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POTENTIAL Of BAJAKAH STEM ENDOPHYTIC FUNGI (*Spatholobus littoralis* Hassk) as ANTIBACTERIA AGAINST *Escherichia coli*

Iva Oktafira.[1*], Kunti Nastiti.[2], Ali Rakhman Hakim.[3], Rohama[3]

[1]*Pharmacy D epartment, Health Faculty, Sari Mulia University, Indonesia*

ivaoktafira18@gmail.com

Abstract

Endophyte fungi isolated from their host plants produce the same secondary metabolic compounds as those produced by their host plants. So that the metabolic compound is no longer taken from the plant directly but from the endophyte fungus. So that the preservation of the plant, especially the bajakah (*Spatholobus littoralis* Hassk), is maintained and can be developed as a drug without worrying about the biodiversity of the plant. To determine the antibacterial activity of the steel-stem endophyte fungus (*Spatholobus littoralis* Hassk) in inhibiting *Escherichia coli* and to identify the content of secondary metabolites of the steel-stem endophyte fungus. True Experimental research method with Post test Only Control Design where there are two randomly selected groups, namely the negative control group of *Escherichia coli* and the positive control of chloramphenicol with the experimental group of endophytic fungi. Endophyte fungal supernatants antibacterial activity test using disc diffusion and identification of secondary metabolites using TLC (Thin Layer Chromatography). The results of an endophyte supernatant study can inhibit *Escherichia coli* bacteria characterized by an average diameter of the endophyte supernatant 24.42±5.28 mm and positive control of chloramphenicol with an average diameter of 27.68±1.95 mm. The endophyte supernatant has the secondary metabolic content of flavonoid. The supernatant of the steel rod endophyte fungus is potentially antibacterial to *Escherichia coli* bacteria and has flavonoid secondary metabolite content.

Keywords: escherichia coli, endophyte, *Spatholobus littoralis* Hassk

Introduction

One of the medicinal plants that are often used empirically as medicine is bajakah tampala (*Spatholobus littoralis* Hassk) where this plant is one of the species of the genus *Spatholobus* which is spread and lives in Asia. From hereditary experience from the surrounding community, boiled water from bajakah tampala stems can be used as a medicine for stomach pain, diarrhea and dysentery. Phytochemical screening of ethanol extract of bajakah tampala proved to have secondary metabolites in the form of flavonoids, saponins, and tannins. Flavonoids and saponins have antibacterial activity against *Escherichia coli* (Maulidie *et al*, 2019). Flavonoids have antibacterial activity by damaging the bacterial cell wall which consists of lipids and amino acids so that it will react with alcohol groups in flavonoid compounds (Aswir & Misbah, 2018). While the mechanism of saponin is by damaging

the permeability of the bacterial cell wall so that it can cause the death of the bacterial cell. Finally, the antibacterial mechanism of tannin is that tannin can wrinkle the bacterial cell wall so that it can disrupt the permeability of the cell itself, causing wall damage (Anggraini et al., 2019). Research on the use of endophytic fungi is still very limited to their use as biocontrol agents in agriculture and agro-industry. The use of endophytic fungi for use in drug development is still very limited or rarely carried out both in terms of research and research funds (Triastuti, 2020) so that strategic steps are needed in its development.

To find out the strength of the bajakah stem endophytic fungus produced, the researcher wants to test its activity against *Escherichia coli* bacteria. According to a survey conducted by the Ministry of Health Basic Health Research in 2016 diarrhea cases in South Kalimantan amounted to 63,257 cases, then increased to 75,046 cases in 2017 and decreased in 2019 and 2020 respectively, namely 59,227 and 35,092 cases. It can be seen that there was an increase in diarrhea cases in 2017 by 11,789 cases of diarrhea that occurred in South Kalimantan (Watulingas et al., 2022). Bacteria that can cause this infection, for example *Escherichia coli*, where *Escherichia coli* bacteria are bacteria that live in the intestines of humans and animals (Maulidie *et al*, 2019).

Indonesia is a developing country, where the level of awareness to maintain health is still lacking, so it is easy to be infected by a disease. One of the efforts to treat various microbial infections can use plants that are medicinal in plant tissue there are living microorganisms called endophytic microbes (Sumampouw, 2014). From the current case, many cases of resistance are increasing so that the active compounds obtained from endophytic fungi are expected to be used as antibacterial against *Escehrichia coli* bacteria.

Based on the description above, it is hoped that this research can produce secondary metabolites from bajakah stem endophytic fungi (*Spatholobus littoralis* Hassk) which have the potential as antibacterial *Escherichia coli*.

Materials and Methods

The research method used in this research is experimental research method. True Experimental with the aim of identifying the antibacterial activity of bajakah endophytic fungi (*Spatholobus littoralis* Hassk) and testing the inhibition of bajakah endophytic fungi (*Spatholobus littoralis* Hassk) against *Escherichia coli* bacteria. This research was conducted at the Microbiology Laboratory of Sari Mulia University.

The tools used in this study are autoclave (GEA YX-280D), Bunsen, Petri dish (pyrex), glass funnel, Erlenmeyer (pyrex), measuring cup (pyrex) 100 mL, object glass, beaker glass (pyrex), incubator (ESCO Isotherm), preparation needle, ose needle, LAF (Laminar Air Flow), optilab microscope, oven, tweezers, volume pipette, tube rack, razor blade, test tube (pyrex), gloves, spatula, stirring rod, spiritus, cell phone camera and analytical balance (Metteler Toledo).

The materials used in this study are bajakah plant stems (*Spatholobus littoralis* Hassk), *Escherichia coli* bacterial culture, Potato Dextrose Agar (PDA) media, Nutrient Agar (NA) media, 95% alcohol, distilled water, chloramphenicol, cotton, aluminum foil and tissue.

Research Procedures

1. Preparation of PDA and NA Media

Potato Dextrose Agar (PDA) was weighed 3.9 grams and chloramphenicol as much as 10 mg then put into Erlenmeyer, dissolved with distilled water until 100 ml then checked the pH to 7.0 ± 0.2. After that, it was heated to boiling and dissolved completely. After dissolving perfectly, it is clogged using cotton and then sterilized in an autoclave for 15 minutes at 121ºC. Weigh Nutrient Agar (NA) as much as 2 grams, then put into an Erlenmeyer which is dissolved with distilled water up to 100 ml. After that, it is heated to a boil and dissolves perfectly. After dissolving perfectly the plug uses cotton and then sterilized in an autoclave for 15 minutes at 121ºC (Indrawati A., *et al*, 2019).

2. Endophytic Fungus Characteristics

The bejakah stem is washed using running water and then cut into small pieces with a size of 3-4 cm. after that it is sterilized with 70% ethanol soaked for 2 minutes. rinse with sterile water three times. Pieces of bajakah stems are planted on Potato Dextrose Agar (PDA) media which has been added with chloramphenicol, then incubated for 3 days at room temperature, namely 25ºC-28ºC or until there is fungal growth (Indrawati A., *et al*, 2019).

3. Endophytic Fungus Isolation

Endophytic fungi that grew on Potato Dextrose Agar (PDA) media were purified on Potato Dextrose Agar (PDA) media and incubated at 25ºC for 7 days until the fungi grew. After that, the mushrooms that have grown are observed for the shape and color of the colonies. Each colony that has a different shape or color will be subcultured again on tilted Potato Dextrose Agar (PDA) media until a pure colony is obtained (Indrawati A., *et al*, 2019).

4. Purification of Endophytic Fungal Isolates

PDA media was taken from the petri dish using an ose needle, then the pieces of media were placed on the object glass. Take the pure culture fungus using an ose needle then the fungal inoculum is placed on top of the media piece on the object glass and then covered with a cover glass. The prepatate was placed on a plastic cross section and then incubated for 3 days. After that, make macroscopic and microscopic observations (Indrawati A., *et al*, 2019).

5. Antibacterial Test of Endophytic Fungi

Nutrient Agar (NA) media was poured aseptically into a sterile Petri dish as much as 15 ml and allowed to solidify. Then take a suspension of *Escherichia coli* bacteria and inoculate it on the surface of Nutrient Agar (NA) media using a sterile swab. Endophytic fungal isolates were suspended using sterile distilled water, after which the paper disk was immersed in the endophytic fungal suspension for 15 minutes. After that, drain and place on Nutrient Agar (NA) media that has been inoculated with test bacteria. Incubate at 37ºC for 24 hours. Then measure the diameter of the inhibition zone measured in millimeters (mm) using a caliper by measuring the distance from the edge of the test well to the boundary of the inhibition zone circle (Indrawati A., *et al*, 2019) (Pangow et al., 2020).

6. Test Bacteria Preparation

One ose of pure *Escherichia coli* culture was inoculated on a slanted agar medium, then incubated at 37ºC for 24 hours. The results of rejuvenation obtained, then taken 1 ose and then suspended into NaCl as much as 10 ml with 0.9% physiological (Pangow et al., 2020).

7. Secondary Metabolite Testing

The identification test of secondary metabolites in the extract of endophytic fungal isolates from bajakah stems (*Spatholobus littoralis* Hassk) was carried out using thin layer chromatography using chloroform: methanol eluent with a ratio of 5: 21 in 1 mL. The eluent was made by taking 195 µL of chloroform and 815 µL of methanol then put it into the chamber and wait until it was saturated. Then the endophytic fungal extracts are totolkan simultaneously on the activated KLT plate with a size of 6 cm x 3 cm which has been given a lower limit of 0.5 cm and an upper limit of 0.3 cm. then the KLT plate that has been bottled is eluted with eluent that has been saturated in the chamber. Then make observations at 366 nm and sprayed using cyto borate spray reagent and for flavonoid tests which are indicated by a greenish-yellow color, dragendrof reagent to detect the presence of alkaloid compounds, Lieberman reagent to test for triterpenes, 85% phosphoric acid reagent for steroid tests, and H^2O^2 reagent to detect the presence of aromatic acids (Vilca *et al*, 2015).

Results and Discussion

1. Bajakah Stem Endophytic Fungus Isolates

Figure 1: First Day Bajakah Stem Endophytic Fungus Isolate Results

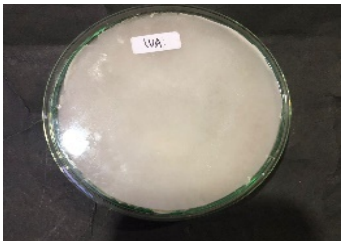

Figure 2: Tenth Day Bajakah Stem Endophytic Fungus Isolate Results

The purpose of isolating endophytic fungi is mainly to obtain biologically active secondary metabolite products (Kuncoro & Sugijanto, 2016). The media used in the isolation of endophytic fungi is Potato Dextrose Agar (PDA) media, because PDA media has carbohydrates that are good for fungal growth. PDA is also one of the good media used to culture a microorganism in the form of fungi (Ngole et al., 2017). Observations were made for 10 days to see the possibility of endophytic fungi growing. On the 10th day of observation, there was uniform growth of endophytic fungi from the three bajakah stems in petri dishes.

On PDA media, chloramphenicol antibiotics are added, the function of chloramphenicol antibiotics added to the PDA medium is intended to inhibit the growth of endophytic bacteria or contaminant bacteria that might contaminate (Hasiani et al., 2015). Research on endophytic fungi is one form of biotechnology development, in this case, increasing the production of secondary metabolites through microbes, especially fungi. This is done to produce secondary metabolite products that are superior and in abundance. Among the various microorganisms that have been developed for their potential as a source of medicinal materials today are secondary as a result of coevolution or genetic transfer (genetic recombination) from their host plants to endophytic microbes. The ability of endophytic microbes to produce certain phytochemical compounds that are also produced by their host plants may be related to the genetic recombination of endophytic microbes with their hosts during their evolutionary time (Kuncoro & Sugijanto, 2016).

2. Purification of Bajakah Stem Endophytic Fungus Isolate

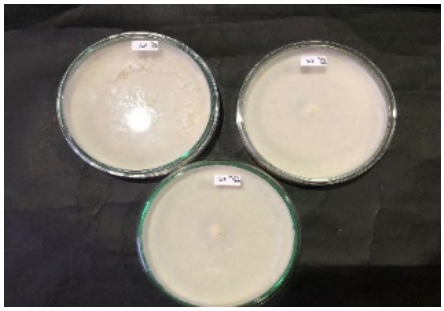

Figure 3: First Day Bajakah Stem Endophytic Fungus Isolate Results

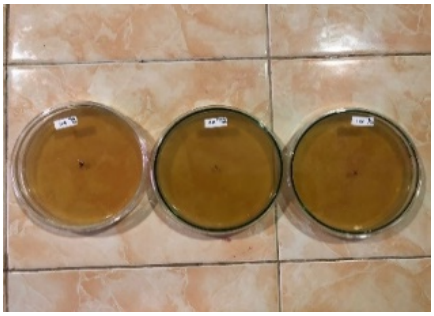

Figure 4: Third Day Bajakah Stem Endophytic Fungus Isolate Results

This purification aims to separate endophytic colonies with different morphologies to be isolated separately. The results of endophytic fungal isolates that have grown on PDA isolation media added with chloramphenicol (PDAC), then gradually purified one by one. Each pure isolate of endophytic fungi obtained was then transferred into the media in PDAC petri dishes. Morphological observations were made again after incubation for 3 days, and if macroscopically different colony growth was still found, it had to be separated again until pure isolates were obtained. Endophytic fungi are incubated at room temperature for 3-5 days according to their growth (Hasan Basri et al., 2021).

3. Endophytic Fungus Characteristics

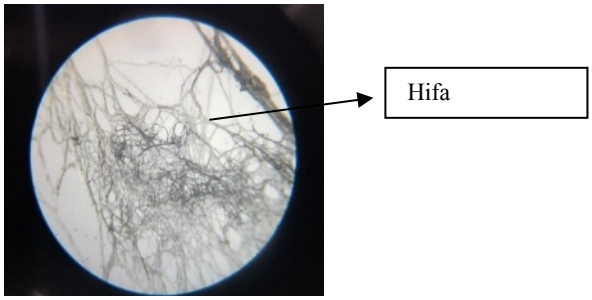

Figure 5: Microscopic Observation Results of Bajakah Stem Endophytic Fungus 40x Magnification

The results of the endophytic fungal isolates were then subjected to macroscopic and microscopic observations at 40□ magnification with a lab optical microscope to see the morphological characterization of endophytic fungi. Microscopic observations were made by taking a few hyphae of endophytic fungi then observed under a microscope.

Purified fungi were macroscopically viewed for visible morphology, namely hyphal color, shape, and texture. The results of macroscopic observations of the three fungi have the same characteristics, namely visible white colonies, cotton-like colonies and have a smooth and uniform texture in all 3 replicates. The results of microscopic observations also only exist from sterile hyphae. The results of observations compared with the characteristics of fungi in the mycology book by Suryani *et al* (2020) this fungus is thought to be similar to the fungus Mycelia sterilia. The results of observations were also compared with research conducted by Hafsari (2017), namely in microscopic observations of endophytic fungi can be seen in Figure 5 in the results of microscopic observations consisting only of hyphae, so that endophytic fungi are thought to belong to Mycelia sterilia fungi.

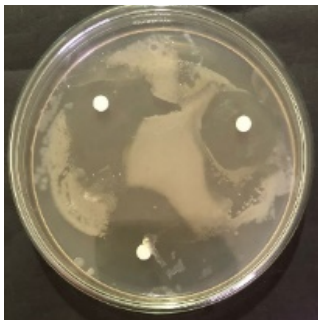

Figure 6: Supernatan Antibacterial Activity Test Results

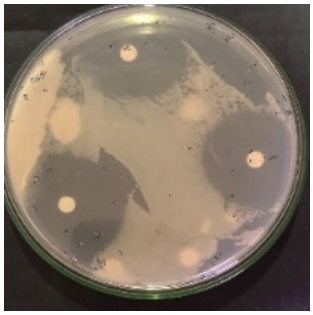

Figure 7: Chloramphenicol Antibacterial Activity Test Results

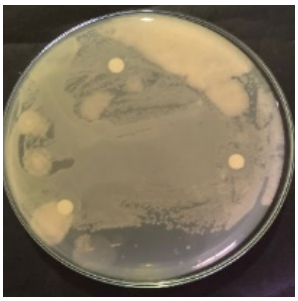

Figure 8: Aquadest Antibacterial Activity Test Results

Table 1: Inhibition Zone Diameter

Sample	Inhibition Zone Diameter (mm)			
	1	**2**	**3**	**Average ± SD**
Supernatan	21,13	21,63	30,51	24,42 ± 5,28
Positive Controls	28,43	25,47	29,14	27,68 ± 1.95
Negatice Controls	0	0	0	0

The results of the endophytic fungal isolates were taken 10 ml from the PDB media then separated between the fungus and the PDB media using centrifugation so that the supernatant was obtained and then tested for antibacterial activity using *Escherichia coli* bacteria with the disc diffusion method (Paper disk) and incubated for 24 hours using an incubator. Supernatant has antibacterial activity with the diameter of the inhibition zone characterized by the presence of a clear zone, this supernatant has antibacterial activity with an average diameter of 24.42 ± 5.28 mm including a very strong category and positive control has greater antibacterial activity with an average diameter of 27.68 ± 1.95 mm including a very strong category. While the negative control of aquadest did not have a diameter of bacterial inhibition zone. This is in accordance with research conducted

by Silva *et al* (2011) that the endophytic fungus Mycelia sterilia showed antibacterial activity against Escherichia coli.

In this study, endophytic fungi suspected to belong to the Mycelia sterilia family were obtained. The supernatant was tested for inhibition against the growth of *Escherichia coli* test bacteria. *Escherichia coli* bacteria are Gram negative bacteria. Based on the measurement results after incubation for 24 hours, it shows that the supernatant can inhibit the growth of test bacteria by forming an inhibition zone around the supernatant. From the measurement of the diameter of the inhibition zone, the supernatant suspected to be the Mycelia sterilia family has potential against *Escherichia coli* bacteria. Antibacterial activity can be caused by the content of secondary metabolites contained in endophytic fungi.

Fungi and bacteria have differences, namely fungi are nucleus cells surrounded by membranes and include eukaryotic cells (have a core membrane) while bacteria are organelles surrounded by membranes, do not have a clearly formed nucleus and include prokaryotic cells (do not have a core membrane (James & Sherman, 2008).

4. Secondary Metabolites

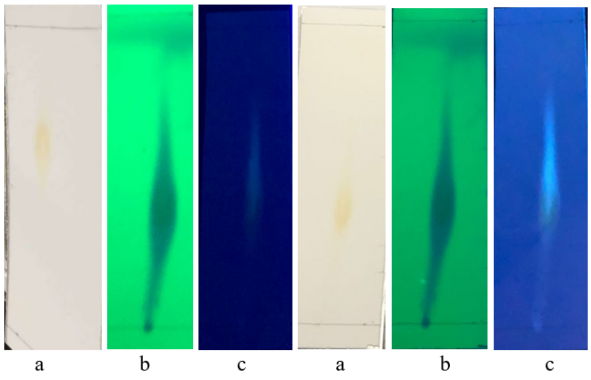

Figure 9: Flavonoid Identification Results using KLT

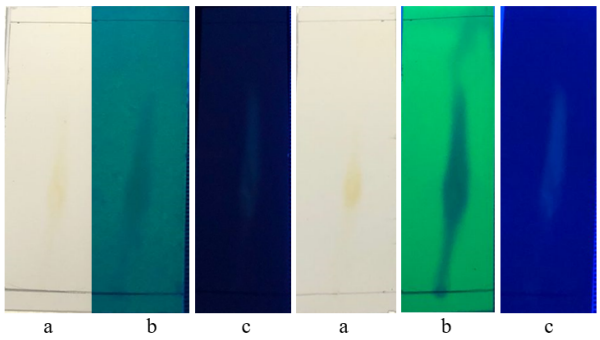

Figure 10: Alkaloid Identification Result using KLT

Identification of flavonoid compounds using KLT (Thin Layer Chromatography) with a mobile phase of methanol: chloroform (6: 2) and stationary phase silica gel plate GF254 detected using cyto borate reagent as detection of flavonoid compounds seen before and after spraying seen that the supernatant positively contains flavonoids characterized by spots become more yellow. The observation results in visible light showed a greenish yellow color, in UV light 254 nm showed a blue color and in UV light 366 nm showed blue fluorosence. This shows that the supernatant is positive for flavonoid secondary metabolites (Raihan et al., 2020).

Identification of alkaloid compounds using KLT (Thin Layer Chromatography) with a mobile phase of methanol: hexan (6: 2) and stationary phase silica gel plate GF254 was detected using dragendorf reagent as detection of alkaloid compounds seen before and after being sprayed, it was seen that the supernatant negatively contained alkaloids characterized by yellow spots and did not change color to orange after being sprayed with Dragendorf reagent. Thus, from the identification of secondary metabolites, the supernatant of endophytic fungi only contains flavonoids and does not contain alkaloids.

Secondary metabolites in bajakah stem endophytic fungi that have an inhibitory mechanism against bacterial growth are flavonoids. The mechanism of action of flavonoids as antibacterial is to form complex compounds with extracellular and soluble proteins so that they can damage the bacterial cell membrane followed by the release of intracellular compounds (Amalia et al., 2017).

Endophytic microbial research can be used as a solution to the discovery of compounds from plants that are efficacious as antibacterials with the advantages of endophytic microbial research in finding new sources of bioactive compounds that have the potential to be developed into antibacterial agents.

Conclusion

Based on the results of the study, the supernatant has the potential to inhibit *Escherichia coli* bacteria which is characterized by the average diameter of the supernatant of 24.42 ± 5.28 mm including the very strong category. Supernatant of endophytic fungi also contains flavonoid secondary metabolites that have the ability to inhibit *Escherichia coli* bacteria.

Reference

Amalia, A., Sari, I., & Nursanty, R. (2017). Aktivitas Antibakteri Ekstrak Etil Asetat Daun Sembung (Blumea balsamifera (L.) DC.) terhadap Pertumbuhan Bakteri Methicillin Resistant Staphylococcus aureus (MRSA). *Jurnal UIN Ar-Raniry*, *5*(1), 387–391.

Anggraini, W., Nisa, S. C., Da, R. R., & Ma, B. (2019). Aktivitas Antibakteri Ekstrak Etanol 96 % Buah Blewah (Cucumis melo L . var . Antibacterial Activity of 96 % Ethanol Extract Cantaloupe Fruit (Cucumis melo L . var . cantalupensis) Against *Escherichia coli* bacteria. *Pharmaceutical Journal of Indonesia*, *5*(1), 61–66.

Aswir, & Misbah, H. (2018). Kajian Potensi Ekstrak Anggur Laut (Caulerpa racemosa) Sebagai Antibakteri Terhadap Bakteri *Escherichia coli* DAN Staphylococcus aureus. *Photosynthetica*, *2*(1), 1–13.

Hafsari, A. R. (2017). Isolasi dan Identifikasi Kapang Pelarut Fosfat dari Fosfat Guano Gua Pawon. *Biota*, *10*(2), 165–180. https://doi.org/10.20414/jb.v10i2.13

Hasan Basri, M., Zulkifli, L., & Syukur, A. (2021). Isolation of Endophytic Fungi from Vitex trifolia L and Antagonism Test against Sclerotium rolfsii and pathogenic bacteria. *Jurnal Biologi Tropis*, *21*(1), 72–80. https://doi.org/10.29303/jbt.v21i1.2340

Hasiani, V. V., Ahmad, I., & Rijai, L. (2015). Isolasi Jamur Endofit dan Produksi Metabolit Sekunder Antioksidan dari Daun Pacar (Lawsonia inermis L.). *Jurnal Sains Dan Kesehatan*, *1*(4), 146–153. https://doi.org/10.25026/jsk.v1i4.32

Indrawati, A., Hartih, N. A., & Muyassara. (2019). Isolasi Dan Uji Potensi Fungi Endofit Kulit Batang Langsat (Lansium domesticum Corr.) Penghasil Antibakteri Terhadap Staphylococcus aureus dan Escherichia coli. *Media Farmasi Poltekkes Makasar*, *15*(1).

James, & Sherman. (2008). *Microbiology: a laboratory manual* (B. Cummings (ed.); 8th ed). Pearson Education.

Maulidie, M., Saputera, A., Widia, T., Marpaung, A., & Ayuchecaria, N. (2019). Konsentrasi Hambat Minimum (KHM) Kadar Ekstrak Etanol Batang Bajakah Tampala (*Spatholobus littoralis* Hassk) Terhadap Bakteri Escherichia Coli. *Jurnal Ilmiah Manuntung*, *5*(2), 167–173.

Pangow, E., Posangi, J., Lolo, W. A., & Bara, R. A. (2020). Uji Aktivitas Antibakteri Jamur Endofit Pada Daun Dan Batang Tumbuhan Kumis Kucing (Orthosiphon aristatus) Terhadap Bakteri *Escherichia coli* dan staphylococcus aureus. *Pharmacon*, *9*, 211–218.

Raihan, M., Taqwa, N., Hanifah, A. R., Lallo, S., Ismail, I., & Amir, M. N. (2020). Skrining Fitokimia Ekstrak Kulit Buah Nangka (Artocarpus heterophyllus) Dan Aktifitas Antioksidannya Terhadap [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] (ABTS). *Majalah Farmasi Dan Farmakologi*, *23*(3), 101–105. https://doi.org/10.20956/mff.v23i3.9400

Silva, M. R. O., Almeida, A. C., Arruda, F. V. F., & Gusmao, N. (2011). Endophytic fungi from brazilian mangrove plant Laguncularia racemosa (L.) Gaertn.(Combretaceae): their antimicrobial potential. *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*, *2*, 1260–1266.

Sumampouw, M. (2014). Uji Efek Antibakteri Jamur Endofit Akar Bakau Rhizophora stylosa Terhadap Bakteri Staphylococcus aureus dan Escherichia coli. *Jurnal E-Biomedik*, *2*(1). https://doi.org/10.35790/ebm.2.1.2014.3694

Suryani, Y., Taupiqurrahman, O., & Kulsum, Y. (2020). *Mikologi* (Issue 1). PT. Freeline Cipta Granesia.

Triastuti, A. (2020). Jamur endofit sebagai sumber obat bahan alam. *Jurnal Ilmiah Farmasi*, *16*(1), 52–73.

Watulingas, D. Y., Agustina, N., & Masyarakat, K. (2022). Analysis Of Factors Related To The Event Of Diarrhea In Toddlers In The Work Area Of The Pekauman Public Health Center, Banjarmasin City In 2021. *An-Nadaa: Jurnal Kesehatan Masyarakat*, *9*(1), 108–112.