

DENTINO
JURNAL KEDOKTERAN GIGI
Vol VI. No 1. Maret 2021

ANTIBACTERIAL EFFECTIVENESS TEST OF RAMANIA LEAVES (*Bouea macrophylla Griffith*) FLAVONOIDS EXTRACT ON *Aggregatibacter actinomycetemcomitans* BACTERIA CAUSING AGGRESSIVE PERIODONTITIS

Noor Khalishah¹⁾, Beta Widya Oktiani²⁾, Rosihan Adhani³⁾

¹⁾ Dentistry Faculty, Lambung Mangkurat University, Banjarmasin

²⁾ Department of Periodontology, Dentistry Faculty, Lambung Mangkurat University, Banjarmasin

³⁾ Department of Dental Public Health, Dentistry Faculty, Lambung Mangkurat University, Banjarmasin

ABSTRACT

Background: Aggressive periodontitis is a disease that is mostly suffered by young patients with minimal local factors. The prevalence of this disease is approximately 8% in Indonesia, where *Aggregatibacter actinomycetemcomitans* bacteria are presented as dominant bacteria in this disease. *Aggregatibacter actinomycetemcomitans* is a gram-negative bacterium that is sensitive to flavonoids. Ramania leaves are natural substances which contain flavonoids. Flavonoids in ramania leaves can be applied as an antibacterial substance. **Objectives:** The objectives of this research are to determine and analyze the minimum inhibitory concentration and the minimum bactericidal concentration of flavonoids extract from ramania leaves at the concentration of 0.1%, 0.3%, and 0.5%, and analyze the antibacterial effectiveness on *Aggregatibacter actinomycetemcomitans*. **Methods:** True experimental design with pre and post-test with control group design was applied in this study. Antibacterial test using broth and agar dilution method was performed in a total of 5 treatment groups with 6 repetitions. **Results:** All extract at 0.1%, 0.3% and 0.5% concentration demonstrated the ability to inhibit bacterial growth, and the minimum bactericidal concentration was found at 0.5% concentration. Result test for One way ANNOVA on minimum inhibitory concentration and Kruskal wallis test on minimum bactericidal concentration exhibited significant value of less than 0.05 presenting significant differences in some groups. **Conclusion:** Minimum inhibitory concentration of ramania leaves flavonoid extract is effective on *Aggregatibacter actinomycetemcomitans* at the concentration of 0.1% and minimum bactericidal concentration of ramania leaves flavonoid extract is more effective at the concentration of 0.5%.

Keywords: *Aggregatibacter Actinomycetemcomitans*, Aggressive Periodontitis, Dilution Method, Flavonoids, Ramania Leaves.

Correspondence: Noor Khalishah, Faculty of Dentistry, Lambung Mangkurat University, Jl. Veteran No 12B, Banjarmasin, South Borneo, E-mail : khalishah.muaidi.khalidah@gmail.com

INTRODUCTION

Indonesia has natural resources such as herbal plants which are often used as therapy in medicine. Treatment using plants is gradually favored by the people because there is changing in habits that use natural ingredients as treatments.¹

Ramania is a native plant of Indonesia that is spread across Sumatra, Java, Borneo, and Maluku.² Ramania leaves are often consumed as vegetables by the public.³ Ramania leaves have many health benefits because they contain several compounds such as triterpenoids, steroids, phenols, tannins, and flavonoids.⁴

Flavonoids are the most common phenolic group found in nature. Flavonoids have potential as anticancer, antibiotic, and antibacterial.^{5,6} The mechanism of flavonoids functioning as an antibacterial in general is to damage the cell wall, inhibit macromolecular synthesis, inhibit the synthesis of nucleic acids, and damage the permeability of bacterial cell membranes.^{7,8,9}

Aggregatibacter actinomycetemcomitans is a facultative gram-negative anaerobic bacterium, in the form of coccobacilli and is an opportunistic pathogen. *Aggregatibacter actinomycetemcomitans* is the dominant bacterium found in patients who have aggressive periodontitis with a prevalence of

40.9% and in patients who have localized aggressive periodontitis with a prevalence of 81.8% when compared with chronic periodontitis which amounted to 28.2%. Therapy given to patients with aggressive periodontitis tends to improve and maintain oral hygiene. This also includes the debridement of root surface, and the use of antibiotics. Inappropriate use of antibiotics can cause bacterial resistance, so it is necessary to develop other alternatives as antibacterial.^{10,11}

Based on the description that has been proposed, researchers are interested to analyze the antibacterial effectiveness of ramania leaves (*Bouea macrophylla Griffith*) flavonoid extract on the growth of *Aggregatibacter actinomycetemcomitans* using the dilution methods in vitro.

MATERIALS AND METHODS

The study began with an ethical clearance approved by the Health Research Ethic Commission of the Faculty of Dentistry, Lambung Mangkurat University No. 003/KEPKG-FKGULM/EC/I/2020. The research design used was a true experimental design with a pretest-posttest with a control group design consisting five groups with six repetitions. Five groups consisted of groups of 0.1%, 0.3%, 0.5% concentration, 0.2% *chlorhexidine gluconate* as the positive control, and aquadest as the negative control.

The tools used were blenders, Erlenmeyer flasks, separating funnels, filter paper, digital scales, test tubes, measuring cups, autoclaves, vacuum rotary evaporators, waterbath, Petri dishes, rounded ose, spiritus lamps, sterile cotton, analytical balance, spectrophotometers, vortex mixer, micropipette, hot plate, colony counter, sterile tip, beaker glass, dropper, and laminatory flow. The ingredients used were 1500 g ramania leaves, aquadest, n-hexane, and ethyl acetate, *Aggregatibacter actinomycetemcomitans*, Nutrient agar (NA) media, Brain Heart Infusion Broth (BHIB) media and a 0.5 McFarland standard.

The research was conducted at Biomedical Laboratory Faculty of Dentistry, Lambung Mangkurat University Banjarmasin, Jamu Pucuk Sirih Factory Banjarmasin, and the FMIPA Laboratory of Lambung Mangkurat University, Banjarbaru on January to March 2020.

Determination Test

Ramania leaves were obtained from Martapura. The selected leaves were dark green in colour and the fourth or the fifth leaves located from the tips. Before going through the extraction stage, a determination test was first to ensure that the leaves are ramania (*Bouea macrophylla*

Griffith) leaves in the Laboratorium FMIPA Lambung Mangkurat University.¹²

Extraction Ramania Leaves

The extraction process of ramania leaves (*Bouea macrophylla Griffith*) carried out at Jamu Pucuk Sirih Factory Banjarmasin by initially cleaning the ramania leaves from dirt and washing them with running water until clean, then the leaves were cut using a knife and then dried using an oven at 50°C for 4 hours and continued by smoothing the leaves using a blender

The extract was made using maceration method by immersing simplicia with 95% ethanol then dissolving it in an Erlenmeyer tube at a ratio of 1:10 weight/volume or 1 cm above the simplicia. This mixture was stirred until homogenous and then closed and kept for 72 hours. The filtrate was stirred every 24 hours using a magnetic stirrer with a speed of 50 rotations per minute (rpm) for 15 minutes. After 72 hours, the mixture was filtered and concentrated with a vacuum rotary evaporator using a 50°C temperature and dried using a Waterbath to obtain a thick extract. The thick extract obtained a total of 74 grams weigh.¹³

The thick extract was poured into a separating funnel and dissolved with distilled water then n-hexane solution was added until it was homogeneous and until two layers were formed. The two layers were then separated leaving the bottom layer that consists of the extract and n-hexane. Ethyl acetate was added and stirred until homogeneous and kept still until two layers were formed. The two layers were separated and the ethyl acetate layer containing flavonoids compound was collected. The collected layer was further evaporated until the solvent was completely eliminated to obtain 8 grams of flavonoids extract.

Antibacterial Test

The antibacterial test was carried out at the Biomedical Laboratory FKG ULM. One milliliter of bacterial suspension, that had been standardized with 0.5 McFarland solution, was inserted into each test tube containing 1 ml of the extract with 3 different concentrations that were the concentration of 0.1%, 0.3%, and 0.5%. The test tube was measured to determine the effect of ramania leaves (*Bouea macrophylla Griffith*) flavonoids on the growth of *Aggregatibacter actinomycetemcomitans* by observing the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) using a spectrophotometer at 450 nm wavelength and colony counter.

The MIC was determined by differentiating the absorbance value after incubation that was

characterized with the reduction. The lowest concentration which inhibits bacterial growth is demonstrated by a negative value, indicating that the concentration of bacteria decreases. Further tests were carried out to determine the MBC by taking 0.2 ml of the concentration that had shown an antibacterial activity added to a petri dish containing sterile NA media, then incubated for 24 hours at 37°C in an anaerobic incubator. The counting of bacterial colony number was using a colony counter which screen identifies spots for the presence of bacteria. Markers can be used for calculating bacteria by touching the surface of the petri dish on colony counter that will present the number of bacterial colony.

If the result for the counting of bacterial colony is 0 (no bacteria or no spots presented on the colony counter screen) then the MBC is obtained.

RESULTS

Research on the effectiveness of ramania leaves flavonoid extract (*Bouea macrophylla Griffith*) against *Aggregatibacter actinomycetemcomitans* bacteria that cause aggressive periodontitis with dilution methods in vitro had been carried out. This research was conducted for 24 hours using the dilution method to determine the MIC and the MBC of the extract toward *Aggregatibacter actinomycetemcomitans*. MIC is obtained from the average difference before and after 24 hours incubation using a spectrophotometer. MBC is obtained by calculating the average bacterial growth on agar using a colony counter.

Table 1 Average MIC value of 0.1%, 0.3%, 0.5% Ramania Leaves Flavonoid Extract, Positive Control and Negative Control toward *Aggregatibacter actinomycetemcomitans*

Groups	Repetition	Mean	Standard deviation
0.1%	6	-0.92888	0.124274
0.3%	6	-0.62667	0.054588
0.5%	6	-0.62167	0.031608
Positive control	6	0.8315	0.20187
Negative control	6	0.45305	0.211579

In the table above, there are negative and positive mean values. A negative value indicates that the 0.1%, 0.3%, and 0.5% ramania leaves flavonoid extract can inhibit the growth of *Aggregatibacter actinomycetemcomitans*. Positive

values indicate no inhibitory activity was found in the positive control group (0.2% chlorhexidine gluconate) and negative control (aquades) on the growth of *Aggregatibacter actinomycetemcomitans*.

Statistical analysis of MIC data began with the *Shapiro wilk* normality test, resulted in all groups having a sig value less than 0.05 which means that the data were normally distributed. Data were subsequently proceeded to determine the homogeneity with the *Levene's test* and resulted in a significance value of 0.059 ($p > 0.05$), which means that the data had a homogeneous variant. These results enabled the data to be processed with *One way ANNOVA* test that revealed a significance of 0.000 ($p < 0.005$). Such result could be interpreted as the presence of significant differences in some groups, therefore *Post hoc Bonferroni* test was conducted to find out which groups contribute to the differences.

Table 2 Results for *Post hoc Bonferroni* Test of 0.1%, 0.3%, 0.5% Ramania Leaves Flavonoid Extract, Positive Control, and Negative Control toward *Aggregatibacter actinomycetemcomitans*

Groups	0.1%	0.3%	0.5%	Positive control	Negative control
0.1%		0.001	0.001	0.000	0.000
0.3%	0.001		1.000	0.000	0.000
0.5%	0.001	1.000		0.000	0.000
Positive control	0.000	0.000	0.000		0.000
Negative control	0.000	0.000	0.000	0.000	

From the table above, it can be seen that the 0.3% group and the 0.5% group show no significant difference ($1.000 > 0.05$), meanwhile, the other groups have a significant difference (< 0.05) that are between the 0.1% group and 0.3% (0.001), 0.1% group and 0.5% (0.001), 0.1% group and positive control (0.000), 0.1% group and negative control (0.000), 0.3% group and positive control (0.000), 0.3% group and negative control (0.000), 0.5% group and positive control (0.000), 0.5% group and negative control (0.000), as well as between positive control group and negative control (0.000). Table above also presents that the 0.3% group and the 0.5% group show no significant difference ($1.000 > 0.05$), while the other groups have a significant difference (< 0.05) between the 0.1% group and 0.3% (0.001), 0.1% group and 0.5% (0.001), 0.1% group and positive control (0.000), 0.1% group and negative control (0.000), 0.3% group and positive control (0.000), 0.3% group and negative control (0.000), 0.5% group and negative control (0.000), 0.5% group and positive control (0.000), 0.5% group and negative control (0.000), as well as between positive control group and negative control (0.000).

group and positive control (0.000), 0.5% group and negative control (0.000), and between positive control group and negative control (0.000).

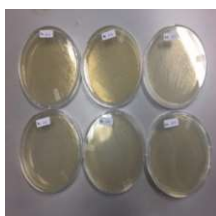
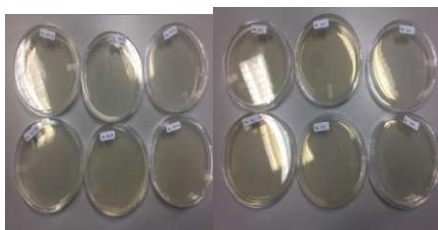
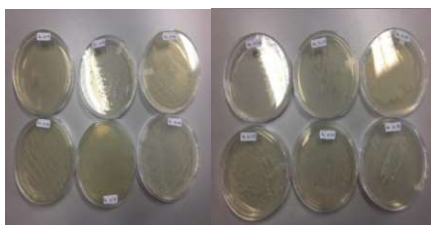


Figure 1 The results for the measurements of *Aggregatibacter actinomycetemcomitans* colonies number in agar dilution

Table 3 Average Values of MBC 0.1%, 0.3%, 0.5% Ramania Leaves Flavonoid Extract, Positive Control, and Negative Control toward *Aggregatibacter actinomycetemcomitans*

Groups	Repetition	Mean	Standard deviation
0.1%	6	1076.17	832.633
0.3%	6	63.83	39.362
0.5%	6	0	0
Positive control	6	0	0
Negative control	6	2715.33	715.749

In the table above the concentration of 0.5% and positive control have a mean value of 0 which indicates an ability to be bactericidal towards *Aggregatibacter actinomycetemcomitans*. In the group of 0.1%, 0.3%, and negative controls there is a mean value of more than zero which indicates that the concentration has not been able to be bactericidal.

The data obtained were carried out to statistical analysis using the *Shapiro wilk*

normality test. There were groups that were resulted with a mean of 0 that are the 0.5% group and a positive control so that the data analysis was performed using a non-parametric test of *Kruskal wallis*. The significance value obtained from the *Kruskal wallis* test was $0 < 0.05$, which means that there were significant differences in some groups and to find them out, the analysis was continued with *Post hoc Mann Whitney* test.

Table 4 Results for *Post hoc Mann Whitney* Test of 0.1%, 0.3%, 0.5% Flavonoids Ramania Leaves Extract, Positive Control, and Negative Control toward *Aggregatibacter actinomycetemcomitans*

Groups	0.1%	0.3%	0.5%	Positive control	Negative control
0.1%		0.004	0.002	0.002	0.016
0.3%	0.004		0.002	0.002	0.004
0.5%	0.002	0.002		1.000	0.002
Positive control	0.002	0.002	1.000		0.002
Negative control	0.016	0.004	0.002	0.002	

From the table above, it can be observed that the 0.5% group and the positive control group show no significant difference ($1.000 > 0.05$), while the other groups have a significant difference (< 0.05) between the 0.1% group and 0.3% (0.004), the 0.1% group and 0.5% (0.002), the 0.1% group and positive controls (0.002), the 0.1% group and negative controls (0.016), the 0.3% group and 0.5% (0.002), 0.3% group and positive control (0.002), 0.3% group and negative control (0.004), 0.5% group and negative control (0.002), as well as the positive control group and negative control (0.002).

DISCUSSION

Minimum inhibitory concentration was obtained at a concentration of 0.1% that inhibited the growth of *Aggregatibacter actinomycetemcomitans* with a decrease in the average absorbance value of 0.92888. According to Yanuhar (2016), the determination of MIC is based on the lowest concentration of ingredients that can inhibit bacteria and can be seen from turbidity.¹⁴ Dechsupa *et al* (2019) stated that ramania was able to inhibit the growth of some gram-negative bacteria such as *A. buamannii*, *P. aeruginosa*, *V. parahemolyticus*, *V. cholera*, *E. aerogenes*, *K. pneumoniae*, *P. mirabilis*, *S. boydii*, *S. enteritidis*, gram-positive such as *S. aureus*, *E. faecalis* because it contains several compounds such as flavonoids.¹⁵

In the *Post hoc* test of MIC data, several groups demonstrated significant differences and other groups revealed no significant differences.

Groups that have no significant differences were the groups of 0.3% and 0.5% concentration while other groups were observed to be significantly different. This insignificant difference may be due to the average absorbance value of the spectrophotometer which is not too contrasting because, according to Armedita *et al* (2018), the antibacterial power of material at high concentrations will become less stable that consequently produce inconsiderable difference in absorbance values.¹⁶

Positive control in this study used 0.2% chlorhexidine gluconate. The measurement of inhibitory levels reveals that there is a positive absorbance value while there is no growth of *Aggregatibacter actinomycetemcomitans* in the measurement of bacteria colony. Based on Limeback *et al* (2012), this may be due to the antibacterial mechanism of 0.2% chlorhexidine gluconate which can release calcium ions in glycoprotein bonds in bacterial cell walls into liquid media. Another cause for the increased in turbidity might be due to changes in pH of the BHIB solution from the 0.2% chlorhexidine gluconate reaction to bacteria so that bacteria are unable to metabolize sugar and then acid will not be formed. Calcification can occur because the pH of the solution is neutral, causing a change in turbidity with an increase in absorbance.^{17,18} The instrument used in the determination of absorbance value is a spectrophotometer which, according to Rambat *et al* (2017), measures the level of turbidity based on light absorbance and demonstrates a disadvantage that assembles its inability to distinguish particles in liquid media.¹⁹

The minimum bactericidal concentration was obtained at a concentration of 0.5%. Determination of the minimum bactericidal concentration was according to the lowest level of antibacterial that demonstrates the ability to be bactericidal. The higher the concentration of antibiotic substances, the faster the bactericidal property can be obtained.^{9,20} This might be due to the presence of flavonoids compounds that can be bactericidal.

Flavonoids exhibited bactericidal potency by inhibiting the nucleic acid synthesis in rings A and ring B in the intercalation process of hydrogen bonds. This results in the buildup of nucleic bases, that instigates the inhibition of bacterial DNA and RNA formation. Flavonoids can also cause damage to the permeability of microsomal and lysosomal bacterial cell membranes. The inhibition of cell membranes function is established by the formation of complex compounds with extracellular and dissolved proteins that damage bacterial cell membranes.²¹

Aggregatibacter actinomycetemcomitans are gram-negative bacteria which, based on Nugraha *et al* (2017), possess a cell wall that consists of one or more than 20 thin peptidoglycan layers and a membrane as an outer layer of the peptidoglycan. Gram-negative bacterial cell walls are more susceptible to physical shocks, such as antibiotics or antibacterial agents (i.e. flavonoids) because they only contain one peptidoglycan layer and no content of teichoic acid. Flavonoids can penetrate the peptidoglycan walls of gram-negative bacteria because they are both polar, and it is different from steroids and triterpenoids which are non-polar compounds.^{22,23} Based on the results of this research, it can be concluded that the minimum inhibitory concentration of flavonoids ramania leaves extract that is effective toward *Aggregatibacter actinomycetemcomitans* is at the concentration of 0.1% and the minimum bactericidal concentration that is effective against *Aggregatibacter actinomycetemcomitans* is at a concentration of 0.5%.

REFERENCES

1. Nonci FY, Daeng AT, Hasnia A. Uji Aktivitas Antimikroba Hasil Fraksinasi Ekstrak Etanol Daun Patikala (*Etilangera Elatior*) Terhadap Beberapa Mikroba Uji. *Jurnal Farmasi UIN Alauddin Makassar*. 2016; 4(2): 35.
2. Taihuttu HN. Identifikasi Karakteristik Lahan Tanaman Gandaria (*Bouea macrophylla Griff*) di Desa Hunuth Kecamatan Banguala Kota Ambon. *Agrologis*. 2013; 2(1): 68-72.
3. Harsono T. Tinjauan Ekologi dan Etnobotani Gandaria. *Jurnal Biosains*. 2017; 3(2): 119-124.
4. Sukalingam K. Preliminary Phytochemical Analysis and in Vitro Antioxidant Properties of Malaysian Kundang (*Bouea macrophylla Griffith*). *Trends in Phytochemical Research*. 2018; 2(4): 261-266.
5. Glio TM. *Membuat Pestisida Nabati Untuk Hidroponik Akuaponik Vertikultur dan Sayuran Organik*. Jakarta: Agromedia Pustaka; 2017. p.20.
6. Fitri L, Taufiqurrahman I, Irnamanda DH. Phytochemical and cytotoxicity testing of ramania leaves (*Bouea macrophylla Griffith*) ethanol extract toward vero cells using MTT assay method. *Dentino (Jurnal Kedokteran Gigi)*. 2018; 3(1): 51-56.
7. Heni, Arreneuz S, Zaharah TA. Efektivitas Antibakteri Ekstrak Kulit Batang Belimbing Hutan (*Baccaurea Angulata Merr.*) Terhadap *Staphylococcus aureus* dan *Escherichia Coli*. *JKK*. 2015;4(1): 84-90.
8. Dyozem JP, Hamamoto H, Ngameni B, Ngadjui BT, Sekimizu K. Antimicrobial Action

- Mechanism of Flavonoids from *Dorstenia* Species. *Drug Discoveries & Therapeutics*. 2013; 7(2): 66-72.
9. Winarsih S, Khasanah U, Alfatah AH. Aktivitas Antibiofilm Fraksi Etil Asetat Ekstrak Daun Putri Malu (*Mimosa Pudica*) Pada Bakteri Methicilin-Resistant *Staphylococcus aureus* Secara *in Vitro*. *Majalah Kesehatan*. 2019; 6(2): 76-85.
 10. Newman. *Carranza Clinical Periodontology*. California: Elsevier; 2018. p.624 – 2550.
 11. Felton S, Alison C. *Basic Guide to Oral Health Education and Promotion*. New Delhi: Wiley Blackwell; 2014. p.48.
 12. Hardinsyah, Windardi IP, Aries M, Damayanthi E. Total Phenolic Content, Quercetin, and Antioxidant Activity of Gandaria (*Bouea macrophylla* Griff.) Leaf Extract at Two Stages of Maturity. *J. Gizi Pangan*. 2019; 14(2): 61-68.
 13. Mulyawan R, Taufiqurrahman I, Edyson. Perbedaan Total Flavonoids Antara Metode Pengeringan Alami dan Pengeringan Buatan Pada Ekstrak Daun Ramania (*Bouea macrophylla* Griffith). *Dentin (Jurnal Kedokteran Gigi)*. 2018; 2(1): 98.
 14. Yanuhar U. *Mikroalga Laut Nannochloropsis Oculata*. Malang: UB Media; 2016. p.126.
 15. Dechsupa N, Kantapan J, Tungjai M, Intorasoot S. Maprang “*Bouea macrophylla* Griffith” Seeds Proximate Composition, HPLC Fingerprint, and Antioxidation, Anticancer and Antimicrobial Properties of Ethanolic Seed Extracts. *Heliyon*. 2019; 5(2052): 1-12.
 16. Armedita D, Verry A, Masyhudi A. Aktivitas Antibakteri Ekstrak Etanol Daun, Kulit Batang, dan Getah Angsana (*Pterocarpus Indicus Willd*) terhadap Pertumbuhan *Streptococcus mutans*. *ODONTO Dental Journal*. 2018; 5(1): 5.
 17. Limeback H. *Comprehensive Preventive Dentistry*. India: John Wiley and Sons; 2012. p.160.
 18. Sakaue Y, Takenaka S, Tatsuya O, Domon H, Terao Y, Noiri Y. The effect of chlorhexidine on dental calculus formation an in vitro study. *BMC Oral Health*. 2018; 18(52): 1-7.
 19. Rambat, Lumimuut G, Olivia W, Paulina N. G. Uji Konsentrasi Hambat Minimum (KHM) Perasan Murni Bawang Putih (*Allium sativum*) Terhadap Pertumbuhan *Candida albicans*. *Jurnal Ilmiah Farmasi*. 2017; 6(1): 21.
 20. Suhailah L, Fuadah M. Uji Sensifitas Filtrat Daun Katuk (*Sauropus Androgynus* L.) Terhadap Bakteri *Staphylococcus aureus* dan *Escherichia coli*. *Journals of Ners Community*. 2017; 8(2): 188-194.
 21. Munfaati PN, Ratnasari E, Trimulyono G. Aktivitas Senyawa Antibakteri Ekstrak Herba Meniran (*Phyllanthus niruri*) terhadap Pertumbuhan Bakteri *Shigella dysenteriae* Secara *in Vitro*. *Lentera Bio*. 2015; 4(1): 64–71.
 22. Nugraha AC, Prasetya AT, Mursiti S. Isolasi, Identifikasi, Uji Aktivitas Senyawa Flavonoids sebagai Antibakteri dari Daun Mangga. *Indonesian Journal of Chemical Science*. 2017; 6(2): 92-96.
 23. Egra S, Mardhiana, Rofin M, Adiwena M, Jannah N, Kuspradini H, et al. Aktivitas Antimikroba Ekstrak Bakau (*Rhizophora mucronata*) dalam Menghambat Pertumbuhan *Ralstonia Solanacearum* Penyebab Penyakit Layu. *AGROVIGOR*. 2019; 12(1): 26-31.