ANTIBACTERIAL EFFECTIVENESS TEST of MINT LEAF EXTRACT (Mentha piperita L.) IN INHIBITING Stretococcus Sanguinis GROWTH

(*UJI EFEKTIVITAS ANTIBAKTERI EKSTRAK DAUN MINT (Mentha piperita L.) DALAM MENGHAMBAT PERTUMBUHAN Streptococcus sanguinis)*

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ABSTRACT

Streptococcus sanguinis (*s.sanguinis*) is one of the normal flora in the oral cavity. These bacteria act as a pioneer in forming plaques that cause most dental and oral diseases. It takes an antibacterial agent to be able to inhibit the clips of the plaque. One of the herbal plants that have been known to have antibacterial power is the mint leaf (*Mentha Piperita L.*). The antibacterial potency of this plant comes from its chemical compound content, such as flavonoids, polyphenols, tannins, and menthols. This study aimed to determine the inhibitory of mint leaf extract on the growth of Sanguinis. An experimental laboratory carries out research. Tests were carried out using the diffusion method in four test groups, namely mint leaf extract with a concentration of 2.5%, 5%, 7.5%, and 10%, and one control group that uses DMSO. Each treatment group repeated five times on the Mueller-Hinton media (MHA) using disk paper and incubated for 24 hours. The inhibition zone can be seen in the transparent area formed around the disk paperand then measured using the Sorong term to find a large diameter. Statistical analysis was carried out using the Kruskal-Wallis test. The study results that the largest inhibitory zone

diameter was obtained in a test group with a concentration of 10% (16.39 mm), while the lowest diameter in the control group (4.89 mm) 10% concentration was the highest among all test groups. The DMSO did not have an antibacterial effect. The higher the concentration of an antibacterial compound, it will also increase its effectiveness.
***Keywords**: antibacterial test, mint leaf extract*

ABSTRAK

Streptococcus sanguinis (S.sanguinis) merupakan salah satu flora normal di rongga mulut. Bakteri ini bertindak sebagai pelopor dalam pembentukan plak yang menyebabkan sebagian besar penyakit gigi dan mulut. Dibutuhkan agen antibakteri untuk dapat menghambat kliping plak. Salah satu tanaman herbal yang telah diketahui memiliki daya antibakteri adalah daun mint (Mentha Piperita L.). Potensi antibakteri tanaman ini berasal dari kandungan senyawa kimianya, seperti flavonoid, polifenol, tanin, dan mentol. Penelitian ini bertujuan untuk mengetahui daya hambat ekstrak daun mint terhadap pertumbuhan Sanguinis. Sebuah laboratorium eksperimental melakukan penelitian. Pengujian dilakukan dengan menggunakan metode difusi pada empat kelompok uji yaitu ekstrak daun mint dengan konsentrasi 2,5%, 5%, 7,5%, dan 10%, serta satu kelompok kontrol yang menggunakan DMSO. Masing-masing kelompok perlakuan diulang sebanyak lima kali pada media Mueller-Hinton (MHA) menggunakan kertas cakram dan diinkubasi selama 24 jam. Zona hambat dapat dilihat pada daerah transparan yang terbentuk di sekitar kertas cakram kemudian diukur menggunakan istilah Sorong untuk mencari diameter yang besar. Analisis statistik dilakukan dengan menggunakan uji Kruskal-Wallis. Hasil penelitian bahwa diameter zona hambat terbesar diperoleh pada kelompok uji dengan konsentrasi 10% (16,39 mm), sedangkan diameter terendah pada kelompok kontrol (4,89 mm) konsentrasi 10% tertinggi di antara semua kelompok uji. Kesimpulan penelitian adalah daun mint memiliki efek antibakteri dimana semakin tinggi konsentrasi suatu senyawa antibakteri maka akan semakin meningkat pula efektivitasnya.

***Kata Kunci**: ekstrak daun mint; uji antibakteri*

INTRODUCTION

Streptococcus sanguinis is a normal flora in the oral cavity, also known as S. sanguinis.[1] These bacteria are often labelled as a pioneer in the process of plaque formation. Definition of dental plaque is soft structured deposits, greyish yellow and glued on hard surfaces of the oral cavity, the denture fixed and restoration.[2] Dental plaque is a biofilm. Dental plaque is an ecosystem that bacteria forms microcolonies. It is surrounded by a protective matrix that encloses the plaque microorganisms consisting of glycoproteins and extracellular polysaccharides.[3] Early plaque formation was initiated when the enamel surface is coated by a pellicle, a thin layer that comes from salivary glycoprotein. This pellicle will be the place for bacteria to attach to the tooth surface. The bacteria were first exposed to salivary pellicle is Streptoccocus sanguinis, so the bacteria is considered a pioneer in plaque formation.[4,5] The adherent Streptococcus sanguinis can initiate the adhesion of other plaque- forming bacteria, namely *Streptococcus mutans, Streptococcus gordonii, Actinomyces naeslundii, Haemophilus parainfluenzae, Veillonella atypica, Prevotella loescheii,* and *Eikenella corrodens*.[6,7] The condition indicates Streptococcus sanguinis transformed into a bacterial pathogen that could be detrimental to oral health.

Oral and dental health is a part of overall body health. The onset of diseases or disorders of the teeth and mouth area will cause a disturbance in the function of speech, mastication and esthetics, which in turn can decrease a person's quality oflife.[8] The result of Health Research (Riskesdas) in 2018 reported that oral disease is still a problem that often occursin people in Indonesia, which reached 57.6%.[9] This condition has increased quite significantly compared to the results of Riskesdas in 2013, which got 25.9%. Two diseases of the oral cavity with the highest prevalence based Riskesdas 2018 are as much as 88.8% of dental caries and periodontal disease 74.1%.[10] Dental plaque is a major factor in the occurrence of both diseases.[3] Dental plaque is not easily removed through wipes or rinsed with water only. It is because plaque has the extracellular matrix that attaches firmly to the tooth surface.[2,11] Bacteria in dental plaque will continue to multiply so that if it is not cleaned, it can cause an increased thick layer of plaque. There areproducts produced by bacteria and occur outside the surface adhesion of bacteria in plaque.[12] Dental plaque will eventually be calcified in conjunction with various molecules present in the mouth to form calculus and cause more severe periodontal disease.[13] Prevention of periodontal disease getting worse can be done by controlling plaque.[14] Plaque control is an effort to maintain oralhygiene by preventing plaque accumulation on the surface of the teeth and gingiva.[14,15] There are two methods used to control plaque (mechanically and chemically). Mechanical plaque control is done by brushing the teeth with the correct technique and using toothpaste. Chemical plaque control is

gargling mouthwash. This method is another alternative to thoroughly cleaning the surface of the oral cavity.

Some of the ingredients in mouthwash have antibacterial and antiseptic properties, which function to reduce plaque bacteria formation.[5,15,16] Synthetic chemical antibacterial ingredient is generally contained in toothpaste such as triclosan, or chlorhexidine in mouthwashes, causing a direct inhibitory effect on plaque formation.[8,5] Mechanically and chemically are considered effective in preventing the accumulation of gram-negative and gram-positive plaque-forming bacteria. However, sometimes they also found no side effects in using synthetic antibacterial agents such as triclosan and chlorhexidine. Several previous studies reported bacterial resistance, mucosal irritation, an idiosyncratic reaction, and allergic reactions.[16,17] This situation can be avoided by choosing the type of mouthwash that is based on herbal plants. Herbal plant extracts that have been researched previously have been shown to have benefits following developed conventional treatments, but with minimal side effects.[18]

Indonesia is one of the countries whose biodiversity is widespread in various regions. Several types of plants in Indonesia can be used as herbal medicine because they can support the health economy, increase the value ofhealth and make the Indonesian nation prosperous.[19] One of the types of plants that can be used as medicinal plants is mint(*Mentha piperita L.*) or mint leaves. Mint plants have been widely used for traditional medicine, such as antispasmodic, aromatic, antiseptic, analgesic and anti-inflammatory for the treatment of various types of diseases such as disorders of the bile ducts, irritable bowel syndrome, indigestion, myalgia, neuralgia, cancer treatment, colds, menstrual cramps, pregnancy, sore throat, inflammation of the oral mucosa, toothache and bad breath. Many benefits can be obtained from mint leaves, especially for oral health. This plant has natural compounds and is safer than chemical compounds, which can cause side effects..[20,21] Natural compounds in mint leaves are flavonoids, polyphenols, tannins, and menthol. Flavonoids have a working mechanism of denaturing protein cells until they cannot function again so that they can damage the permeability of the bacterial cell walls, then for polyphenol compounds to disrupt the stability of the bacterial cell membrane, their technical tannins shrink and bind to proteins that can reduce protein synthesis of gram-negative and gram-positive bacterial cells and synthesis of bacterial cell walls as well as to menthol eliminate bacterial pathogens.[22–25] Golestannejad et al. 2017 concluded that there was a significant antibacterial effect on the essential oil of *Mentha piperita L*. against *Streptococcus mutans* compared to the two essential oils of *Foeniculum vulgare* and *M. Mentha arvensis*. The concentrations used in this study were 0.312%, 0.625%, 1.25%, 2.5%, 5% and 10% which were observed for three days. The highest inhibition zone was at a concentration of 10% at 11.25 mm, and the lowest zone of inhibition was 6.40 mm at a

concentration of 0.312%, 0.625%, 1.25%. The results were obtainedfrom the main content of menthol, which has good antibacterial activity.[26] Mint leaves as a medicinal plant with many benefits and good antibacterial power against various bacteria in the oral cavity. Until now, it has not been studied regarding its effectiveness against *Streptococcus sanguinis* bacteria. The research conducted on the effectiveness of mint (*Mentha piperita L.*) leaf extract with a concentration of 2.5%, 5%, 7.5% and 10% in inhibiting the growth of *Streptococcus sanguinis*.

METHOD

This research was purely experimental research with the post-test only control group design in vitro. This research design can measure the effect of treatment on the experiment by comparing the group with the control group.[27] The research will be conducted using the agar diffusion method to determine the effectiveness of the extract of mint leaves (*Mentha piperita L.*) in inhibiting the growth of *Streptococcus sanguinis* bacteria. The object study was certified *Streptococcus sanguinis* (ATCC 10556). It was obtained from pure isolates and identified microscopically in the Microbiology Laboratory of the Faculty of Medicine, General Achmad Yani University.

Mint leaves are obtained from Manoko Lembang, West Java. Then the plant was determined to match the morphological characteristics of the mint plant. The mint leaves were made into mint leaf extract at the Plant Determination Laboratory of the Biological Research Center for Botanical Sector (LIPI) Cibinong. The procedure for making extracts was carried out by selecting leaf samples (leaves still fresh and mature and dark green). The sorted mint leaves dried at room temperature for three days. The dried leaves were crushed into a powder using a blender, then stored in a clean, tightly closed container. In mint leaf extract, the powder was macerated with 96% ethanol as a solvent. The ratio of the material to the solvent was 1: 5, then it was macerated for 24 hours which aims to mix it evenly or homogeneously. After 24 hours, the filter results were separated. While the dregs were soaked in ethanol, repeat it until a clear solution is obtained. Collect the maceration results, then concentrate using a rotary evaporator so that a thick extract is obtained, then extracted using an oven temperature between 60-80oC. The final result of this process obtained a concentrated extract of mint leaves and weighing it.[20,26,28]

This research requires a mint leaf extract concentration of 2.5%, 5%, 7.5% and 10%. The extract concentration was determined by weighing the mint leaf extract made according to the concentrations, namely 0.25g, 0.5g, 0.75g and 1g and then added 10 mL of DMSO solution.

The procedure begins with taking the *Streptococcus sanguinis* bacteria, rejuvenated on the blood agar medium. Then enter the bacteria using sterile ose into a tube containing 2 ml of physiological NaCl, then standardize it with the standard 0.5 McFarland solution. The total density of bacteria used was the same, namely 1.5 x 10^8

CFU / ml. The instrument was a spectrophotometer with a wavelength of 625 nm to obtain an absorbance of 0.08–0.10.[4,6,29] Testing the effectiveness of the antibacterial inhibition of mint leaves extract was carried out by the agar diffusion method (Kirby-Bauer), using paper disc diffusion on Trypton Soya Agar (TSA) media. In the agar media that has been made, the suspension of *Streptococcus sanguinis* bacteria is carried out by streaking evenly on the surface of the media using a sterile loop. After that, the paper disks were immersed in the respective concentrations of mint leaf extract 2.5%, 5%, 7.5% and 10% and DMSO for 15 minutes. Then the paper disk is placed on the media's surface to use tweezers with light pressure so that it can stick. Each petri dish was labelled with a name according to the treatment given and wrapped in plastic wrap. After that, it was incubated at 35ºC for 24 hours in an incubator. Then, observations and measurements were made using a calliper in millimetres (mm) to determine the width of the inhibition area of each paper disk against bacterial growth. The width of the inhibition area is measured from the diameter of the clear zone formed around the paper disk on agar media.

RESULT

Inhibition can be determined by looking at the formed inhibition zone, which is the clear area that forms around the paper disk. The clear area developed was measured using a calliper to determine how much inhibition the mint leaf extract. (Table 1)

Table 1. Results measurement of inhibition zone diameter in all treatment groups against *Streptococcus sanguinis* bacteria

Group	Inhibition zone diameter (mm)					Mean (mm)	Antibacterial Strength Criteria
	1	2	3	4	5		
K	4.00	6.00	3.45	5.00	6.00	4.89	Poor
P1	16.25	15.70	14.60	15.25	13.30	15.02	Strong
P2	16.45	16.80	15.25	15.00	13.30	15.36	Strong
P3	16.80	16.25	16.80	15.10	13.20	15.63	Strong
P4	17.00	17.00	14.60	15.25	13.30	16.39	Strong

Based on the data in Table 1, it can be seen that in all test groups, a clear inhibition zone was formed, which meant that all of these groups had antibacterial properties that inhibit the growth of *Streptococcus sanguinis*.

The mean of the zone of inhibition of each concentration compared one another using the Kruskall-Wallis test to see the significant differences in the comparisons of each group.

Table 2 Pairwise comparison test

Group	K	P1	P2	P3	P4
K		0.00*	0.00*	0.00*	0.00*
P1	0.00*		0.99	0.94	0.46
P2	0.00*	0.99		1.00	0.71
P3	0.00*	0.94	1.00		0.88
P4	0.00*	0.46	0.71	0.88	

* p<0,05 (there are significant differences)

In the Kruskall-Wallis test and the pairwise comparison test, the comparison between the control group and all the groups tested showed a significant difference because DMSO had no antibacterial effect. In comparing each test group at each concentration, there was no significant difference because all of the test groups contained beneficial compounds from mint leaves that were antibacterial, only different from the concentration.

DISCUSSION

All mint leaf extract groups from the lowest to the highest concentrations have antibacterial power, which effectively inhibits the growth of

Streptococcus sanguinis bacteria. The 10% concentration had the highest average inhibition power, namely 16.39 mm, and the 2.5% concentration had the smallest average inhibition power, namely 15.10 mm. Based on the classification of the antibacterial strength of Davis and Stout, all concentrations in the test group had strong antibacterial power with a diameter scale between 10-20 mm. The average inhibition power of the control group was 4.89 mm, which means it was classified as a weak criterion because it had a diameter scale of <5 mm.[30,31]

The test group with a concentration of 10% had the greatest inhibitory power because a higher concentration of mint leaf extract could increase the antibacterial activity found in the menthol content of mint leaf extract.[26,28] This follows the theory of Pelczar et al. (Year??), namely, the higher the concentration of antibacterial ingredients, the stronger the antibacterial activity.[32] Setiawan (2016) concluded that the greater the concentration of an antibacterial substance, the greater the inhibitory power against bacterial growth.[33]

The clear zone around the paper disk of the control group using DMSO, which was formed with a mean of 4.89 mm, cannot be considered a zone of inhibition. DMSO has no antibacterial effect. It is usually used as a solvent to dilute a test material and can be used as a control.[34,35] The clear zone formed on the control is caused by the paper disk that has not dried when pressed will cause the DMSO liquid to spread. The inoculum on the media will mix with the liquid, so that in the middle the inoculum becomes thin. The inoculum attached to the media was released away from the paper. The disk looks like an inhibition zone has formed. In the diffusion method, the concentration of an inoculum will affect the clear zone formed if it is too dilute.[36] Rahmadani (2015) stated that a clear zone would be formed in the negative control group if the paper disk was not dry, and it would cause a clear zone to form from DMSO fluid that spreads around the paper disk.[37]

The antibacterial activity of active chemical compounds in mint leaf extract is obtained from essential oils containing flavonoids, tannins, polyphenols and menthol.[20]

Essential oils were considered secondary metabolites and had an important role in plant defence which has antibacterial properties.[38] The mechanism of essential oils affected cell membranes. The composition in essential oils attacked the cytoplasmic membrane resulting in damage to membrane permeability, electron transport function, nutrient absorption, nucleic acid synthesis and also ATP enzyme activity.[27]

Flavonoids are active compounds denatured proteins cell irreversibly and damaged the permeability of bacterial cell walls, microsomes, and lysosomes.[23,24,39,40] Tannins in mint leaf extract are polyphenolic compounds. They have four mechanisms to inhibit bacterial growth. Inhibiting nucleic acid synthesis by inhibiting reverse transcriptase and DNA topoisomerase enzymes, bacterial cells cannot form. Activate adhesins and microbial cell enzymes, disrupt protein transport.

And degrade bacterial cell walls by poisoning cell wall polypeptides, which cause osmotic and physical pressure of bacterial cells to die.[39] Polyphenols are water-soluble glycosides that have a mechanism of action by forming complexes with hydrolytic enzymes to disrupt cell membranes, non-specific relationships with carbohydrates, and other relationships that deactivate adhesives will disrupt the stability of bacterial cell membranes.[41] Menthol have an antibacterial effect against gram negative and gram positive by eliminating pathogenic bacteria.[42]

CONCLUSION

Based on the results of research on the effectiveness of mint leaf extract (*Mentha piperita L.*) on the growth of *Streptococcus sanguinis*, it can be concluded that mint leaf extract has antibacterial effectiveness in inhibiting the growth of the bacteria *Streptpcoccus sanguinis*. Mint leaf extract with a concentration of 10% had the highest inhibitory power in inhibiting the growth of *Streptococcus sanguinis* with an average of 16.39 mm and the lowest inhibition at a concentration of 2.5% with an average of 15.02 mm.

CONFLICT OF INTEREST

We declare that there is no conflict of interest in the scientific articles we write.

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REFERENCES

1. Zhu B, Macleod LC, Kitten T, Xu P. *Streptococcus sanguinis* biofilm formation & interaction with oral pathogens. Futur Med 2018;13(8):915–32.
2. Newman T, Carranza K. Newman and carranza's clinical periodontology. 3rd ed. Elsevier; 2019. p. 19–126.
3. Harvey JD. Periodontal microbiology. Dent Clin North Am 2017;61(2):253–69.
4. Zakki M. Uji aktivitas antibakteri ekstrak cathechin teh putih terhadap streptococcus sanguinis. O Dent J 2017;4(2):108.
5. Suherna IS, Setiawan A, Pertiwi P, Halim M. Gambaran efek pasta gigi yang mengandung herbal terhadap penurunan indeks plak. https://www.researchgate.net/. 2015. [Diunduh tanggal 19 Maret 2015].
6. Andayani R, Chismirina S, Kumalasari I. Pengaruh ekstrak buah belimbing wuluh (*Averrhoa bilimbi*) terhadap interaksi *Streptococcus sanguinis* dan *Streptococcus mutans* secara in vitro. Cakradonya Dent J 2014;6(2):678–744.
7. Kolenbrander PE, Andersen RN, Blehert DS, Egland PG, Foster JS, Palmer RJ. Communication among oral bacteria. Microbiol Mol Biol Rev 2002;66(3):486–505.
8. Oroh ES, Posangi J, Wowor VNS.

Perbandingan efektivitas pasta gigi herbal dengan pasta gigi non herbal terhadap penurunan indeks plak gigi. e-GIGI 2015;3(2):573–8.

9. KKRI. Hasil utama riskesdas 2018. Badan Penelitian dan Pengembangan Kesehatan. Jakarta; 2018.
10. KKRI. Laporan nasional riskesdas 2018. Badan Penelitian dan Pengembangan Kesehatan. Jakarta; 2018.
11. Lang NP, Lindhe J. Clinical periodontology and implant dentistry. 6th ed. Wiley Blackwell; 2013. p. 169–179.
12. Ladytama RS, Nurhapsari A, Baehaqi M. Efektivitas larutan ekstrak jeruk nipis (*Citrus aurantifolia*) sebagai obat kumur terhadap penurunan indeks plak pada remaja usia 12 – 15 tahun - studi di SMP nurul islami, mijen, Semarang. ODONTO Dent J 2014;1(1):39.
13. Pranata N. Dental calculus as the unique calcified oral ecosystem a review article. Ocean Biomed J 2019;2(2):52.
14. Egi, M et al. Efek berkumur sari buah tomat (*Solanum lycopersicum L.*) terhadap indeks plak gigi. Sound Dent 2018;3(2):70–84.
15. Saputri D, Novita CF, Zakky M. Perbandingan tindakan menjaga kebersihan rongga mulut dan status oral hygiene pada anak usia sekolah dasar di daerah perkotaan dan pedesaan. J Syiah Kuala Dent Soc 2016;2(2):90–6.
16. Balagopal S, Arjunkumar R. Chlorhexidine: the gold standard antiplaque agent. J Pharm Sci Res 2013 Dec 1;5:270–4.
17. Dwipriastuti D, Putranto RR, Anggarani W. Perbedaan efektivitas chlorhexidine glukonat 0,2% dengan teh hijau (*Camellia sineresis*) terhadap jumlah porphyromonas gingivalis. O Dent J 2017;4(1):50–4.
18. Oktanauli P, Taher P, Prakasa AD. Efek obat kumur beralkohol terhadap jaringan rongga mulut (kajian pustaka). J Ilm dan Teknol Kedokt Gigi 2017;13(1):4.
19. BPOM RI. Peraturan badan pengawas obat dan makanan Republik Indonesia nomor 13 tahun 2014 tentang pedoman uji klinik obat herbal.
20. Singh R, Shushni MAM, Belkheir A. Antibacterial and antioxidant activities of *Mentha piperita L*. Arab J Chem 2015 May 1;8(3):322–8.
21. Fayed MAA. *Mentha piperita L.* - a promising dental care herb mainly against cariogenic bacteria. Univers J Pharm Res 2019;4(3):31–6.
22. Setiawan A, Kunarto B, Sani EY. Ekstraksi daun peppermint (*Mentha piperita L.*) menggunakan metode microwave assisted extraction terhadap total fenolik, tanin, flavonoid dan aktivitas antioksidan. J Petrol 2013;369(1):1689–99.
23. Carolia N, Noventi W. Potensi ekstrak daun sirih hijau (*Piper betle L.*) sebagai alternatif terapi *Acne vulgaris*. Majority 2016;Vol. 5(1):140-145.
24. Rahayu SR, Diarti MW. Uji daya hambat

filtrat daun ciplukan (*Physalis angulata linn*) terhadap pertumbuhan bakteri *Staphylococcus aureus*. J Anal Med Bio Sains 2018;5(2):101-106.

25. Prama P, Sukrama I, Handoko S. Uji efektifitas antibakteri ekstrak buah jeruk nipis (*Citrus aurantifolia*) terhadap pertumbuhan *Streptococcus mutans* in vitro. Bali Dent J 2019;3(1):45.
26. Golestannejad Z, Gavanji S, Mohammadi E, Motamedi A, Bahrani M, Rezaei F, et al. comparison of antibacterial activity of essential oils of *Foeniculum vulgare mill, Mentha arvensis and Mentha piperita* against *streptococcus mutans*. Adv Herb Med 2017;3(1):3–13.
27. Masturoh I, Anggita T N. Metodologi penelitian kesehatan. KKRI; 2018. hal. 142.
28. Widyastuti, Fantari HR, Putri VR, Pertiwi I. Formulasi pasta gigi ekstrak kulit jeruk (*Citrus sp.*) dan daun mint (*Mentha piperita L.*) serta aktivitas terhadap bakteri *Streptococcus mutans*. J Pharmascience 2019;6(2):111.
29. Tri Budi S H. Pengaruh seduhan teh hijau (*Camellia sinensis*) terhadap hambatan pertumbuhan bakteri *Streptococcus sanguis* penyebab karies (in vitro). Surakarta: Fakultas Kedokteran Gigi Universitas Muhammadiyah Surakarta. 2017.
30. Novita W. Uji Aktivitas antibakteri fraksi daun sirih (*Piper Betle L*) terhadap pertumbuhan bakteri *Streptococcus mutans* secara in vitro. JMJ 2016;4(2):140–55.
31. Nurfitri Saridewi M, Bahar M, Anisah A. Uji efektivitas antibakteri perasan jus buah nanas (*Ananas comosus*) terhadap pertumbuhan isolat bakteri plak gigi di puskesmas Kecamatan Tanah Abang periode april 2017. Biog J Ilm Biol 2017;5(2):104–10.
32. Pelczar Jr MJ, Chan ECS, Krieg NR. Dasar-dasar mikrobiologi. Jakarta: Universitas Indonesia; 1988.
33. Setiawan B. Daya hambat konsentrasi enzim bromelin dari ekstrak bonggol nanas (*Ananas comosus* (*L.*) *Merr*) *Streptococcus sanguinis*. Makassar: Fakultas Kedokteran Gigi Universitas Hasnuddin. 2016.
34. S. Esath Natheer. Evaluation of antibacterial activity of *Morinda citrifolia*, *Vitex trifolia* and *Chromolaena odorata*. African J Pharm Pharmacol 2012;6(11):783–8.
35. Utomo SB, Fujiyanti M, Lestari WP, Mulyani S. Uji aktivitas antibakteri senyawa c-4-metoksifenilkaliks[4]resorsinarena termodifikasi hexadecyltrimethylammonium-bromide terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. JKPK 2018;3(3):201.
36. Vandepitte, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CG. Prosedur laboratorium dasar untuk bakteriologi klinis. 2nd ed. Penerbit Buku Kedokteran EGC; 2011. hal. 97–114.

37. Rahmadani F. Uji aktivitas antibakteri dari ekstrak etanol 96% kulit batang kayu jawa (*Lannea coromandelica*) terhadap bakteri *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*, *Pseudomonas aeruginosa*. Jakarta: Fakultas Kedokteran dan Ilmu Kesehatan UIN Syarif Hidayatullah Jakarta. 2015.
38. Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Front Microbiol 2012;3:1–24.
39. Iqbal, Rustam N, Kasman. Analisis nilai absorbansi kadar flavonoid daun sirih merah (*Piper crocatum*) dan daun sirih hijau (*Piper betle L*). Gravitasi 2017;15(1):1–8.
40. Fitriyana A. Optimasi formula salep ekstrak etanolik daun sirih (*Piper betle linn*.) dengan menggunakan basis larut air. Surakarta: Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Sebelas Maret. 2012.
41. Maulana IA, Triatmoko B, Nugraha AS. Skrining fitokimia dan uji aktivitas antibakteri ekstrak dan fraksi tanaman senggugu (*Rotheca serrata* (*L*.) *Steane* & *Mabb*.) terhadap *Pseudomonas aeruginosa*. JPSCR J Pharm Sci Clin Res 2020;5(1):01.
42. Bupesh G, Amutha C, Nandagopal S, Ganeshkumar A, Sureshkumar P, Murali K. Antibacterial activity of *Mentha piperita L*. (peppermint) from leaf extracts - a medicinal plant. Acta Agric Slov 2008;89(1):73–9.