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RESEARCH ARTICLES

Antibacterial Activity of Legundi (*Vitex trifolia L.*) Fruit Extract Against *Lactobacillus gasseri* ATCC 19992: Study In Vitro

Assyifa Cindykia[1*], Humairah Medina Liza Lubis[2]

[1] Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Jalan Gedung Arca No.53 Medan 20217 Sumatera Utara, Indonesia

[2] Department of Anatomical Pathology, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Jalan Gedung Arca No. 53 Medan 20217 Sumatera Utara, Indonesia

Email correspondence: syifacindy02@gmail.com

Abstract: *Lactobacillus* species are the most frequent bacterial colonies in the human microbiome that can be found in the gastrointestinal tract, urinary tract, and vaginal microbiota. *Lactobacillus gasseri* is an important constituent of the female urogenital tract. if there is excessive proliferation of *Lactobacillus*, there will be changes in the homeostasis of the human microbiome. Homeostasis of the human microbiome can undermine the symbiotic relationship between host and microorganisms, induce physiological changes in the individual, and lead to the development of diseases such as to the development of diseases such as cancer. Legundi fruit extract was found to be cytotoxic against several lines of cancer cells and has antimicrobial activity. This research aims to determine the diameter of antibacterial inhibition of Legundi fruit extract, and determine the minimum inhibition concentration (MIC), minimum kill concentration (MKC) against *Lactobacillus gasseri*. This research used solid and liquid dilution and diffusion methods with concentrations of 10%, 20%, 25%, 50%, 75%, 100%, positive and negative controls**.** Extract of Legundi fruit can't inhibit bacterial growth at concentrations of 10% and 20%. The longest diameter was in the positive control with streptomycin. The higher the concentration of Legundi fruit ethanol extract given, the higher the inhibition ability**.**

Keywords: Antibacterial, cervical cancer, extract of legundi fruit, in vitro, lactobacillus gasseri

INTRODUCTION

Lactobacillus is a lactic acid bacteria known as beneficial bacteria. Lactobacillus has a low pH because it has organic acid lanilla that can maintain a pH below 4.5 (ranging from 3.8-4.5). Optimum temperature, high salt tolerance, habitat and potential pathogenicity are different.[1]

Lactobacillus gasseri is one of the normal flora bacteria in the vagina which has

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a rod shape and is a bacterium that can produce hydrogen peroxide and bacteriocins. This hydrogen peroxide can cause toxic properties to other organisms. The production of H_2O_2 in lactic acid bacteria causes *Lactobacillus sp*.[2] Bacteria to be found more than the number of obligate anaerobic bacteria with minimal catalase enzyme.[1]

Physiologically and under normal circumstances, the normal bacterial flora residing in the vagina plays a role in maintaining a dynamic balance, when excessive proliferation occurs, it can be the cause of various diseases, including multiple gynecological diseases, namely choleitis, and high-grade cervical intraepithelial neoplasia (CIN) and cervical cancer.[3] Normal flora found in vagina include Mycoplasma genitalium, *Lactobacillus* bacteria species namely L. crispatus, L. iners, L. jensenii and L. gasseri, Staphylococcus epidermidis, Enterococcus, Escherichia coli, and species of Bacteriodes.[4]

Vaginal *Lactobacillus* plays a role in controlling pathogen growth and development by producing hydrogen peroxide, lactic acid, bacteriocins, and the presence of a competitive in the vaginal epithelium adhesion in a competitive manner. Activation of the complement system and local immune system response.[2,5]

In the female reproductive tract, the role of *Lactobacillus* is prominent, and when the dynamic balance of the microbial environment is disturbed, the negative effects of anaerobic bacteria will counteract the positive effects of Lactobacillus. Microbial dysregulation can release a large number of pro-inflammatory cytokines and chemokines, resulting in a local inflammatory response.[6] The link between vaginal microbes and cancer has also been widely studied. Some studies have also shown that lactic acid bacteria have cytotoxic effects on cervical cancer cells.

Legundi fruit (*Vitex trifolia L*) is a small-leaved shrub plant that can be found in Asian countries including Indonesia. Legundi fruit is often used in traditional Chinese medicine to treat dizziness, headaches, migraines, cancer and TB (Tuberculosis).[7,8] In addition, Legundi fruit can also be used for the treatment of gum swelling and pain, malignant tumors, and others. Legundi fruit extract was found to be cytotoxic against several cancer cell lines and has antimicrobial activity.[9]

Based on the taxonomic level of Legundi fruit can be classified as follows Kingdom: *Plantae*; Division: *Spermatophyta*; Sub division: *Angiosperms*; Class: *Dicotylendonae*; Order: *Lamiales*; Family: *Verbenaceae*; Genus: *Vitex*; Species: *Vitex trifolia*.[10]

Some chemical compounds found in Legundi include flavonoids (Casticin; 3,6,7-trimethyl quercetin; vitexin; Artemetin; 5 methyl Artemetin; 7-desmethyl Artemetin; Luteolin; Luteolin-7-O-²-D-glucuronide; luteolin-3-O-²- D-glucuronide and Isoorientin), Terpenoids, and Sterols (²-

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sitosterol and ²-sitosterol-²-D-glucoside). Other research proves that in the legundi fruit, the compounds sabinene (13.17%), eucalyptol (23.07%), terpinen-4-ol (4.26%), α-terpineol (5.19%), d-Limonene (7.25%), and caryophyllene (14.72%) were found.[11] The active compound of the legundi fruit is the terpenoid group, namely Labdane type-diterpene, found in the form of 1,1,4a-Trimethyl-5-6 dimethylenedecahydronaphthalene.[12]

Flavonoid compounds can be used as antibacterials that work together with extracellular proteins to form complex compounds that can cause protein denaturation in bacterial cells and damage cell membranes in bacteria. Terpenoids can also be antibacterial because of the mechanism of action that resembles flavonoid compounds, namely by damaging the cell membrane in bacteria.[13] Alkaloid compounds can work by disrupting the components of the peptidoglycan cell wall in bacteria and changing the amino acid composition in bacteria.[14]

In vitro research concluded that the concentration of legundi (*Vitex trifolia L.*) extract that most effectively inhibits the growth of Staphylococcus epidermidis bacteria starts from 10% concentration with an inhibition of 8 mm, 15% concentration with an inhibition of 8.84 mm, 20% concentration with an inhibition of 9.84 mm, 25% concentration with an inhibition of 10.54 mm, this indicates that the greater the concentration, the greater the inhibition of bacterial growth.[15]

This research is an initial study that is useful for testing the effectiveness of Legundi fruit extract (*Vitex trifolia L.*) as an antimicrobial effect in reducing the progressivity of cervicovaginal microbiome biomarker *Lactobacillus gasseri*. The results of the study are expected to be data that can be used for further research on alternative treatments based on herbal medicine in cervical cancer. So far there has been no research on the effectiveness of legundi fruit extract as an antimicrobial aimed at *Lactobacillus gasseri* bacteria.

METHOD

This research was conducted at the Laboratory of Biochemistry and Microbiology, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara. Which is based on the approval of the Health Research Ethics Commission from the Faculty of Medicine of Universitas Muhammadiyah Sumatera Utara No. 1052/KEPK/FKUMSU/2023. This research is experimental study. In vitro studies are measured by assessing the Minimum Inhibitory Level, Minimum Kill Level and the diffusion method.

Extract preparation and Sample preparation. Two thousand five hundred grams of Legundi fruit were cleaned, washed, dried and pulverized. Simplisia is made into extracts by the maceration method by soaking the simplisia in 96% ethanol (1:10) for 3 days with several stirrings in a dark place. several times stirred in a dark place storage. Filtered with vaccum filtration

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and Whatman filter paper no. 40, evaporated with rotary evaporator T=62^{0}C, P=80 mBar.

Phytochemical screening. Tannin test (96% ethanol extract of Legundi fruit is dripped onto a drip plate by adding 1% FeCl3 solution). Alkaloid test (Ethanol extract of 96% Legundi fruit 0.5 g was added with 1 mL of 2N HCL and 9 ml of distilled water, then heated for 2 minutes and cooled. Separate the filtrate into 2 tubes, tube 1 was given Dragendorff's reagent (potassium tetraidobismuthate) and tube 2 was given Mayer's reagent.), triterpenoid and steroid test (Ethanol extract of 96% Legundi fruit as much as 0.5 g was added with 2 mL of ethanol and then heated. Add 0.5 mL of anhydrous acetic acid and 2 mL of sulfuric acid then homogenize).[16] Flavonoid test (Ethanol extract of 96% of legundi fruit is given Mg powder and 1 mL of concentrated HCl, and add ethanol, then shake vigorously and wait until it separates. If an orange color is found, the extract is positive for containing flavonoids), saponin test (Ethanol extract of 96% Legundi fruit as much as 0.5 g is added with 10 mL of distilled water then heated and cooled. Shake vigorously for 10 seconds, if foam is formed that can last for 10 minutes add 1 drop of 2N HCL, if there is still foam then saponin is positive).[16]

The dilution method is divided into 2 types: liquid dilution and solid dilution. Type liquid dilution is done by assessing the level of turbidity in a test tube that is filled with liquid media in the form of aquadest or extract solution and a certain amount of the tested microbe. The tubes are incubated at ±36^{0}C for 18-24 hours.[17]

While the solid dilution type is carried out on agar media according to the microbes that is cultured. This is done to assess the presence or absence of bacteria.[17]

Diffusion method This method is used to assess the size of the zone of inhibition after administering antimicrobial or an extract in a solid medium. In this method which is assessed in the form of a clear zone on solid media. In this method, bacteria inoculated in solid media and disc paper is used which is attached to the solid media. The stronger the antibacterial activity, the wider the inhibition, which is described by the wider the clear area. Inhibition which is described by the width of the clear area.[18]

The materials used in this study are Legundi fruit, 96% ethanol, aquabidest, aquadest, anhydrous acetic acid, HCL 2N, FeCl3, Mg powder, dragendorff reagent, mayer reagent, *Lactobacillus gasseri* ATCC 19992, Steptomycin (S), blank disc, Man Rogosa Sharpe Agar (MRSA), dan Man Rogosa Sharpe Broth (MRSB).

The tools needed in this study are chromatography columns Amberlite FPA900 UPS Cl, laminar air flow, oven, autoclave, hotplate, scales, rack, erlenmeyer, magnetic strirer, tweezers, Bunsen lamp, petri dish, measuring cup and beacker, drip pipette, spatula, destroying drops. and beacker, dropper pipette, spatula, distillator, rotary evaporator, macerator container, shaker.

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RESULT

The results of the test on the extract of the legundi fruit, the results of the phytochemical test, and the results of the in vitro test with the treatment of the legundi fruit by dilution and diffusion can be seen in more detail below.

Legundi Fruit Extract (*Vitex trifolia L.*) Test

After sample preparation, 2500 grams of legundi fruit produces 5.7 liters of liquid extract using the maceration method. With the organoleptic testing method, the extract was thick, had a distinctive odor, was blackish brown in color, and had a bitter taste. Using the evaporation and water bath methods, a thick extract of 40.07 grams was obtained and the extract yield was 4.45%.

Phytochemical Test Results

The results of the phytochemical test can be seen in table 1.

Table 1. Phytochemical Test Result

Component	Description	Result
Tannin	A blackish green color is formed	+
Alkaloid	Mayer: Yellowish precipitate formed	+
	Dragendorff: A brick red precipitate is formed	+
Triterpenoid/ Steroid	Purple color formed	+ (terpenoid)
Flavonoid	Forms a greenish black color	+
Saponin	there appears to be foam	+

In vitro analysis results of liquid dilution method with fruit treatment Legundi

The results of liquid dilution method with fruit treatment Legundi can be seen in table 2.

Table 2. Legundi fruit extract minimum inhibitory Result

No	Concentration	Observation result
1	Concentration 10%	Turbid
2	Concentration 20%	Turbid
3	Concentration 25%	Clear
4	Concentration 50%	Clear
5	Concentration 75%	Not assessable
6	Concentration 100%	Not assessable
7	Positive Control	Clear
8	Negative Control	Turbid

Note: Not assessable→ Extracts are too concentrated and dark in color, making it difficult to assess.

Figure 1. Legundi fruit extract minimum inhibitory level

The results of in vitro analysis with the liquid dilution method with Legundi fruit treatment can be concluded that at concentrations of 25%, 50% and the positive control no turbidity was found so that the ability of Legundi fruit to inhibit *Lactobacillus gasseri* bacteria is at that

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concentration, with the smallest concentration capable of inhibiting bacteria is 25%, while at a concentration of 75%, and 100% cannot be assessed.

In vitro analysis results of solid dilution method with fruit treatment Legundi

The results of solid dilution method with fruit treatment Legundi can be seen in table 3.

Table 3. Legundi fruit extract minimum kill level Result

No	Type of tube	Observation result
1	Concentration 10%	Microbial growth
2	Concentration 20%	Microbial growth
3	Concentration 25%	No microbial growth
4	Concentration 50%	No microbial growth
5	Concentration 75%	No microbial growth
6	Concentration 100%	No microbial growth
7	Positive Control	No microbial growth
8	Negative Control	Microbial growth

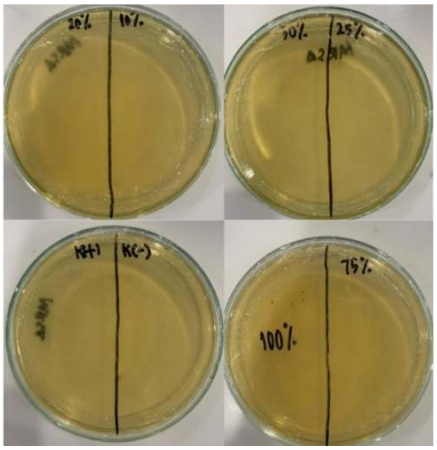

Figure 2. Legundi fruit extract minimum kill level

The results of in vitro analysis with the solid dilution method with Legundi fruit treatment can be concluded that at concentrations of 25%, 50%, 75%, 100% and the positive control no bacterial growth was found so that the ability of Legundi fruit to kill *Lactobacillus gasseri* bacteria is at that concentration, with the smallest concentration capable of killing bacteria is at a concentration of 25%.

In vitro diffusion method analysis results with Legundi fruit treatment

The results of the diffusion method were obtained from the administration of different concentrations of legundi fruits as well as positive control using the antibiotic streptomycin and negative control using aquabidest can be seen can be seen in table 4.

Table 4. Inhibitory power at the concentration of Legundi fruit extract and the control group

Repetition	Diameter of *Lactobacillus gasseri* growth inhibition zone (mm)							
	Concentration of Legundi fruit extract (*Vitex trifolia L.*)						Control Group	
	10 %	20 %	25 %	50 %	75 %	100 %	-	+
Repetition 1	13,68	14,72	17,21	20,33	23,58	25,12	0	28,15
Repetition 2	13,37	15,03	17,02	19,65	24,07	25,85	0	28,35
Repetition 3	13,07	14,09	17,55	18,03	23,39	26,03	0	29,05

It was also shown that in repetition 1, 10% concentration inhibition 13.68 (strong), 20% concentration inhibition 14.72 (strong), 25% concentration inhibition 17.21 (strong), 50% concentration inhibition 20.33 (very strong), 75% concentration inhibition 23.58 (very strong), 100% concentration inhibition

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25.12 (very strong), and positive control 28.15 (very strong). Repetition 2 showed 10% concentration inhibition 13.37 (strong), 20% concentration inhibition 15.03 (strong), 25% concentration inhibition 17.02 (strong), 50% concentration inhibition 19.65 (strong), 75% concentration inhibition 24.07 (very strong), 100% concentration inhibition 25.85 (very strong), and positive control 28.35 (very strong). In repetition 3, 10% concentration showed 13.07 (strong) inhibition, 20% concentration 14.09 (strong) inhibition, 25% concentration 17.55 (strong) inhibition, 50% concentration 18.03 (strong) inhibition, 75% concentration 23.39 (very strong) inhibition, 100% concentration 26.03 (very strong) inhibition, and positive control 29.05 (very strong).

DISCUSSION

Legundi fruit extract (*Vitex trifolia L.*) contains secondary metabolite compounds including flavonoids, terpenoids, alkaloids, tannins, and saponins. This is in accordance with the results of the phytochemical screening test contained in the table 1.

Based on Table 2 and 3, the assessment of the bacterial inhibition zone by calculating the diameter of the clear zone on the control disk provided. This can occur because legundi fruit extract (*Vitex trifolia L.*) contains secondary metabolite compounds including flavonoids, terpenoids, alkaloids, tannins, and saponins.[15] The most commonly used extraction method is maceration method because it can extract thermolabile materials (without heating). In addition, maceration is a simple extraction method because it can penetrate the cell wall and enter the cell cavity containing the active substance.[19] The active substance will dissolve and the difference in concentration between the active substance solution inside and outside the cell causes the concentrated solution to come out until there is a balance of concentration between the solution inside and outside the cell.[20]

The results of the *in vitro* analysis that have been carried out prove that the greater the concentration used, the higher the inhibition on bacteria and the higher the ability of Legundi fruit extract to kill and stop the progressivity of bacterial growth, this is in accordance with the research of Fatmawati D et al, (2020) which proves that ethanol extract of Legundi fruit against *Staphylococcus aureus* bacteria with concentrations of 25%, 50%, 75%, and 100% shows that the higher the concentration used, the greater the inhibition zone produced.[13] This is evident in the results of *in vitro* studies on the dilution method and diffusion method in this study.

This is evidenced in the results of in vitro research on the dilution method and diffusion method. The dilution method shows that at concentrations of 10% and 20% there is still turbidity and bacterial growth, so that at these concentrations *Lactobacillus gasseri* bacteria can still grow, while at concentrations of 25%, 50%, 75%, and 100% there is no turbidity. Similarly, with the diffusion method, the greater the

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concentration, the longer the diameter of the inhibition zone. So, this proves that the in vitro research conducted is in accordance with previous research. The dilution method shows that at concentrations of 10% and 20% there is still turbidity and bacterial growth, so at these concentrations *Lactobacillus gasseri* bacteria can still grow, while at concentrations of 25%, 50%, 75%, and 100% there is no turbidity. Similarly, with the diffusion method, the greater the concentration, the longer the diameter of the inhibition zone.

Indriatie & Masruri 2023 research, proving *in vitro* research with the diffusion method, showed that legundi was able to inhibit the growth of *Staphylococcus aureus* bacteria well. This is in line with the in vitro research diffusion method contained in table 2 that 96% ethanol extract of Legundi fruit is able to inhibit the growth of different bacteria, namely *Lactobacillus gasseri* with strong to very strong categories.[11]

CONCLUSION

Legundi fruit extract (*Vitex trifolia L.*) contains secondary metabolite compounds including flavonoids, terpenoids and alkaloids. Flavonoid compounds can be used as antibacterials that work with extra-cellular proteins to form complex compounds that can cause protein denaturation in bacterial cells and damage cell membranes in bacteria. Similarly, terpenoids can also be antibacterial because the mechanism of action resembles flavonoid compounds, namely by damaging cell membranes in bacteria, while alkaloid compounds can work by disrupting the components that make up the peptidoglycan cell wall in bacteria and changing the amino acid composition in bacteria.

Based on the ability of minimum inhibition concentration (MIC), minimum kill concentration (MKC) and the diameter of inhibition on *Lactobacillus gasseri*, it was found that legundi fruit extract was unable to inhibit *Lactobacillus gasseri* at concentrations of 10% and 20%. The diameter of inhibition was found to be longer when the concentration given was higher. So that legundi fruit extract is very potential as an herbal treatment for cervical cancer in reducing the growth progressivity of *Lactobacillus gasseri.*

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This research is only limited to research on bacterial culture, so research is needed on test animals that are directly aimed at the target organ.

REFERENCES

1. Kovachev SM. Cervical cancer and vaginal microbiota changes. *Arch Microbiol*. 2020;202(2):323-327. doi:10.1007/s00203-019-01747-4
2. Curty G, de Carvalho PS, Soares MA. The role of the cervicovaginal microbiome on the genesis and as a biomarker of premalignant cervical

E-ISSN: 2528-410X

intraepithelial neoplasia and invasive cervical cancer. *Int J Mol Sci*. 2020;21(1). doi:10.3390/ijms21010222

3. Zhou ZW, Long HZ, Cheng Y, Luo HY, Wen DD, Gao LC. From Microbiome to Inflammation: The Key Drivers of Cervical Cancer. *Front Microbiol*. 2021;12(November):1-15. doi:10.3389/fmicb.2021.767931
4. Yang X, Da M, Zhang W, Qi Q, Zhang C, Han S. Role of *Lactobacillus* in cervical cancer. *Cancer Manag Res*. 2018;10:1219-1229. doi:10.2147/CMAR.S165228
5. Chee WJY, Chew SY, Than LTL. Vaginal microbiota and the potential of *Lactobacillus* derivatives in maintaining vaginal health. *Microb Cell Fact*. 2020;19(1):1-24. doi:10.1186/s12934-020-01464-4
6. Wu F, Kong Y, Chen W, et al. Improvement of vaginal probiotics *Lactobacillus* crispatus on intrauterine adhesion in mice model and in clinical practice. *BMC Microbiol*. 2023;23(1):1-13. doi:10.1186/s12866-023-02823-y
7. Aye MM, Aung HT, Sein MM, Armijos C. A review on the phytochemistry, medicinal properties and pharmacological activities of 15 selected myanmar medicinal plants. *Molecules*. 2019;24(2). doi:10.3390/molecules24020293
8. Lubis HML. Kajian Molekuler p53 Pemanfaatan Tanaman Herbal Buah Legundi (*Vitex trifolia L*) terhadap Massa Tumor Jaringan Kulit Molecular Study of p53 , Utilization of Legundi Fruit Herbs (*Vitex trifolia L*) in Skin Tissue Tumor Mass. *Bul FARMATERA J Kedokt Univ Muhammadiyah Sumatera Utara*. 2018;3(1):41-48.
9. Gong G, Shen YL, Lan HY, et al. The Cyr61 Is a Potential Target for Rotundifuran, a Natural Labdane-Type Diterpene from *Vitex trifolia L.*, to Trigger Apoptosis of Cervical Cancer Cells. *Oxid Med Cell Longev*. 2021;2021. doi:10.1155/2021/6677687
10. Khairul U, Vemithasa C. Potensi *Vitex trifolia* (Verbenaceae) sebagai insektisida botani untuk mengendalikan hama Crocidolomia pavonana (Lepidoptera: Crambidae). *PROS SEM NAS MASY BIODIV INDON*. 2018;4(Heyne 1987):169-172. doi:10.13057/psnmbi/m040212
11. Indriatie R, Masruri M. Antibacterial Activity of Legundi Leaf (*Vitex trifolia*) Essential Oil Using in-vitro and in-silico Methods. *J Pure App Chem Res*. 2023;12(April):26-37. doi:10.21776/ub.jpacr.2023.012.001.738
12. Medina H, Lubis L, Purwoningsih E. Anti-Cervical Cancer Study of a Labdane-type Diterpene Obtained from Legundi Fruit (*Vitex triolia L* .) Targeting the Bcl-2 Gene. *Bul FARMATERA J Kedokt Univ Muhammadiyah Sumatera Utara*. 2022;7(3):41-49.
13. Fatmawati D, Mahmudati N, Wahyuni S, Rahardjanto A, Fatmawati D. Ekstrak buah legundi (*Vitex trifolia Linn.*)dapat menghambat pertumbuhan bakteri Staphylococcus aureus. *Pros Semin Nas V*. Published online 2020.
14. Wulansari ED, Lestari D, Khoirunissa MA. Kandungan Terpenoid Dalam

E-ISSN: 2528-410X

Daun Ara (Ficus Carica L.) Sebagai Agen Antibakteri Terhadap Bakteri Methicillin-Resistant Staphylococcus aureus. *Pharmacon*. 2020;9(2):219. doi:10.35799/pha.9.2020.29274

15. Marpaung JK, Sitorus P, Nasution P, Yanti RD. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Legundi (Vitextrifolia L.) Terhadap Pertumbuhan Staphylococcus epidermidis. *J Farmanesia*. 2020;7(2):9-14. doi:10.51544/jf.v7i2.2770
16. Sulistyarini I, Sari DA, Wicaksono TA. Skrining Fitokimia Senyawa Metabolit Sekunder Batang Buah Naga (Hylocereus polyrhizus). *J Ilm Cendekia Eksakta*. Published online 2019:56-62.
17. Fitriana YAN, Fatimah VAN, Fitri AS. Aktivitas Anti Bakteri Daun Sirih: Uji Ekstrak KHM (Kadar Hambat Minimum) dan KBM (Kadar Bakterisidal Minimum). *Sainteks*. 2020;16(2):101-108. doi:10.30595/st.v16i2.7126
18. Elzuhria A N, Kaffah NS, N NR, et al. Antibiotics Sensitivity Test Diffusion and Dilution Methods. *J Res Pharm Pharm Sci*. 2023;2(1):38-47. doi:10.33533/jrpps.v2i1.7027
19. Gori A, Boucherle B, Rey A, Rome M, Fuzzati N, Peuchmaur M. Development of an innovative maceration technique to optimize extraction and phase partition of natural products. *Fitoterapia*. 2021;148(November 2020). doi:10.1016/j.fitote.2020.104798
20. Rahmi N, Salim R, Miyono M, Rizki MI. Pengaruh Jenis Pelarut Dan Metode Ekstraksi Terhadap Aktivitas Antibakteri Dan Penghambatan Radikal Bebas Ekstrak Kulit Kayu Bangkal (Nauclea subdita). *J Penelit Has Hutan*. 2021;39(1):13-26. doi:10.20886/jphh.2021.39.1.13-26