of attachment, and tooth mobility. Periodontitis caused by a complex interaction between the body's immune response and microorganisms in dental plaque. One of the main agents in the etiology of chronic periodontitis is *Porphyromonas gingivalis*. Jackfruit (*Atrocarpus heterophyllus* Lam.) leaf contains active compounds such as saponin, flavonoid, and tannin.

Objective: This study is aimed to determine the inhibition of jackfruit leaf extract (*Atrocarpus heterophyllus* Lam.) on the growth of *Porphyromonas gingivalis* bacteria.

Method: This research is an in vitro laboratory experiment. Jackfruit leaf extraction was carried out by maceration method with 70% ethanol solvent, then diluted serially by dilution to obtain various concentrations. Concentrations used include 100%; 50%; 25%; 12.5%; 6.25%; 3.125%; 1.56%; and 0.78%. Inhibition test against *Porphyromonas gingivalis* bacteria was carried out by spectrophotometric method and colony count.

Result: Jackfruit leaf extract with a concentration of 12.5%; 25%; 50%; and 100% can kill *Porphyromonas gingivalis* bacterial colonies resulting in 0 colonies. A concentration of 6.25% inhibited the growth of bacteria by 91.77%. The results of statistical tests obtained a significance value of 0.001 (P <0.05).

Conclusion: Jackfruit leaf extract (*Atrocarpus heterophyllus* Lam.) has an inhibitory effect on the growth of *Porphyromonas gingivalis* bacteria with MIC values at a concentration of 6.25% and MBC at a concentration of 12.5%.

Keywords: Jackfruit leaf; *Porphyromonas gingivalis*; Inhibitory activity; Spectrophotometric; colony counts

1. Introduction

Periodontitis is an inflammation that affects the periodontal tissue, causing pocket formation, loss of attachment, and tooth mobility. Periodontitis caused by a complex interaction between the body's immune response and microorganisms in dental plaque. Periodontitis always begins from gingivitis but gingivitis does not necessarily end in periodontitis. According to Riskesdas 2018 data, 57.6% of the Indonesian population has some kind of problems with their teeth and mouth. Of the Indonesian population who experienced dental and oral problems, only 10.2% received treatment from dental medical personnel while the other 89.2% did not receive any treatment. The prevalence of periodontitis in people over 15 years of age is 67.8%. This means that 7 out of 10 people in Indonesia suffer from periodontal disease [1]. There are two classifications of periodontitis, namely aggressive periodontitis and chronic

* Corresponding author: Eka Fitria Augustina

periodontitis. The bacterium *Aggregati bacter actinomycete mcomitans* is a pathogen that causes aggressive periodontitis that often occurs in younger people [2]. Chronic periodontitis caused by a red complex bacterial component consisting of *Treponema denticola*, *Porphyromonas gingivalis*, and *Tannerella forsythia*, which are obligate anaerobic gram-negative bacteria that cause chronic periodontitis that often occurs in adults [3].

Porphyromonas gingivalis is the main agent in the etiology of chronic periodontitis which is most often found in the subgingival plaque of patients with chronic periodontitis, with a percentage of 85.75% [4]. These bacteria require heme or hemin and vitamin K in their nutritional environment and obtain metabolic energy by fermenting amino acids, which are needed by these bacteria caused by the lack of sugar in the periodontal pocket [5]. *Porphyromonas gingivalis* bacteria are not the initial aggressors of the inflammatory response, but are opportunistic bacteria by cross-talking with the host and destroying the host's defense mechanisms. *Porphyromonas gingivalis* serves as secondary colonizers of dental plaque, often following primary colonizers such as *Streptococcus gordonii* and *Porphyromonas intermedia* [5, 6].

Indonesia, a country rich in biodiversity including nutritious plants, where the use of plants as a medicine is widely used by the community and also known to be more affordable. One of the medicinal plants is jackfruit (*Atrocarpus heterophyllus* Lam.). Jackfruit leaves are useful in the treatment of fever, ulcers, skin diseases, antidiarrheal, analgesic and immunomodulatory. Jackfruit leaves are known to contain saponin (1.36%), flavonoid (0.92%) and tannin (3.08%) which act as antibacterial compounds [7, 8].

Saponins have anti-bacterial properties which reduce the surface tension of the cell wall resulting in leakage in the cell wall [9]. Flavonoids inhibit the function of cell membranes by forming complex compounds with extracellular and dissolved proteins so that they can damage the bacterial cell membrane and followed by the release of intracellular compounds [10]. In addition, it is known that the mechanism of flavonoids inhibits the function of cell membranes by interfering with the permeability of cell membranes by inhibiting enzyme binding [11].

Tannins have several antibacterial mechanisms, namely inhibiting the formation of bacterial extracellular enzymes, directly inhibiting oxidative phosphorylation, and forming a complex of tannin bonds to metal ions which can increase the toxicity of tannins. The monomers of tannins which can be hydrolyzed have a higher potency than the oligomeric forms [12, 13].

2. Material and methods

This study was conducted from August 2022 until September 2022 at the Airlangga University Dental Medicine Research Center. Jackfruit (*Atrocarpus heterophyllus* Lam.) leaf were extracted in the Balai Penelitian dan Konsultasi Industri (BPKI) Surabaya according to the protocol of the laboratory.

This study is an experimental analytical laboratory research with post-test only control group design consisting of 10 treatment groups: Jackfruit leaf with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, positive control, and negative control. After doing the calculation using the Federer formula, it is found that the repetition should be held 3 times at a minimum, therefore the total sample would be 30.

2.1. Materials and Equipment Preparation

The materials used in this experiment are jackfruit leaf extract, 70% ethanol, sulfuric acid, acetic acid, pure *Porphyromonas gingivalis* isolate, Brain Heart Infusion Broth (BHIB) media, Muller-Hinton Agar (MHA), McFarland standard 0.5, and sterile aquades. The equipment used are a maceration vessel, stirring rod, plastic container, strainer, filter paper, funnel, volume pipette, analytical balance, measuring glass, blender, vacuum rotary evaporator, water bath, micropipette, test tube, petri dish, incubator, round ossicle, autoclave, bunsen, Uv-Vis BKD-560 spectrophotometer, vortex mixer, colony counter, eppendorf, erlenmeyer flask.

2.2. Preparation of Jackfruit Leaf Extract

Jackfruit leaves were washed thoroughly then cut into pieces. Jackfruit leaves that have been cut are dried using an oven with a temperature of 50^{o}C. Jackfruit leaves are dried until the leaves are easily torn. The dried leaves were crushed finely and sieved to obtain a fine powder.

Jackfruit leaf powder was weighed as much as 600 grams and maceration was carried out with 2.5 L of 70% ethanol. The 70% ethanol filtrate was separated from the residue using a Buchner funnel and filter paper. The filtrate was collected in Erlenmeyer and stored at room temperature. The residue was given another 2.5L of 70% ethanol until the residue was buried in the jar. The jar is placed on a digital shaker, the speed is set at 50 rpm and carried out for 24 hours.

The extract was filtered using a filter and collected in an Erlenmeyer. The results of the first and second extracts were mixed and evaporated using a rotary evaporator. The time for evaporation is 4 hours. The extract obtained was evaporated with a water bath for 2 hours. The extract obtained was then tested using phytochemical screening.

2.3. MIC & MBC Test on *Porphyromonas gingivalis*

The preparation of the pure isolates of *Porphyromonas gingivalis* bacteria obtained from the Research Center of the Faculty of Dental Medicine, Airlangga University. The pure isolates were then grown on Muller-Hinton Agar (MHA) media and incubated anaerobically into an incubator for 1x24 hours at 37 °C to create bacterial cultures. After the incubation, the suspension was made by taking *Porphyromonas gingivalis* from the culture medium using an ossicle, then putting it in a test tube containing 11 ml of sterile Brain Heart Infusion Broth (BHIB) and then putting it in an incubator, and incubated anaerobically for 1x24 hours at 37 °C. Next, the dilution was carried out by adding sterile aquades and homogenized until the turbidity was comparable to the standard Mc Farland 0.5 (1.5x108). Then 1 ml of the bacterial suspension that had been standardized with Mc Farland turbidity 0.5 (1.5x108) was put into each test tube containing 5 ml jackfruit leaf extract with 8 different concentrations, namely 100%, 50%, 25%, 12 .5%, 6.25%, 3.125%, 1.56%, 0.78%, test tubes containing bacteria culture and chlorhexidine as positive controls, and test tubes containing bacteria culture and media as negative controls. After that, each test tube was incubated for 24 hours at 37°C. After that, 0.1 ml from each test tube is taken and planted on petri dishes containing Muller Hinton Agar (MHA) then incubated for 48 hours at 37°C. The test tubes were then measured to determine the inhibitory effect of jackfruit leaf extract on the growth of *Porphyromonas gingivalis* by observing MIC and MBC using a UV-Vis spectrophotometer BKD-750 (lambda = 750 nm) and The petri dishes were then measured to determine the inhibitory effect of jackfruit leaf extract on the growth of *Porphyromonas gingivalis* by observing MIC and MBC using colony counter with spreading technique.

3. Results

Based on the results of the phytochemical screening that has been carried out, it shows that the percentage of the content contained in jackfruit leaf extract (*Atrocarpus heterophyllus* Lam.) is flavonoid 7.05%; tannins 8.36%; and saponins 4.90%.

Table 1 Phytochemical Screening Result

Compound	Percentage
Tanin	8.36%
Flavonoid	7.05%
Saponin	4.90%

A spectrophotometry test was conducted to determine whether jackfruit leaf extract actually has antibacterial activity against *Porphyromonas gingivalis*, by observing the differences in general between all the absorbances.

Based on (Figure 1), it can be seen that the highest spectrophotometric test results in *Porphyromonas gingivalis* bacteria was 0.986 OD with an average of 0,961 OD, namely when given negative control treatment. The lowest spectrophotometric test results was when the positive control treatment was given, namely 0.018 OD with an average of 0,25 OD.

The spectrophotometry results concluded that the concentration level of the jackfruit leaf extract is directly related to the decrease in the number of the *Porphyromonas gingivalis* bacterial colonies. The increasing levels of the concentration of the jackfruit leaf exctract that was given can be seen closely related to the decrease of the bacterial colonies which can be seen from the reduction in the value of optical density along with the.

After conducting the spectrophotometry test, the dilution test results were then cultured into Muller-Hinton Agar to do a colony count test. Each plate represents each tube with the concentration. The MIC and the MBC value were determined based on the average number of bacterial colonies growing on MHA media. The results of the colony count test can be seen in Figure 2.

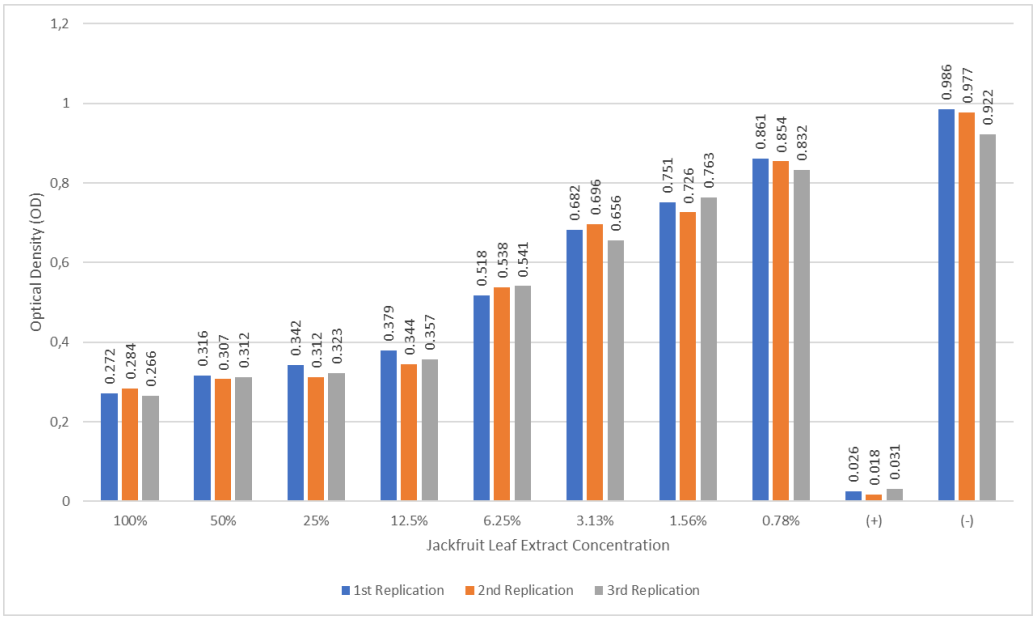

Figure 1 Absorbance Results Using a Spectrophotometry (OD)

Based on (Figure 2), it can be seen that the highest colony count test result in *Porphyromonas gingivalis* bacteria was 171 CFU/mL with an average of 170 CFU/ml when negative control was given. The lowest average colony count test results were when the concentration of jackfruit leaf extract was 100%, 50%, 25%, and 12.5%, and negative control where the colony count test result was 0 CFU/ ml.

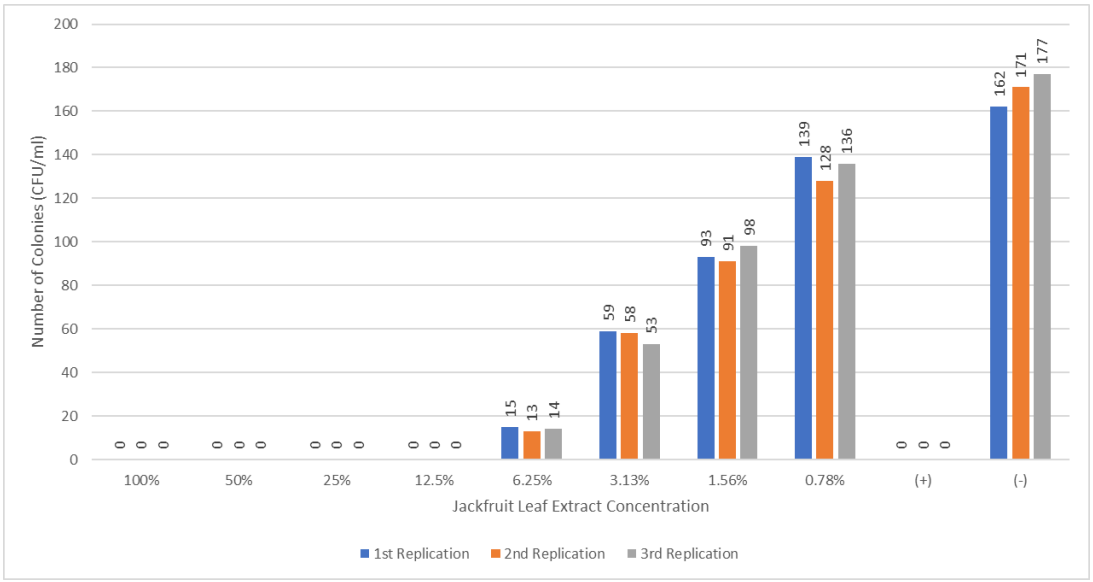

Figure 2 Colony Count Results with Colony Counter (CFU/ml)

In order to acknowledge the MIC and the MBC value, the calculation of the bacterial inhibition percentage and average colony growth was conducted. The table following contains the calculation result:

In this study, statistical data analysis and determination of MIC and MBC were done using the data from bacterial colony growth on Muller-Hinton Agar (MHA) media. The data obtained was then tested for statistical analysis with the normality test using the Shapiro-Wilk test. The test results show that all treatment groups have a value $p > 0.05$, thus the data is normally distributed. The test is continued with the homogeneity test, using the Levene's test. The test obtained a p-value of 0.001 ($p > 0.05$), which means that the data is not homogeneous. With a non-parametric test, Kruskal Wallis, the p result is 0.001, which means the data has a significant difference. The result of the post hoc Mann

Whitney test (Table 2) showed that concentrations of 6.25%, 3.125%, 1.56%, and 0.78% have significant differences with concentrations of 100%, 50%, 25%, and 12.5%.

Table 2 Average Colony Growth and Bacterial Inhibition Percentage

Group		Average Colony Growth (CFU/ml)	Bacterial Growth Percentage (%)
Concentrations	0.78%	134.3	79
	1.56%	94	55.29
	3.125%	56.67	33.33
	6.25%	14	8.23
	12,5%	0	0
	25%	0	0
	50%	0	0
	100%	0	0
Positive Control		0	0
Negative Control		170	100

Table 3 The Result of Post Hoc Mann Whitney test

Groups	100%	50%	25%	12.5%	6.25%	3.125%	1.56%	0.78%	Control (+)	Control (-)
100%	-									
50%	1	-								
25%	1	1	-							
12.5%	1	1	1	-						
6.25%	*0.037	*0.037	*0.037	1	-					
3.125%	*0.037	*0.037	*0.037	*0.037	0.5	-				
1.56%	*0.037	*0.037	*0.037	*0.037	0.5	0.5	-			
0.78%	*0.037	*0.037	*0.037	*0.037	0.5	0.5	0.5	-		
Control (+)	1	1	1	1	*0.037	*0.037	*0.037	*0.037	-	
Control (-)	*0.037	*0.037	*0.037	*0.037	0.5	0.5	0.5	0,5	*0.037	-

*Shows significant differences

4. Discussion

Periodontal disease has a high prevalence in Indonesia and can be found in all groups and age groups. Periodontal disease if not treated immediately will get worse and will damage the alveolar bone structure and cause deep periodontal pockets which will end in loss of teeth [2]. One of the bacteria that causes periodontal disease is the bacterium *Porphyromonas gingivalis*. These bacteria are very influential in the process of initiation and severity of periodontal disease.

Besides being used as an alternative medicine for several diseases, such as fever, boils, skin diseases, and anti-diarrhea, jackfruit leaves are known to be used as an antibacterial, analgesic and immunomodulator. The antibacterial properties of jackfruit leaves are obtained from the active compounds contained in jackfruit leaves, namely, tannins, flavonoids, and saponins [7, 8]. Tannin is the active compound with the most content in jackfruit leaves. The antibacterial mechanism of tannins is inhibiting the formation of bacterial extracellular enzymes, inhibiting oxidative

phosphorylation directly, and forming a complex of tannin bonds to metal ions which can increase the toxicity of tannins against bacteria [12, 13]. The mechanism of flavonoids inhibits cell membrane function by interfering with cell membrane permeability by inhibiting enzyme binding [11]. Saponins have a mechanism of reducing the surface tension of the cell wall which results in damage to the cell wall [9].

Based on research conducted by Kusumawati *et al.* that there was a decrease in the number of *Escherichia coli* bacteria colonies after being given jackfruit leaf extract [7]. Another study conducted by Majid *et al.* showed a decrease in the growth of *Staphylococcus aureus* bacteria after being given jackfruit leaf extract [14]. The results of this study are also in line with previous studies, namely jackfruit leaf extract has antibacterial properties against bacteria which is *Porphyromonas gingivalis* bacteria.

The process of extracting jackfruit leaves (*Atrocarpus heterophyllus* Lam) is carried out using the maceration method, because this method is quite simple, without heating so that the active substances contained in jackfruit leaf extract are not damaged due to high heating [15]. The maceration technique was carried out using 70% ethanol solvent. The choice of 70% ethanol as a solvent in the jackfruit leaf extraction process is because 70% ethanol binds more polar compounds which tend to be more soluble and ethanol is inert so it does not easily react with other components. In addition, ethanol has a low boiling point (78.37°C) so it is easy to separate from oil in the distalization process and the content of active compounds that have potential as antibacterials can be maximized [16, 17].

In this study, the absorbance value using spectrophotometry was not carried out statistical analysis, because the data did not have a significant difference between concentration groups which could be affected by the concentration level of the jackfruit leaf extract concentration which caused light to not be absorbed properly, making it very difficult to differentiate between living and dead bacterial cells. Therefore, in this study statistical data analysis and determination of MIC and MBC used data from the growth of bacterial colonies on Muller Hinton Agar (MHA) media.

5. Conclusion

The conclusion is jackfruit leaf extract has an inhibitory effect on the growth of *Porphyromonas gingivalis* bacteria with MIC value at the concentration of 6.25% and MBC value at the concentration of 12.5%.

Compliance with ethical standards

Acknowledgments

We would like to express our gratitude towards our teachers, family, and friends for all the support during the process of this study.

Disclosure of conflict of interest

The authors have no conflict of interest to declare.

Statement of ethical approval

This research study was approved ethically by Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearance Commission, with an ethical clearance letter number: 690/HRECC.FODM/IX/2022.

References

[1] Suratri MA, Jovina TA, Andayasari L, Edwin VA, Ayu GA. Pengaruh hipertensi terhadap kejadian penyakit jaringan periodontal (periodontitis) pada masyarakat Indonesia (data riskesdas 2018). Buletin Penelitian Kesehatan. 2020 Dec 30;48(4):228.

[2] Newman MG, Takei H, Klokkevold PR, Carranza FA. Newman and Carranza's Clinical periodontology E-book. Elsevier Health Sciences; 2018 May 29.

[3] Nayak A, Bhat K, Shivanaikar S, Pushpa P, Kugaji M, Kumbar V. Detection of red complex organisms in chronic periodontitis by multiplex polymerase chain reaction. Journal of Advanced Clinical and Research Insights. 2018 Sep 1;5(5):139-44.

[4] Datta HK, Ng WF, Walker JA, Tuck SP, Varanasi SS. The cell biology of bone metabolism. Journal of clinical pathology. 2008 May 1;61(5):577-87.

[5] Bostanci N, Belibasakis GN. *Porphyromonas gingivalis*: an invasive and evasive opportunistic oral pathogen. FEMS microbiology letters. 2012 Aug 1;333(1):1-9.

[6] How KY, Song KP, Chan KG. *Porphyromonas gingivalis*: an overview of periodontopathic pathogen below the gum line. Frontiers in microbiology. 2016 Feb 9;7:53.

[7] Kusumawati, E., Apriliana, A. and Yulia, R., 2017. Kemampuan Antibakteri Ekstrak Etanol Daun Nangka (Atrocarpus heterophyllus Lam.) Terhadap Escherichia coli. Jurnal Sains dan Kesehatan, 1(7), pp.327-332.

[8] Firdaus AA, Kriswandana F. Potensi Ekstrak Daun Nangka sebagai Biolarvasida Nyamuk Culex sp. GEMA LINGKUNGAN KESEHATAN. 2018 Apr 1;16(1).

[9] Madduluri S, Rao KB, Sitaram B. In vitro evaluation of antibacterial activity of five indigenous plants extract against five bacterial pathogens of human. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5(4):679-84.

[10] Nuria MC, Faizatun A. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Jarak Pagar (Jatropha Curcas L) Terhadap Bakteri Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, dan Salmonella typhi ATCC 1408. Mediagro: Jurnal Ilmu-Ilmu Pertanian. 2009;5(2).

[11] Li H, Wang Z, Liu Y. Review in the studies on tannins activity of cancer prevention and anticancer. Zhong yao cai= Zhongyaocai= Journal of Chinese medicinal materials. 2003 Jun 1;26(6):444-8.

[12] Sieniawska E, Baj T. Tannins [Internet]. Pharmacognosy: Fundamentals, Applications and Strategy. Elsevier Inc.; 2017. 199–232 p. Available from: http://dx.doi.org/10.1016/B978-0-12-802104-0.00010-X.

[13] Zulkarnain Z, Muthiadin C, Nur F. Potensi Kandungan Senyawa Ekstraksi Daun Patikan Kebo (Euphorbia hirta L.) sebagai Kandidat Antibiotik Alami. Teknosains: Media Informasi Sains dan Teknologi. 2021 Aug 19;15(2):190-6.

[14] Majid NS, Yamlean, PV, Citraningtyas G. Formulasi Dan Uji Efektivitas Krim antibakteri Ekstrak Daun Nangka (*Artocarpus Heterophyllus* Lam.) Terhadap Bakteri S*taphylococcus aureus*," *PHARMACON*. 2019; 8(1), pp. 225.

[15] Badaring DR, Sari SPM, Nurhabiba S, Wulan W, Lembang SAR. Uji Ekstrak Daun Maja (Aegle Marmelos L.) terhadap Pertumbuhan bakteri *Escherichia coli* Dan *Staphylococcus aureus*. *Indonesian Journal of Fundamental Sciences*. 2020; 6(1), p. 16.

[16] Mubarak Z, Chismirina S, Qamari CA. Aktivitas antibakteri ekstrak kayu manis (Cinnamomum burmannii) terhadap pertumbuhan Enterococcus faecalis. Cakradonya Dental Journal. 2016;8(1):1-0.

[17] Riwanti P, Izazih F, Amaliyah A. Pengaruh Perbedaan Konsentrasi Etanol pada Kadar Flavonoid Total Ekstrak Etanol 50, 70 dan 96% Sargassum polycystum dari Madura. Journal of Pharmaceutical Care Anwar Medika (J-PhAM). 2020 Jun 30; 2(2): 82-95.