Al-Kimia, 11-1-2023, 47-56
Available online at: http://journal.uin-alauddin.ac.id/index.php/al-kimia

Antibacterial Activity Test of Secang Wood (*Caesalpinia sappan L.*) Ethanol Extract Against *Streptococcus mutans*

Febri Intan Listiana, Mutista Hafshah*, Rais Nur Latifah

Program Studi Kimia, Fakultas Sains dan Teknologi, Universitas Islam Negeri Walisongo Semarang, Jawa Tengah, Indonesia

**Corresponding Author: mutista.hafshah@walisongo.ac.id*

Received: April,14,2023 /Accepted: June,12,2023
doi: 10.24252/al-kimiav11i1.37136

Abstract: *Dental caries are caused by the Streptococcus mutans bacteria, which cause a food deposit to harden and adhere to the tooth surface. Cavities will develop if food residue is not removed. Herbal plants, specifically secang wood, can be used as an antibiotic. The purpose of this study was to observe the secondary metabolite content of secang wood extract, antibacterial activity, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of secang wood extract against Streptococcus mutans bacteria. This research method includes the maceration of secang wood extract with 96% ethanol solvent, phytochemical screening of secang wood extract, inhibition zone test with paper disc method, and MIC and MBC tests. Qualitative testing revealed that the secang wood extract contained alkaloids, flavonoids, tannins, and saponins. Flavonoids, tannins, and saponins are all constituents of plants. Secang wood extract indicate antibacterial activity against Streptococcus mutans, as evidenced by the formation of inhibition zones at concentrations of 20%, 40%, 60%, 80%, and 100%, namely 8.75±0.354; 11±1.41;, 12.75±0.354; 16.25±0.354; and 17.5±0.000 mm. Secang wood ethanol extract had a MIC of 12.5% against Streptococcus mutans and an MBC of 25%.*

Keywords*: antibacteri, MBC, MI, secang wood, Streptococcus mutans.*

INTRODUCTION

Indonesians as a whole frequently experience issues with their teeth and mouth (Maulanti & Laksono, 2021). According to Riskesdas data, 57.6% of Indonesians experience these issues (Riskesdas, 2019). Dental caries is one of the most prevalent oral and dental conditions. Dental caries are a condition that affects the teeth's hard tissues, including the enamel, dentine, and cementum. It is conducted by the activity of microorganisms that break down food particles in poorly cleaned teeth (Carrascosa et al., 2021). Dental caries are primarily appeared by three factors: the host (teeth), the agent (microorganisms), and the substrate (food residue on the teeth). *Streptococcus mutans, Streptococcus sanuis, Streptococcus mitis,* and *Streptococcus salivarius* are examples of microorganisms that cause dental caries. Streptococcus mutans is the dominant bacteria involved in the development of dental caries (Mayasari & Sapitri, 2019).

Brushing and flossing on a regular basis can assist you overcome dental and oral problems (Abdellatif et al., 2021; Löe, 2000). Furthermore, the use of antiseptic mouthwash can inhibit the growth of bacteria that cause dental caries (Brookes et al., 2020). Commercial antiseptic mouthwashes with a high alcohol content that are applied on a regular basis can increase the risk of oral cancer (Aceves Argemí et al., 2020; Ustrell-Borràs et al., 2020). As a

result, it is necessary to investigate other natural active ingredients with antibacterial activity against bacteria that cause dental caries.

Indonesia has a rich biodiversity, including plant species used as traditional medicinal ingredients. The sappan tree is one of the plants applied in traditional medicine. Wood chips or shavings are a traditional medicinal ingredient made from the sappan plant (*Caesalpinia sappan* L). As they contain secondary metabolites, these plants can be applied as a source of medicine (Vardhani. Afifah K, 2019). Secondary metabolites found in secang wood include flavonoids, tannins, alkaloids, saponins, phenylpropane, and terpenoids. Secang wood contains brazilin, which plays a role in providing a red color and is non-toxic to living organisms, making it safe for daily use, the quantitative analysis test results reveal that the concentration of brazilin in secang wood is measured at 8.54% w/w (N. K. Utami et al., 2022). Secang wood is reported to exhibit a range of pharmacological activities such as enzyme inhibition, antibacterial, antioxidant, anti-inflammatory, antifungal, anthelmintic, hepatoprotective, cytotoxic, wound healing, analgesic, anticonvulsant, hypolipidemic, insecticidal, antiplasmodial, and others due to its secondary metabolite content (Kekuda et al., 2021). *Streptococcus aureus* growth has been shown to be inhibited by secang wood (Cahyaningtyas et al., 2019).

Secang wood is a potential natural material to study and further investigate based on the content of secondary metabolites in secang wood and previous research data regarding secang wood's ability to inhibit bacterial growth. The purpose of this research is to determine the secondary metabolite content of secang wood atanol extract and its antibacterial potential against *Streptococcus mutans*.

RESEARCH METHODS

Materials and Tools

The materials applied in this study were secang wood, distilled water, Mc Farland's solution, concentrated hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), chloroform, $FeCl_3$, NaCl powder, *Streptococcus mutans* bacteria, Nutrient Agar, Mueller Hinton Agar (MHA), Brain Heart Infusion Borth (BHIB), and 96% ethanol.

The tools and materials used are rotary vacuum evaporator, analytical balance, Beaker glass (pyrex), petri dish (Herma, Pyrex, Anumbra), test tube (Iwaki), hot plate, bunsen, Erlenmeyer (pyrex), stir bar, measuring cup (Pyrex), aluminum foil, incubator, filter paper, autoclave, loop needle, UV-Vis Spectrophotometer, LAF (Laminar Air Flow), micropipette, tip, spatula spreader.

Procedures

Secang Wood Extract

The maceration method was used to create an ethanol extract from secang wood samples. A 96% ethanol solvent was used to macerate secang wood powder. In a maserate container, 100 g of sawdust was mixed with 1000 mL of 96% ethanol. After stirring for the first 6 hours, the mixture was allowed to stand for 2x24 hours. To obtain an ethanol extract of secang wood, the mixture was filtered through filter paper. To obtain a thick extract, the extract was concentrated using a Rotary Vacuum Evaporator at 500°C. The yield value was calculated after weighing the viscous extract (Cacique et al., 2020).

Phytochemical Screening of Secang Wood Ethanol Extract (Rajkumar et al., 2022)

Alkaloids

To determine the alkaloid content, 1 g of the test sample was mixed with 2-3 drops of Wagner's reagent. Such a brown precipitate forms, it indicates the presence of alkaloids. Wagner's reagent can be made with 2 g of KI and 1 g of I2 dissolved in 50 mL of water.

Tannins

One gram of the test sample was put in a test tube, followed by 2-3 drops of 1% FeCl3 solution. If the solution turns black-blue or black-green, it indicates the presence of tannins.

Flavonoids

Flavonoids can be examined by taking 1 g of the test sample and adding magnesium powder and 3 drops of concentrated hydrochloric acid. Flavonoids are present if the color changes from orange to red-purple.

Saponins

One gram of sample was dissolved with 10 mL of distilled water and vigorously shaken for 10 seconds, or until frothy. For 10 minutes, the tube is placed upright. If it is still foaming, it is saponin positive. To ensure that the foam formed is the result of saponins, add 3 drops of 2N HCl solution and observe the foam's resistance; if the foam is stable, the presence of saponins is confirmed.

Testing the Antimicrobial Potential of Secang Wood Ethanol Extract
Tool Sterilization

The tools are sterilized by first washing them with soap. It is dried in the open air after washing. When the tool is dry, it is wrapped in paper. Cotton is used to plug the test tube and Erlenmeyer flask's mouths. All tools should be sterilized in an autoclave at 121°C for 15 minutes. Heat the loop in a Bunsen burner to sterilize it.

Preparation of Streptococcus mutans Bacterial Stock Culture (Syahrani et al., 2021)

MHA medium was poured into three reaction tubes. Bacterial colonies were streaked on agar media. Bacterial culture stock was incubated at 290°C for 1x24 hours.

Preparation of Streptococcus mutans Bacterial Suspension (Aviany & Pujiyanto, 2020)

One ose of Streptococcus mutans bacterial culture stock was removed from the slanted media and suspended in BHIB media solution. The bacterial suspension was homogenized before being incubated at 370°C for 24 hours. This is conducted so that the bacteria can regenerate. The turbidity of the suspension was adjusted to meet the McFarland standard of 0.5. If the bacterial suspension appears turbid, gradually add 0.9% NaCl solution until the turbidity matches the standard Mc Farland turbidity of 0.5.

Antibacterial Activity Test Using Paper Disc Diffusion Method (Kirby & Bauer)

Disc paper was used to test antibacterial activity. The concentrations used were 100%, 80%, 60%, 40%, and 20%, with sterile distilled water serving as the negative control and chloramphenicol serving as the positive control. MHA agar medium was poured into a petri dish and allowed to solidify. By immersing a sterile cotton bud in the bacterial suspension, the test bacteria were spread on the solidified media. The surface of the agar

medium is rubbed with a cotton bud that has been moistened with the bacterial suspension and allowed to dry. The disc paper was then placed on the agar medium's surface, and 10 L of the extract was dripped on top. Cover the cup and leave it at room temperature for 24 hours (Olivia, 2018). The formation of clear areas around the disc paper revealed antibacterial activity. The diameter of the inhibition zone was measured twice on the horizontal and vertical sides with a vernier caliper, then added up and averaged.

MIC Value Determination (Sieberi et al., 2020)

There were a total of 12 sterile test tubes prepared. Each test tube is labeled 1-10, with tube 11 serving as a positive control, containing Streptococcus mutans equivalent to 0.5 McFarland turbidity. Tube 12 K(-) serves as the negative control, containing a 100% ethanol extract of secang wood. The test solution was an ethanol extract of secang wood that had been diluted in stages to achieve concentrations of 100, 50, 25, 12.5, 6.25, 3,12, 1.56, 0.78, and 0.38%. Fill tubes 1-10 with 2 mL of the test solution and 1 mL of the bacterial suspension that has been equated with the McFarland turbidity standard of 0.5. The mixture's initial absorbance value was determined using a UV-Vis spectrophotometer, and it was then incubated for 24 hours at 37ºC. Following incubation, the mixture was visually examined for turbidity and the absorbance value was determined using Uv-Vis spectrophotometry. If the final absorbance value (after incubation) of each tube exceeds the initial absorbance value (before incubation), it can be concluded that bacterial growth is still taking place. Bacterial growth is inhibited if the final absorbance value does not differ from the initial absorbance value, or if the final absorbance value is less than the initial absorbance value.

MBC test

The MBC value was determined by taking 100 L of the test solution, pouring the prepared MHA media into a petri dish, and flattening it with a sterile spreader. The concentration of the test solution used refers to the determined MIC value. The mixture was then incubated at 370°C for 24 hours. The presence or absence of bacterial growth on the agar media is determined by the presence or absence of white spots on the agar media (Rollando et al., 2019)

RESULTS AND DISCUSSION

The ethanol extract of secang wood obtained is thick, brownish red in color, and has a distinct secang wood aroma. A total of 10.47 g of condensed extract was obtained, with a yield of 10.47%.

Results of Phytochemical Screening

A phytochemical screening was performed to identify the secondary metabolite compounds. Secondary metabolite analysis includes alkaloids, tannins, flavonoids, and saponins. Table 1 reveals the results of the phytochemical screening.

Antibacterial activity test results with the paper disc method

The paper disc method with MHA media was used to test antibacterial activity in this study. The diameter of the clear zone around the disc paper after it has been dipped in the test solution and measured with a vernier caliper is the parameter of this test. This antibacterial test used concentrations of 20%, 40%, 60%, 80%, and 100%.

Table 1. Phytochemical screening results

Secondary metabolites		Analysis
Alkaloid	+	Brown precipitate
Flavonoid	+	Purplish red
Tanin	+	Blackish green
Saponin	+	Stable foam

Table 2. Antibacterial test

Concentration (%)	Inhibition zone diameter (mm)	Category (Sakul et al., 2020)
20	8.75 ± 0.354	currently
40	11 ± 1.414	strong
60	12.75 ± 0.354	strong
80	16.25 ± 0,354	strong
100	17.5 ± 0.000	strong
Negative Control	0 ± 0.000	weak
Positive Control	50.25 ± 2.475	very strong

Secang Wood Extract Control (+) Control (-)

According to the result of the study, secang wood extract has antibacterial activity against S. *mutans*. The presence of inhibition zones that form around the disc paper at each test concentration indicates the antibacterial activity (Figure 1). According to Table 1, the growth inhibition zones of *Streptococcus mutans* produced from different concentrations of secang wood extract, namely 20%, 40%, 60%, 80%, and 100%, have different diameter values and antibacterial strength categories. The inhibition zone formed increased in size from the lowest to the highest concentration. The inhibition zone formed by secang wood extract falls into the moderate to strong inhibition category. According to the average data obtained, the higher the concentration of the extract, the larger or wider the inhibition zone formed. The antibacterial effect on bacterial growth is affected by increasing the concentration of the extract (Fitriyanti et al., 2019; Parisa et al., 2019).

The presence of secondary metabolites in the extract that act as antimicrobials causes the formation of a clear zone around the disc paper. Secang wood contains alkaloids, flavonoids, tannins, and saponins as metabolites. Alkaloid compounds are heterocyclic

nitrogen compounds found in many plants that are used as antimicrobial agents due to their ability to kill bacteria by damaging their DNA (Oktirisma, 2018).

Saponins have an antibacterial effect by interfering with the stability of the bacterial cell membrane, resulting in bactericidal action in the cell. Saponins work by inhibiting the permeability of the bacterial cell membrane, resulting in cell membrane damage and, ultimately, the inability of the bacterial cell to grow and develop (Armedita et al., 2018).

Secang wood also contains antibacterial flavonoid compounds. Flavonoids work by damaging the cytoplasmic membrane. The bacterial cytoplasmic membrane controls input of food components and nutrients. Bacterial metabolites are released when the cytoplasmic membrane is damaged. Because nutrients cannot combine the energy formation process, bacterial cells cannot grow, resulting in cell death (Mayasari & Sapitri, 2019).

Secondary metabolites, such as tannins, have also been shown to have antibacterial properties. Tannin's antibacterial mechanism is that it can deactivate microbial cells on the cell surface via enzymes associated with cell membranes and cell wall polypeptides. Tannins can damage bacterial cell membranes, and astringent tannin compounds can induce the formation of complex compounds against enzymes or microbial substrates, as well as the formation of a tannin complex bond to metal ions, which can increase the tannin's toxicity (Rahman et al., 2017)**.**

Minimum Inhibitory Concentration Test Results for Streptococcus mutans

The MIC value was calculated using the liquid dilution method and Uv-Vis spectrophotometry. The adsorption value before and after incubation are the parameters used. The dilution method has the advantage of producing extremely homogeneous media, test material, suspension, and bacteria. It can also save media and test materials due to less usage (Mutammima, 2017).

In this MIC test, the concentrations of secang wood ethanol extract were 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, 0.38%, and 0.19%. According to Table 4.4, the MIC value of the ethanol extract of secang wood is 12.5% because the absorbance value after incubation decreased for the first time at this concentration. The absorbance value decreases after incubation at a concentration of 100% -12.5%, indicating that this concentration can inhibit the growth of Streptococcus mutans bacteria. Meanwhile, at a concentration of 6.25% -0.19%, the absorbance value increased after incubation. This indicates that bacterial growth is still occurring at these concentrations. Because secondary metabolites are abundant in the extract, the higher the concentration of the extract used, the greater the activity in inhibiting bacterial growth. These findings are in line with previous research (E. R. Utami, 2021) The more concentrated the extract, the more active the antibacterial compounds it contains, and the greater the ability to inhibit the growth of microorganisms.

The MIC value provides a means to assess the capability of an extract in suppressing a specific microorganism, enabling subsequent actions to be pursued. The research indicates that the secang wood extract exhibits a MIC value of 12.5% against Streptococcus mutans bacteria. These findings align with outcomes obtained through the employment of UV-Vis spectrophotometry, a method known for its enhanced precision.

Tabel 3. MIC test

Concentration (%)	Before incubation	After incubation	Description
100	3.85 ± 0.044	2.205 ± 0.020	Down
50	2.789 ± 0.221	1.5045± 0.278	Down
25	2.5175 ± 0.057	2.198 ± 0,215	Down
12.5	1.6385 ± 0.233	1.149 ± 0,091	Down
6.25	0.7875 ± 0.077	0.8505± 0,128	Up
3.12	0.3765 ± 0.004	0.4095± 0,001	Up
1.56	0.352 ± 0.011	0.736 ± 0,143	Up
0.78	0.289 ± 0.081	0.825 ± 0,092	Up
0.36	0.274 ± 0,085	0.817 ± 0,013	Up
0.19	0.2125 ± 0,006	0.648 ±0,014	Up
Control (-)	3.995 ± 0,075	2.789 ± 0,041	Down
Control (+)	0.144 ± 0,007	0.5635 ± 0,063	Up

Minimum Bactericidal Concentration Test Results for Streptococcus mutans

The MBC value was determined by testing the antibacterial activity in agar media with different concentrations of the MIC results. The concentration of the test solution used was varied, with concentrations of 100, 50, 25, and 12.5%. Because the MIC value of the obtained ethanol extract is 12.5%, the MIC value is determined at MIC concentrations and higher. The spread plate method is applied to determine the MBC, which involves taking a sample that has been tested for MIC up to 100 and then pouring it into the agar media and flattening it with a sterilized spatula spreader before incubating the plate for 24 hours at 37°CThe MBC value of the ethanol extract of secang wood is 25%, as shown in Table 3. The media is still clean at a concentration of 100% -25%, indicating that no bacterial growth has occurred. Meanwhile, bacterial growth is still detected at 12.5% -6.25% concentrations. Because the MBC value is 25%, secang wood has the potential to kill Streptococcus mutans bacteria because it contains antibacterial secondary metabolites

CONCLUSION

The secang wood ethanol extract obtained is red-brown in color, thick, and has a distinct sappan odor. The yield was 10.47%. According to the phytochemical test, the ethanol extract of secang wood contains secondary metabolites of alkaloids, tannins, saponins, and flavonoids. The presence of an inhibition zone indicates that the ethanol extract of secang wood has inhibitory activity against the growth of Streptococcus mutans. The inhibition zones of the test results at 20%, 40%, 60%, 80%, and 100% concentrations were 8.75 ± 0.354 mm, 11 ± 1.414 mm, 12.75 ± 0.354 mm, 16.25 ± 0.354 mm, 17.5±0.000 mm, respectively. The greater the inhibition, the higher the concentration of secang wood extract used. Secang wood extract had MIC and MBC values of 12.5% and 25% against Streptococcus mutans, respectively.

REFERENCES

Abdellatif H., Alnaeimi, N., Alruwais, H., Aldajan, R., & Hebbal, M. I. 2021. Comparison between water flosser and regular floss in the efficacy of plaque removal in patients after single use. *Saudi Dental Journal*, *33*(5), 256–259. https://doi.org/10.1016/j.sdentj.2021.03.005

Aceves Argemí, R., González Navarro, B., Ochoa García-Seisdedos, P., Estrugo Devesa, A., & López-López, Jos. O. S. 2020. Mouthwash With Alcohol and Oral Carcinogenesis: Systematic Review and Meta-analysis. *Journal of Evidence-Based Dental Practice*, *20*(2), 1–9. https://doi.org/10.1016/j.jebdp.2020.101407

Armedita, D., Asfrizal, V., & Amir, M. 2018. Aktivitas Antibakteri Ekstrak Etanol Daun, Kulit Batang, dan Getah Angsana (Pterocarpus Indicus Willd) terhadapPertumbuhan *Streptococcus mutans*. *ODONTO Dental Journa*, *5*(1).

Aviany, H. B., & Pujiyanto, S. 2020. Analisis Efektivitas Probiotik di Dalam Produk Kecantikan sebagai Antibakteri terhadap Bakteri *Staphylococcus epidermidis*. *Jurnal Berkala Bioteknologi*, *3*(2), 24–31.

Brookes, Z. L. S., Bescos, R., Belfield, L. A., Ali, K., & Roberts, A. 2020. Current uses of chlorohexidine for management of oral disease: a narrative review. *Jpurnal of Dentistry*, *103*(January), 1–9.

Cacique, A. P., Barbosa, É. S., de Pinho, G. P., & Silvério, F. O. 2020. Maceration extraction conditions for determining the phenolic compounds and the antioxidant activity of catharanthus roseus (L.) g. don. *Ciencia e Agrotecnologia*, *44*, 1–12. https://doi.org/10.1590/1413-7054202044017420

Cahyaningtyas, D. ., Puspawati, N., & Binugraheni, R. 2019. Uji Aktivitas Antibakteri Ekstrak Etanolik Kayu Secang (Caesalpinia sappan L.) terhadap Staphylococcus aureus. *Biomedia*, *12*, 205–216.

Carrascosa, C., Raheem, D., Ramos, F., Saraiva, A., & Raposo, A. 2021. Microbial biofilms in the food industry—a comprehensive review. *International Journal of Environmental Research and Public Health*, *18*(4), 1–31. https://doi.org/10.3390/ijerph18042014

Fitriyanti, F., Hendrawan, M. N. R., & Astuti, K. I. 2019. Antibacterial Activity Test of Ethanol Extract Pineapple (Ananas comosus (L.) Merr.) Peel against Growth of Propionibacterium acnes. *Borneo Journal of Pharmacy*, *2*(2), 108–113. https://doi.org/10.33084/bjop.v2i2.928

Kekuda, P. T., Vinayaka, K., & Raghavendra, H. 2021. *Medicinal and Aromatic Plants : Traditional Uses , Phytochemistry and Pharmacological Potential* (Issue March).

Löe, H. 2000. Ural hygiene in the prevention of caries and periodontal disease. *International Dental Journal*, *50*(3), 129–139. https://doi.org/10.1111/j.1875-595X.2000.tb00553.x

Maulanti, T., & Laksono, A. D. 2021. Oral Health Problem in Indonesia: An Ecological Analysis. *Indian Journal of Forensic Medicine & Toxicology*, *July*. https://doi.org/10.37506/ijfmt.v15i3.16307

Mayasari, U., & Sapitri, A. 2019. Uji Aktivitas Antibakteri Daun Sereh Wangi (Cymbopogon nardus) Terhadap Pertumbuhan Bakteri *Streptococcus mutans*. *Klorofil*, *3*(2), 15–19.

Mutammima, N. 2017. Uji aktivitas antijamur, penentuan konsentrasi hambat minimum

(KHM) dan konsentrasi bunuh minimum (KBM) serta KLT-bioautografi ekstrak etanol daun plethekan *(Ruellia tuberosa L.) terhadap Candida albicans*. Universitas Islam Negeri Maulana Malik Ibrahim.

Oktirisma, M. 2018. Potensi Antibakteri Ekstrak *Wedelia biflora (L) DC. Terhadap* Pertumbuhan *Streptococcus mutans*. (STKIP) PGRI Seumatera Barat.

Olivia, D. 2018. Aktivitas Antimikroba dari Ekstrak Daun Surian *(Toona sureni Merr.)* terhadap *Strepcoccus mutans dan Candida albicans*. Universitas Sumatera Utara.

Parisa, N., Islami, R. N., Amalia, E., Mariana, M., & Rasyid, R. S. P. 2019. Antibacterial Activity of Cinnamon Extract (*Cinnamomum burmannii*) against *Staphylococcus aureus and Escherichia coli* In Vitro. *Bioscientia Medicina : Journal of Biomedicine and Translational Research*, *3*(2), 19–28. https://doi.org/10.32539/bsm.v3i2.85

Rahman, F. A., Haniastuti, T., & Utami, T. wahyu. 2017. Skrining fitokimia dan aktivitas antibakteri ekstrak etanol daun sirsak (Annona muricata L.) pada *Streptococcus mutans* ATCC 35668. *Majalah Kedokteran Gigi Indonesia*, *3*(1), 1–7.

Rajkumar, G., Panambara, P. A. H. R., & Sanmugarajah, V. 2022. Comparative Analysis of Qualitative and Quantitative Phytochemical Evaluation of Selected Leaves of Medicinal Plants in Jaffna, Sri Lanka. *Borneo Journal of Pharmacy*, *5*(2), 93–103. https://doi.org/10.33084/bjop.v5i2.3091

Riskesdas, T. 2019. Laporan Nasional Riskesdas 2018. In *Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan RI* (p. 126). https://doi.org/10.12688/f1000research.46544.1

Rollando, R., Prasetyo, Y. S. A., & Sitepu, R. 2019. Uji antimikroba minyak atsiri masoyi (Massoia aromatica) terhadap bakteri *Streptococcus mutans*. *Majalah Farmasi Dan Farmakologi*, *23*(2), 52–57.

Sakul, G., Simbala, H., & Rundengan, G. 2020. ji Daya Hambat Ekstrak Etanol Daun Pangi (Pangium edule Reinw. ex Blume) Terhadap Bakteri *Staphylococcus aureus, Escherichia coli* dan *Pseudomonas aeruginosa*. *Pharmacon*, *9*(2), 275–283.

Sieberi, B. M., Omwenga, G. I., Wambua, R. K., Samoei, J. C., & Ngugi, M. P. 2020. Screening of the Dichloromethane: Methanolic Extract of Centella asiatica for Antibacterial Activities against *Salmonella typhi, Escherichia coli, Shigella sonnei, Bacillus subtilis, and Staphylococcus aureus*. *Scientific World Journal*, *2020*. https://doi.org/10.1155/2020/6378712

Syahrani, H. D., Manalu, K., & Tambunan, E. P. S. 2021. Uji Efektivitas Antimikroba Ekstrak Bunga Kecombrang (*Etlingera elatior*) Terhadap Pertumbuhan *Streptococcus mutans* dan *Candida albicans*. *BEST Journal (Biology Education, Sains and Technology)*, *4*(2), 367–373.

Ustrell-Borràs, M., Traboulsi-Garet, B., & Gay-Escoda, C. 2020. Alcohol-based mouthwash as a risk factor of oral cancer: A systematic review. *Medicina Oral Patologia Oral y Cirugia Bucal*, *25*(1), e1–e12. https://doi.org/10.4317/medoral.23085

Utami, E. R. 2021. Aktivitas Antibakteri Ekstrak Etanol Daun Pandan Wangi (*Pandanus Amaryfolius*) Terhadap *Staphylococcus aureus*. *Jurnal Kesehatan: Jurnal Ilmiah Multi Sciences*, *11*(01), 61–71.

Utami, N. K., Amperawati, M., & Rizki, M. I. 2022. Quantitative Analysis of Secondary

Metabolites and Solvent Optimization of Secang Wood Extract (*Caesalpinia Sappan L/BiancaeaSappan*) as Disclosing Agent. *Jurnal Kesehatan Gigi*, *9*(1), 70–74. https://doi.org/10.31983/jkg.v9i1.7844

Vardhani. Afifah K. 2019. *Caesalpinia sappan* L.: Review Article. *Proceedings of International Conference on Applied Science and Health*, *4*, 300–305.