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Research Article

Antibacterial Effects of Ethanolic Extract of Bidara *(Ziziphus mauritiana Lam)* Leaf Against *Porphyromonas gingivalis*

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Abstract

The bidara plant (*Ziziphus mauritiana Lam)* is widely distributed in various Asian countries. Bidara leaves contain secondary metabolites, the main content of which is flavonoids. As a gram-negative anaerobic bacteria, *Porphyromonas gingivalis* is one of the normal flora of the oral cavity. However, over quantities of this bacteria can promote chronic periodontitis. This research aims to analyze the bidara leaf ethanolic extract as an inhibitory agent of *Porphyromonas gingivalis.* This research design is experimental laboratory research with a *post-test* controlled group of *Porphyromonas gingivalis* inhibition. A total of 25 samples consisted of 5 groups of ethanol extract of bidara leaves at concentrations of 1%, 3%, 9%, positive control betel leaves, and negative control aquadest. Bacteria incubation was held for 48 hours, and the free bacterial zone was analyzed by the *One Way ANOVA* test. The results of the analysis showed that there was a significant difference between the control group and the treatment group. This study concludes that the ethanol extract of bidara leaves had a strong inhibitory effect on *Porphyromonas gingivalis*.

Keywords: antibacterial; bidara leaf; *porphyromonas gingivalis*

INTRODUCTION

The Global Burden of Disease Study (2016) states that periodontal disease is the 11th most common disease globally.[1] In Indonesia, the prevalence of periodontal disease in all age groups is still relatively high at 96.58%.[2] The most common type of periodontal disease is chronic periodontitis.[3]

Periodontitis is an inflammation of supporting tissue surrounding the teeth caused by microorganisms and leads to damage to the periodontal ligament, alveolar bone, pocket formation, recession, or.both.[4] Periodontitis can be classified into two: aggressive periodontitis and chronic periodontitis.[5] Chronic periodontitis is a type of periodontitis in which the rate of disease progression is slow to moderate. The main cause of chronic periodontitis is bacteria that accumulate in plaque. Porphyromonas gingivalis is commonly found in dental plaque and can cause pathological changes in periodontal tissues by activating host immune and inflammatory responses. [4]

Several types of anaerobic gram-negative bacteria are found in subgingival plaque, such as *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans*, and *Treponema denticola* which can cause and exacerbate periodontal infections.[5,6] Based on research on the subject population of 128 people with chronic periodontitis, it

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is known that the most dominant bacteria in chronic periodontitis is *Porphyromonas gingivalis*, with a prevalence of about 80.5%.[6]

Porphyromonas gingivalisis a melanogenic bacterium and belongs to the colonies of black-pigmented gram-negative anaerobes. *Porphyromonas gingivalis* usually colonizes oral tissues and can be seen in culture media with the characteristics of forming a colony with a diameter of 1-2 mm, convex and smooth, and has a characteristic with the darker color in the center.[7,8] *Porphyromonas gingivalis* can release harmful substances, such as lipopolysaccharide, as a virulence factor that triggers the host inflammatory response, resulting in periodontal tissue destruction in chronic periodontitis.[4]

Treatment of chronic periodontitis is scalinggandnroottplanning (SRP) and using antibiotics as adjunctive therapy.[9] Antibiotics consumed in excess and inappropriately can make *Porphyromonas gingivalis* resistant. Natural herbal medicine can be another alternative in treating chronic periodontitis, which is considered safer as it has fewer side effects than antibiotics.[10]

One of the plants that are believed to have many benefits is the bidara tree (*Ziziphus mauritiana Lam*). The Bidara tree consists of roots, stems, leaves, fruit, and seeds. It is known that bidara leaves have pharmacological effects as it belongs to the group of alkaloids, saponins, flavonoids, tannins, terpenoids, and steroids, which have antibacterial, anti-inflammatory, and antifungal properties.[11,14]

Studies on the bidara plant as an antibacterial have been discussed by several researchers. Maulana Siregar, 2020 stated that bidara leaves have analgesic, antipyretic and anti-inflammatory properties due to the flavonoid content so they can play an important role in periodontitis.[12]

In this study, betel leaf extract mouthwash was used as a positive control as it contains active ingredients from herbs and has a secondary metabolite content that is almost the same as bidara leaves as follows: tannins, saponins, and flavonoids.[13]

Based on the description above, this research was conducted to observe the effectiveness of the antibacterial extract of bidara leaves at concentrations of 1%, 3%, and 9% on the growth of *Porphyromonas gingivalis.*

MATERIALS AND METHODS

Analytical, experimental research with observation method using the disk diffusion method was conducted to analyze differences in the antibacterial effectiveness of ethanol, extracts of bidara leaves. A total of 25 samples were divided into five groups. The treatment group with a concentration of 1%, 3%, 9%, negative control of aquadest, and positive control of betel leaves extract mouthwash. This research protocol has been approved by the Research Ethics Commission of the Faculty of Dentistry, Sultan Agung Islamic University, with No.305/B.1-KEPK/SA-FKG/IX/2021.

Porphyromonas gingivalis Bacteria Samples

Porphyromonas gingivalis ATCC 33277 bacteria were cultured on Mueller Hinton agar media (MHA) with brain heart infusion broth (BHIB). The positive control used mouthwash with betel leaf extract from Mustika Ratu, while the negative control used sterile aquadest.

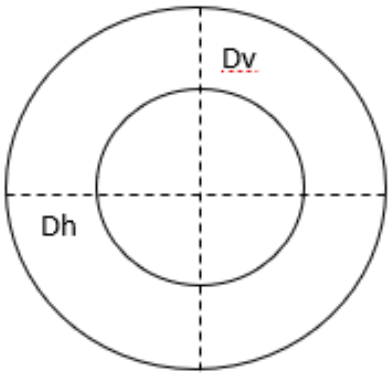
Making Bidara Leaf Extract

The fresh green bidara leaves were picked directly from the tree, then wet sorting and dry sorting were carried out, then cut into small pieces and mashed using a blender to obtain bidara leaf powder.[11] The initial maceration process was carried out by weighing 300 g of bidara leaf powder. The powder of bidara leaf that had been weighed was then put into a maceration container and macerated with 1 liter of 96% ethanol solvent.[11] Bidara leaf

extract (filtrate) was stored in a place protected from sunlight and allowed to stand for 72 hours at room temperature, stirring occasionally. After three days, the filtrate was filtered and macerated using 1 liter of 96% ethanol. Measurements were carried out five times with the same amount of solvent. Each filtrate was filtered using paper Whatman then combined from the maceration and remaceration results.[17] After the filtrate was combined, it was evaporated using a rotary evaporator to get a thick extract of bidara leaves.[17] The extract was then prepared to a concentration of 1%, 3% and 9% with sterile distilled water as a solvent.

Porphyromonas gingivalis Inhibitory Test

Preparation of suspension of *Porphyromonas gingivalis* was carried out by taking the preparation of *Porphyromonas gingivalis* with a sterile one needle, then inserting it into a test tube containing BHIB and homogenizing it with a vortex. The bacterial turbidity was equalized to 0.5 Mc Farland (approximately $1\text{-}2\times10^8$ CFU/ml). The next step was to test the effectiveness of the extract. bidara leaf ethanol on growth of *Porphyromonas. gingivalis.* It was done by spreading the suspension of *Porphyromonas gingivalis* bacteria using a sterile cotton swab evenly on the surface of the MHA media. Each paper disc dripped 10 µm ethanol extractkofnbidara leaves with concentrations of 1%, 3%, and 9%, respectively. As a negative control, the paper disc was dripped with distilled water and betel leaf extract mouthwash as a positive control.[11] It was then incubated at 37°C for 48 hours. After 48 hours, the diameter of the clear zone (free bacterial zone) formed around the paper disc was measured using a caliper.[14] The inhibition zone measurement can be conducted using the formula.[15]



$$\frac{Dv + Dh}{2}$$

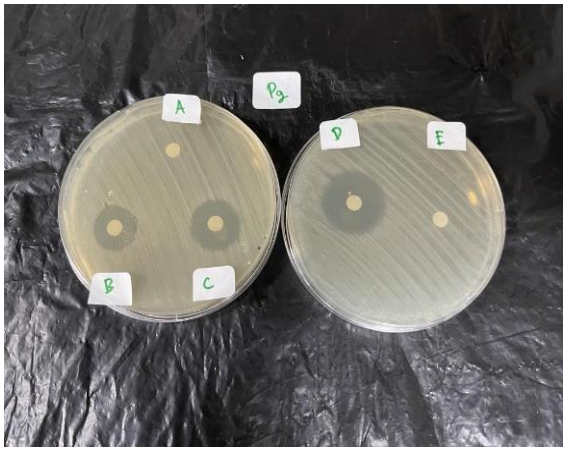
Note:
□ = inhibitory zone
Dv = diameter of vertical
Dh = diameter of horizontal

Statistics

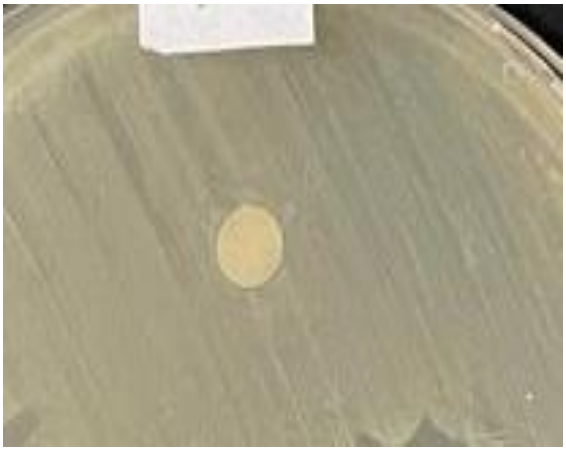
This data was processed using the SPSS computer software program. In addition, a parametric statistical test was carried out with the *One Way Annova* test to analyze the significance of bidara leaves ethanol extract at several concentrations in inhibiting the growth of *Porphyromonas gingivalis*.

RESULT

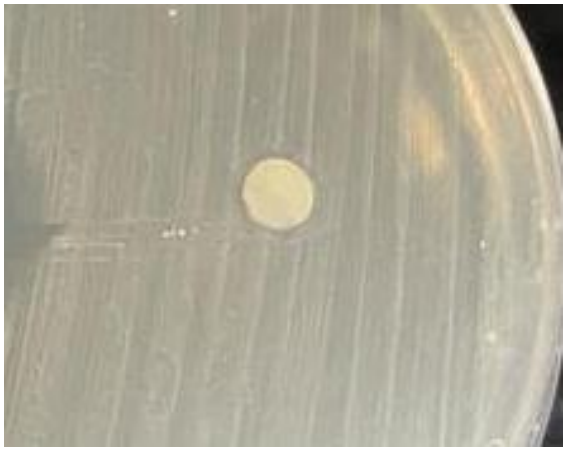
Bidara leaf extract concentrations of 3%, 9%, and positive control mouthwash of betel leaf extract showed antibacterial activity of *Porphyromonas gingivalis*. In comparison, bidara leaves extract with a concentration of 1% and aquadest did not show any inhibition against *Porphyromonas gingivalis*.



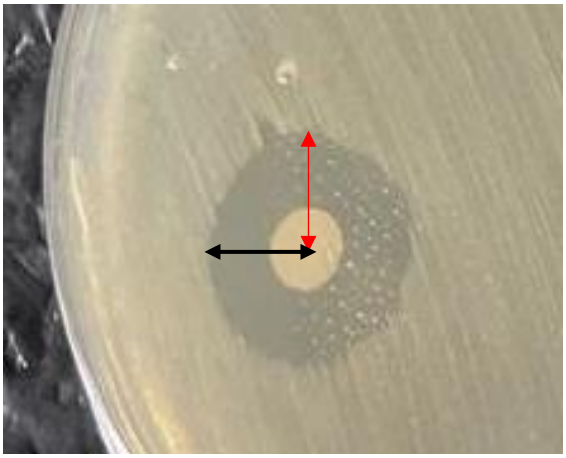
Picture 1. Inhibitory zone on MHA by the method of disk diffusion



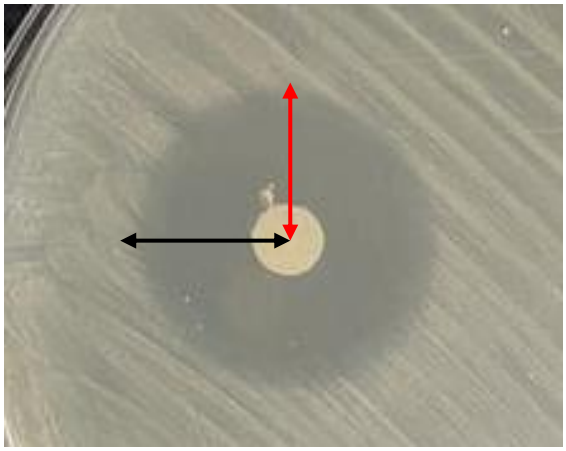
Picture 4. Bidara leaves extract 1% concentration



Picture 2. Negative control of aquadest



Picture 5. Bidara leaves extract 3% concentration



Picture 3. Positive control of betel leaves extract mouthwash

Picture 6. Bidara leaves extract 9% concentration

Vertical diameter (Dv) ↔

Horizontal diameter (Dh) ↔

Table 1. Inhibitory effect analysis of bidara leaf extract at several concentrations on the growth of *Porphyromonas gingivalis*

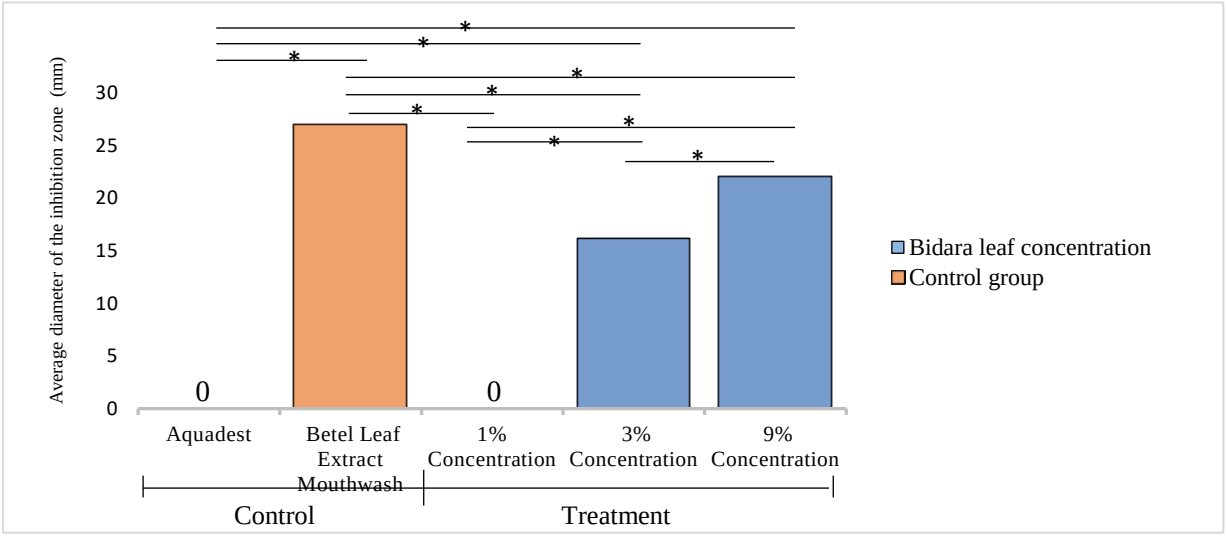
No	The concentration of Bidara Leaves Ethanol Extract			Control Group	
	1%	3%	9%	Aquadest (negative)	Betel Leaf Extract Mouthwash (positive)
1	0	16.8 mm	22.2 mm	0	27.4 mm
2	0	16.05 mm	22 mm	0	27.05 mm
3	0	14.95 mm	20.8 mm	0	26.4 mm
4	0	16.4 mm	21.95 mm	0	27.2 mm
5	0	16.2 mm	22.6 mm	0	27.15 mm
Average	0	16.08 mm	21.91 mm	0	27.04 mm

Table 2. Analysis of normality and homogeneity among groups of *Porphyromonas gingivalis* inhibition test of bidara leaves extract (p>0.05)

Group	Shapiro-Wilk			Levene Sig
	Statistics	df	Sig.	
1% Concentration	-	5	-	0.059
3% Concentration	0.9	5	0.42	
9% Concentration	0.87	5	0.29	
Aquadest Control	-	5	-	
Betel Leaf Extract Mouthwash Control	0.85	5	0.2	

Table 3. One-WayyAnnova analysis among groups of *Porphyromonas gingivalis* inhibition test of bidara leaves extract (p<0.05)

Group	Mean ±Std. Dev	One-way ANOVA(Sig.)
1% Concentration	0.00±0.00	.000
3% Concentration	16.08±0.69	
9% Concentration	21.91±0.67	
Aquadest Control	0.00±0.00	
Betel Leaf Extract Mouthwash Control	27.04±0.37	



Picture 6. Significance of differences between the control and treatment groups

_____ * = Significance

The inhibition test results showed that the ethanol extract of bidara leaves had high inhibitory activity at concentrations of 3% and 9% compared to 1%. Test for normality and homogeneity with *Sapphiro-Wilk* and *Levene* p>0.05 showed that the data were normally distributed and homogeneous (Table 2). Further analysis by the One-Way Anova test (Table 3) showed that the p-value on the One-Way Annova test = 0.000 (p<0.05), indicating that there was a significant difference between each group in inhibiting the growth of *Porphyromonas gingivalis*. Picture 2 shows a significant difference between the control group and the treatment group, namely aquadest with positive control mouthwash of betel leaf extract, aquadest with bidara leaf extract (*Ziziphus mauritiana Lam*) with a concentration of 3% and 9%, and mouthwash with betel leaf extract with a concentration of 3%.

DISCUSSION

The antibacterial activity of *Porphyromonas gingivalis* was shown by measuring the inhibitory zone after 48 hours of incubation at room temperature (37℃). The negative control of sterile distilled water and 1% bidara leaves extract had no antibacterial effect on *Porphyromonas gingivalis*, which was indicated by the absence of an inhibition zone (0 mm). Positive control of betel leaf extract mouthwash showed the formation of the largest clear zone compared to the three groups of bidara leaves concentration (Table 1). The positive control mouthwash of betel leaf extract had an inhibition zone of 27 mm and was more significant than the aquadest control (Table 3).

The 3% bidara leaf extract had an inhibition zone of 16.08 mm and a concentration of 9% of 22 mm (Table 1). However, the positive control of betel leaves extract mouthwash with 9% bidara leaves extract had a significant difference, although both groups had an inhibition zone of >20 mm (Table 3). Thus, it can be concluded that the mouthwash of betel leaf extract with 9% bidara leaves extract is included in the powerful category in inhibiting the growth of *Porphyromonas gingivalis* bacteria. This result is in accordance with the research conducted by Agrianto (2016) regarding the assessment of inhibitory power using the Davis and Stout categories. It was found that the average inhibition zone of clove flower extract was 13.01 mm, which was classified as having a strong inhibitory effect on the growth of *Porphyromonas gingivalis.*[15]

The area of inhibition can be observed by the presence of a clear zone between the growth of bacteria (free bacterial zone). The inhibition zone marked by the clear zone (Figure 1) in the 3% and 9% bidara leaves extract appeared due to antibacterial activity, which was considered to be produced from secondary metabolites, namely alkaloids, saponins, flavonoids, tannins, terpenoids, and steroids which had antibacterial properties, anti-inflammatory, and antifungal agents.[16] The antibacterial effect of bidara leaves ethanol extract on the growth of *Escherichia coli* and showed the presence of an inhibition zone at 9% concentration with an average inhibition zone diameter of 15.66 mm. The antibacterial effectiveness of bidara leaves is due to the active substance. Higher concentrations of bidara extract can increase the number of active ingredients that act as antibacterial agents. Thus, the ability of microbial growth inhibition is more significant.[11]

The effectiveness of pure 9% bidara leaves extracts actively inhibits the growth of *Porphyromonas gingivalis*. The betel leaves extracts used in this study contained a mixture of antibacterial ingredients of 0.5% betel leaves and 0.04% mint leaf oil (*Mentha piperita oil*). In contrast, the bidara leaves extract in this study was pure extract without a mixture of other ingredients for further research. Therefore, it is recommended that bidara leaf extract can be combined with other antibacterial herbs

or other active components to obtain maximum results when used as an antibacterial ingredient.

CONCLUSION

Bidara leaves extract concentrations of 3% and 9% effectively inhibited the growth of *Porphyromonas gingivalis* bacteria. In addition, 9% of bidara leaf extract with mouthwash control of betel leaf extract had a diameter of inhibition zone above 20 mm, indicating a potent inhibition against the growth of *Porphyromonas gingivalis*.

REFERENCES

1. Kementrian Kesehatan RI. Faktor Risiko Kesehatan Gigi dan Mulut. Pus Data dan Inf Kementeri Kesehat RI. 2019;1–10.
2. Dinyati M, Adam AM. Kuretase gingiva sebagai perawatan poket periodontal. Makassar Dent J. 2016;5(2):58–64.
3. Alibasyah ZM, Andayani R, Farhana A. Potensi Antibakteri Ekstrak Jahe (Zingiber officinale Roscoe) terhadap Porphyromonas gingivalis secara In Vitro. J Syiah Kuala Dent Soc. 2016;1(2):147–52.
4. Carranza FA, Newman MG, Klokkevold PR, Takei HH. Newman and Carranza's Clinical Periodontology. Thirteenth. Newman and Carranza's Clinical Periodontology. 2019. 19 p.
5. Al Qahtani NA, Joseph B, Deepthi A, Vijayakumari BK. Prevalence of chronic periodontitis and its risk determinants among female patients in the Aseer Region of KSA. J Taibah Univ Med Sci. 2017;12(3):241–8. https://doi.org/10.1016/j.jtumed.2016.11.012
6. Quamilla N. Stres dan Kejadian Periodontitis (Kajian Literatur). J Syiah Kuala Dent Soc. 2016;1(2):161–8.
7. Andriani I, Chairunnisa FA. Periodontitis Kronis dan Penatalaksaan Kasus dengan Kuretase. Insisiva Dent J Maj Kedokt Gigi Insisiva. 2019;8(1):25–30. https://doi.org/10.18196/di.8103
8. Mahalakshmi K, Krishnan P, Chandrasekaran SC, Panishankar KH, Subashini N. Prevalence of periodontopathic bacteria in the subgingival plaque of a south indian population with periodontitis. J Clin Diagnostic Res. 2012;6(4 SUPPL. 2):747–52.
9. Alibasyah ZM, Ningsih DS, Ananda SF. Daya Hambat Minuman Probiotik Yoghurt Susu Sapi terhadap Porphyromonas Gingivalis Secara In Vitro. J Syiah Kuala Dent Soc. 2018;2(3):65–75.
10. Nakayama K. Porphyromonas gingivalis and related bacteria: From colonial pigmentation to the type IX secretion system and gliding motility. J Periodontal Res. 2015;50(1):1–8. https://doi.org/10.1111/jre.12255
11. Hermanto NR, Syaify A, Sudibyo. Pengaruh Aplikasi Gel Aloe Vera Sebagai Bahan Tambahan Scaling Dan Root Planing Terhadap Penyembuhan Jaringan Periodontal Pada Perawatan Periodontitis Kronis. J Kedokt Gigi. 2015;6(3):307–14.
12. Sapara TU, Waworuntu O, Juliatri. Efektivitas Antibakteri Ekstrak Daun Pacar Air (Impatiens Balsamina L.) Terhadap Pertumbuhan Porphyromonas Gingivalis. J Pharmacon. 2016;5(4):10–7. https://doi.org/10.35790/eg.4.2.2016.14161
13. Taufiq. Aktifitas Efek Antimikroba Ekstrak Etanol Daun Bidara Laut (Ziziphus Mauritiana Lam.) terhadap Pertumbuhancandida Albicans dan Escherichia Coli. J Kesehat. 2018;3(1):1–8.
14. Yahia Y, Benabderrahim MA, Tlili N, Bagues M, Nagaz K. Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two Ziziphus Mill. Species. Plos One J.

2020;15(5):1–16. https://doi.org/10.1371/journal.pone.0232599

15. Siregar M. Berbagai Manfaat Daun Bidara (Ziziphus mauritiana lamk) bagi Kesehatan di Indonesia. J Pandu Husada. 2020;2(1):75–81. https://doi.org/10.30596/jph.v1i2.4415
16. Vifta RL, P MAW, Hati AK. Aktivitas antibakteri salep ekstrak etanol daun sirih hijau (Piper betleL .) Terhadap infeksi bakteri Staphylococcus aureus. J Ilm Farm. 2017;5(2):56–61. https://doi.org/10.26874/kjif.v5i2.117
17. Chairunnisa S, Wartini NM, Suhendra L. Pengaruh Suhu dan Waktu Maserasi terhadap Karakteristik Ekstrak Daun Bidara (Ziziphus mauritiana L.) sebagai Sumber Saponin. J Rekayasa Dan Manaj Agroindustri. 2019;7(4):551. https://doi.org/10.24843/JRMA.2019.v07.i04.p07
18. Ningrum WA, Rahmatullah S. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Bidara (Ziziphus Mauritiana Lamm) Dalam Formulasi Sediaan Sabun Cair Sebagai Antiseptik Terhadap Bakteri Stapylococcus Aureus Atcc 25923. Med Sains Junal Ilm Kefarmasian. 2020;5(1):89–98. https://doi.org/10.37874/ms.v5i1.161
19. Paliling A, Posangi J, Anindita PS. Uji Daya Hambat Ekstrak Bunga Cengkeh (Syzygium aromaticum) terhadap Bakteri Porphyromonas gingivalis. J e-GIGI. 2016;4(2). https://doi.org/10.35790/eg.4.2.2016.14159
20. Abdallah EM, Elsharkawy ER, Ed-dra A. Biological activities of methanolic leaf extract of Ziziphus mauritiana. J Biosci Biotechnol Res Commun. 2016;9(4):605–14. https://doi.org/10.21786/bbrc/9.4/6