Infectious diseases one of the main health problem, especially in developing countries and have been recorded to cause more than 3 million people to die every year. Therapy used is antibacterial, but currently there are many occurrences of resistance to antibacterials, for example by the bacteria Salmonella typhi, Escherichia coli, and Staphylococcus aureus, so it’s necessary to find antibacterial alternatives to treat infectious diseases. Non-pharmacological therapy used is the Ulin plant (Eusideroxylon zwageri), such as it’s fruit which contains secondary metabolites that function as antibacterial. This research aimed to identify the antibacterial activity of the Ulin fruit extract against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria. The type of research used is True Experimental with a Posttest-Only Control Group design. Screening antibacterial activity of Ulin fruit extract against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria using the well diffusion method and determining antibacterial activity test using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods, then the data were analyzed using One Way Anova Test. The results show Ulin fruit extract has antibacterial activity with highest inhibition zone at concentration of 10 mg/ml against Salmonella typhi 19.07 mm, Escherichia coli 12.57 mm, and Staphylococcus aureus 12.31 mm and each has strong antibacterial activity. MIC at concentration of 5 mg/ml. The results of statistical analysis showed significance value of 1,000 (p>0.05), meaning that there was no significant difference. So, Ulin fruit extract (Eusideroxylon zwageri) has antibacterial activity with Minimum Inhibitory Concentration (MIC) value of 5 mg/ml.
bacteria, viruses, parasites, and fungi (Mahdiyah et al. 2021). The infection kills 3.5 million people every year, the victims are living in low to middle income countries (WHO 2014).

Infection is caused by the presence of pathogenic bacteria, including types of microorganisms called bacteria such as Salmonella typhi, Staphylococcus aureus, and Escherichia coli that cause diarrhea, fever, inflammation, skin diseases, respiratory tract infections, urinary tract infections, root canal infections, and others (Akçaya et al. 2019; Kurniawan et al. 2019).

To treat infectious diseases, antibiotics are needed. However, currently there are many occurrences of resistance to antibacterials, for example by the bacteria Salmonella typhi, Escherichia coli, and Staphylococcus aureus. The development and spread of multidrug-resistant (MDR) bacteria around the world needs high attention, where currently there are very limited effective antibiotics to treat infections caused by MDR bacteria (Mahdiyah et al. 2020).

The high incidence of bacteria that are resistant to antibiotics is an opportunity to discover new drugs that are relatively safe and effective to treat bacterial infections. One alternative is to explore medicinal plants that have the potential as antibacterial, this is supported by the fact that people have used plants as traditional medicines to treat various diseases caused by pathogenic bacteria (Dzoyem et al. 2017).

The content of medicinal plants that are proven to be able to overcome the problem of infectious diseases in humans caused by pathogenic bacteria is due to having secondary metabolites such as steroids, terpenoids, phenolics, alkaloids, and other compounds (Khameneh et al. 2019). Ulin plant (Eusideroxylon zwageri) is one of the plants that has efficacy as a natural medicine. Ulin plant (Eusideroxylon zwageri) as one of the native plant species of Kalimantan, especially the fruit part is used by the people of Amuntai, South Kalimantan and West Kalimantan as traditional medicine to treat diabetes mellitus and heart disease. Empirical evidence from the people of Amuntai, South Kalimantan and West Kalimantan states that Ulin fruit (Eusideroxylon zwageri) is often consumed by people with diabetes mellitus and heart disease. Ulin fruit is proven to be effective in lowering blood sugar levels and improving heart performance (Faisal et al. 2021). In addition, the Ulin plant is also used by the Uud Danum Dayak community who live around the Ambalau River, Ambalau District, Sintang Regency, West Kalimantan as a traditional medicine to treat diseases such as fever, allergies, diarrhea, and stamina enhancer (tonic) (Mariani et al. 2016).

In this regard, data on the antibacterial bioactivity of the Ulin plant (Eusideroxylon zwageri) are still limited. Information that has been published about the Ulin plant is only on the bark, wood, and leaves, while the Ulin fruit has not been explored for its antibacterial activity.

The great potential possessed by the Ulin plant (Eusideroxylon zwageri) as a medicinal plant for the treatment of infectious diseases caused by pathogenic bacteria, it is necessary to test the antibacterial activity of Ulin fruit extract against pathogenic bacteria. So with this research, it is expected to be able to increase knowledge about alternative antibiotics or natural antibacterials from medicinal plants as a treatment for diseases caused by pathogenic bacteria.

**METODOLOGI**

**Materials**

The material used is Ulin fruit extract (Eusideroxylon zwageri) which is the result of extraction by researchers (Faisal et al. 2021) as research samples, test bacteria Salmonella typhi, Escherichia coli, and Staphylococcus aureus, soxhletation solvent used 96% ethanol, sterile distilled water, 70% ethanol, amoxicillin and fosfomycin as comparison antibiotics, Tryptone Soya Agar (TSA), Mueller Hinton Agar (MHA), Nutrient Broth (NB),
Methods

Type of research
The research method used in this research is experimental research, namely True Experimental with Posttest-Only Control Group Design research design (Hidayat 2010).

Research location
This research was conducted at the Microbiology Laboratory, Sari Mulia University, Banjarmasin City, South Kalimantan.

Population and sample
The population used in this study was Ulin fruit (Eusideroxylon zwageri) found in Amuntai, South Kalimantan. The sample used in this study was Ulin fruit extract (Eusideroxylon zwageri) which was the result of the researcher’s extraction (Faisal et al. 2021). The Ulin fruit used is the Ulin fruit with the species Eusideroxylon zwageri T. et. B. Determined by the STKIP PGRI Banjarmasin Botanist on September 13, 2021.

Research tools
The tools used in this research are Biological Safety Cabinet (Thermo Scientific), incubator (ESCO Isotherm), autoclave (GEA YX-280D), hot plate (Thermo Scientific-Cimarec), colony counter, refrigerator, analytical balance (AciS AD- 600i), micropipette, erlenmeyer (Pyrex), measuring cup (Pyrex), test tube (Pyrex), L rod, test tube rack, dropper, stirring rod, magnetic stirrer, sterile cork borer, beaker glass (Pyrex), petri dish , ose needles, syringes, tweezers, filter paper, aluminum foil, plastic wrap, labels, caliper and spirit.

Research procedure
The first thing to do in this research is to prepare samples with concentrations of 1 mg/ml, 3 mg/ml, 5 mg/ml, 7 mg/ml, and 10 mg/ml as well as sterilize the tools and materials to be used. Sterilization will be carried out using an autoclave at 121°C for approximately 15 minutes, while the ose needles are sterilized by burning directly on a spirit fire. Besides that, the media also made Tryptic Soy Agar (TSA), Muller Hinton Agar (MHA), and Nutrient Broth (NB) (Napitupulu et al. 2019; Utomo et al. 2018). Bacterial rejuvenation was carried out on a slanted agar to increase the bacterial stock (Ismail et al. 2017). Making positive control with antibiotics fosfomycin 1 gr/100 ml aquadest and amoxicilin 0.5 gr/100 ml aquadest and negative control with sterile aquadest (Wangkanusa et al. 2016). Preparation of the test bacteria suspension that will be used in the antibacterial activity test by the dilution method (Henaulu and Kaihena 2020). Standard solution of MC Farland 0.5 is also needed in the study as a reference to adjust the turbidity of the bacterial suspension so that the number of bacteria is within the given range to standardize the test microbe (Rosmania and Yanti 2020). Antibacterial activity testing begins with screening for antibacterial activity using the well diffusion method to see the sensitivity of the test bacteria to the antibacterial agents used by liquid dilution method (Fitriana et al. 2020). Then after screening, the antibacterial activity test was continued with the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods with the well method (Mahdiyah et al. 2020).

Data analysis
Data analysis was carried out analytically. Minimum Inhibitory Concentration (MIC) research data obtained are normally distributed and homogeneous, so parametric test is used. The parametric test used is the One Way Anova test because in this study there were more than two groups of variables. The distribution of data was analyzed by normality test, namely using the One Sample Shapiro Wilk Homogenity Test because the samples used were less than 100 samples. If the data
significance value is $>0.05$ ($p>0.05$) then the data is normally distributed, but if the data significance value is $<0.05$ ($p<0.05$) then the data is not normally distributed. Then to determine the homogeneity of the data, the homogeneity test of variance was carried out using the Lavene Test of Homogeneity of Variance. Homogeneous data if the obtained significance value $>0.05$ ($p>0.05$) (Hidayat 2010).

**HASIL DAN PEMBAHASAN**

**Antibacterial activity screening**

In the research that I have done, the extract of Ulin fruit (*Eusideroxylon zwageri*) in the antibacterial screening test using the well diffusion method showed an inhibition zone formed around the wellbore which was measured with a caliper and the results were in units (mm). Inhibition zones were found at concentrations of 5 mg/ml, 7 mg/ml, and 10 mg/ml, while at concentrations of 1 mg/ml and 3 mg/ml there was no clear zone around the well, so the results only had an inhibition zone. The reference for the barrier response classification used for the research results is as shown in Table I.

**Table I. Classification of Bacterial Growth Inhibitory Responses**

<table>
<thead>
<tr>
<th>Diameter of bright zone</th>
<th>Growth inhibition response</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&gt;$ 20 mm</td>
<td>Strong (Sensitive)</td>
</tr>
<tr>
<td>16 – 20 mm</td>
<td>Medium (Intermediate)</td>
</tr>
<tr>
<td>10 – 15 mm</td>
<td>Weak (Resistant)</td>
</tr>
<tr>
<td>$&lt;$ 10 mm</td>
<td>None</td>
</tr>
</tbody>
</table>

The results of the screening study for the antibacterial activity of Ulin fruit extract (*Eusideroxylon zwageri*) against *Salmonella typhi, Escherichia coli, and Staphylococcus aureus* bacteria can be seen in Table III.

**Table III. Average diameter (mm)**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Concentration (mg/ml)</th>
<th>Positive control</th>
<th>Negative control</th>
<th>Sterile aquadest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>10</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>15.01</td>
<td>17.78</td>
<td>19.07</td>
<td>17.64</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>3.27</td>
<td>10.27</td>
<td>12.57</td>
<td>17.09</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>6.63</td>
<td>11.99</td>
<td>12.31</td>
<td>16.78</td>
</tr>
</tbody>
</table>

The results of the screening study for the antibacterial activity of Ulin fruit extract (*Eusideroxylon zwageri*) against *Salmonella typhi, Escherichia coli*, and *Staphylococcus aureus* bacteria can be seen in Table III.

The results of the antibacterial activity screening were determined using a caliper. The results were indicated by the presence of an inhibition zone or clear zone around the wellbore in a petri dish containing Ulin fruit extract (*Eusideroxylon zwageri*) and positive controls Amoxicillin and Fosfomycin, whereas in the negative control sterile distilled water no inhibition zone was formed around the wells in the petri dish.

The average diameter of the inhibition zone obtained from testing 3 variations in the concentration of Ulin fruit extract (*Eusideroxylon zwageri*) was a concentration of 5 mg/ml, 7 mg/ml, and 10 mg/ml while the concentrations of 1 mg/ml and 3 mg/ml did not have obstacles zone. The results of the diameter of the inhibition zone against *Salmonella typhi, Escherichia coli*, and *Staphylococcus aureus* were 15.01 mm, 3.27 mm, 6.63 mm (concentration 5 mg/ml), 17.78 mm, 10.27 mm, 11.99 mm (concentration 7 mg/ml), 19.07 mm, 12.57 mm, 12.31 mm (concentration 10 mg/ml). The results of this study are reinforced by previous research on the Ulin plant, in the study of Wila et al. (2018).
showing that the ethanol extract of the bark of the Ulin plant against Salmonella typhi bacteria has an inhibition zone diameter of 14.33 mm with a weak barrier response category and against Escherichia coli bacteria of 24.33 mm with a strong inhibition response category.

The results of the research on the diameter of the inhibition zone on Ulin fruit extract (Eusideroxylon zwageri) were also strengthened by the research by Mariani et al. (2020) methanol extract of Ulin plant leaves with a concentration of 15 mg/ml against Salmonella typhi bacteria had an inhibition zone diameter of 12.33 mm. with a weak inhibitory response category, a concentration of 200 mg/ml against Escherichia coli has an inhibition zone diameter of 22.67 mm with a strong inhibitory response category, and a concentration of 15 mg/ml against Staphylococcus aureus has an inhibition zone diameter of 9.67 mm with a response category no obstacles.

In the positive control Amoxicilin the diameter of the inhibition zones obtained against Salmonella typhi, Escherichia coli, and Staphylococcus aureus were 17.64 mm, 17.09 mm, 16.78 mm, respectively, with a moderate response category. While the positive control Fosfomycin against Salmonella typhi, Escherichia coli, and Staphylococcus aureus were 20.15 mm, 11.94 mm, 10.90 mm, respectively, with weak to strong inhibitory responses (Greenwood 1995). In the negative control using aquadest, no inhibition zone was formed, this is in accordance with the research of Henaulu (Henaulu and Kaihena 2020) which showed that there was no response to the growth inhibition of the test bacteria that was dripped with aquadest. This is due to the neutral nature of the aquadest compound, so it does not have an effect on bacterial growth.

(MIC) Minimum Inhibitory Concentration and (MBC) Minimum Bactericidal Concentration

In this study, the antibacterial activity test of Ulin fruit extract (Eusideroxylon zwageri) to determine the Minimum Inhibitory Concentration against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria was carried out using concentrations that previously had an inhibitory zone, namely Ulin fruit extract with a concentration of 5 mg/ml, 7 mg/ml, and 10 mg/ml, while those that did not have an inhibitory zone, namely Ulin fruit extract with concentrations of 1 mg/ml and 3 mg/ml were not continued to test the inhibitory power. Inhibition test (MIC) was carried out using the liquid dilution method and the results were observed visually. In the study of the antibacterial activity test to see and determine the Minimum Bactericidal Concentration (MBC) of Ulin fruit extract (Eusideroxylon zwageri) against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria using extracts that previously started from those with a MIC value of 5 mg/ml then concentrations of 7 mg/ml and 10 mg/ml. The results of the bactericidal power (MBC) will be observed visually and count the number of colonies using a colony counter.

The results of observations MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) Ulin fruit extract (Eusideroxylon zwageri) against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria can be seen in Table V.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Concentration (mg/ml)</th>
<th>MIC (Minimum Inhibitory Concentration)</th>
<th>MBC (Minimum Bactericidal Concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replication</td>
<td>p-value</td>
<td>Replication</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>5</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>10</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

Notes:
C : Clear or there is a minimum inhibition marked by a clear test tube
GB : Grow Bacteria or no minimum bactericidal power marked by the growth of bacteria
ª : the significance value of the One Way Anova test.
On the data from the Minimum Inhibitory Concentration (MIC) test, statistical tests were carried out using parametric statistical tests using One Way Anova. Based on the results of statistical tests between the concentration variation groups of 5 mg/ml, 7 mg/ml, and 10 mg/ml with a positive control which can be seen in Table VII, it is known that there is no significant difference with a significance value of 1,000 (p>0.05). Therefore, it can be stated that the concentration variation starts from the concentration that has the Minimum Inhibitory Concentration (MIC) of Ulin fruit extract (Eusideroxylon zwageri) which is 5 mg/ml, 7 mg/ml, and 10 mg/ml with positive control having no difference against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria.

Antibacterial activity testing was continued to determine the bactericidal value or Minimum Bactericidal Concentration (MBC) of Ulin fruit extract (Eusideroxylon zwageri) against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria due to the inhibitory power of Ulin fruit extract.

The lowest concentration of the sample that did not show any bacterial growth in the petri dish was the value of bactericidal power or Minimum Bactericidal Concentration. Based on Table VIII, it can be seen that the extract of Ulin fruit (Eusideroxylon zwageri) did not have a bactericidal power value or Minimum Bactericidal Concentration (MBC) against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria in all variations in the concentration of extract samples. This was indicated by the presence of bacterial growth on solid media in petri dishes at all variations in the concentration of the extract that could be seen visually and counted using a colony counter. In contrast to the results of research by Mariam et al. (2020) which showed the results of the Minimum Bactericidal Concentration (MBC) test from the extract of the bark of the Ulin wood tree were positive to have a minimum bactericidal power against the bacterium Aggregatibacter actinomycetemcomitans which had a
Gram-negative strain at a concentration of 20%. While the research on the bark extract of the Ulin plant, Ulin wood extract, and the leaves of the Ulin plant (Eusideroxylon zwageri) against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria with a concentration of 1-15 mg/ml proved to have inhibitory power but the research was not continued to the test. Minimum Bactericidal Concentration (MBC) because the inhibitory results obtained are in the medium-strong category (Darussalam 2017; Wila et al. 2018; Mariani et al. 2020). The difference in the results of the Minimum Bactericidal Concentration (MBC) test with the results of the Minimum Bactericidal Concentration test by Mariam (Mariam et al. 2020) is because the bacteria used are different and the concentration of the Ulin plant bark extract (Eusideroxylon zwageri) used is higher than the concentration used in this study so that in this study there was no MBC. This study used the lowest concentration because it was the first study for the antibacterial activity of Ulin fruit extract (Eusideroxylon zwageri).

In this study the extract of Ulin fruit (Eusideroxylon zwageri) did not have a Minimum Bactericidal Concentration value against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria. The value of bactericidal power that was not found against Salmonella typhi and Escherichia coli bacteria was probably caused by Salmonella typhi and Escherichia coli bacteria which are Gram-Negative strains having complex cell wall structures. Gram-negative bacteria generally have a fairly high natural resistance due to the presence of an outer membrane consisting of two layers of phospholipids and a lipopolysaccharide membrane. This causes natural antibacterials to only pass through the outer membrane in small amounts, so that antibacterial compounds will be able to penetrate but it takes a long time (Mariani et al. 2020). The value of bactericidal power was also not found against Staphylococcus aureus bacteria which had Gram-positive strains. Gram-positive bacteria have very thick cell walls consisting of peptidoglycan which provides rigidity to maintain the integrity of bacterial cells (Darussalam 2017). It’s possible that the extract of Ulin fruit (Eusideroxylon zwageri) cannot penetrate the cell wall of Staphylococcus aureus bacteria which is very thick so that in this study there is no minimum bactericidal power. The ability of Ulin fruit extract (Eusideroxylon zwageri) in inhibiting the growth of bacteria that cause human infection Salmonella typhi, Escherichia coli, and Staphylococcus aureus due to the content of secondary metabolites contained therein such as alkaloids, tannins, flavonoids, phenolics, terpenoids, and steroids which have the ability to act as antioxidants antibacterial (Faisal et al. 2021). These secondary metabolites in plants play an important role as antibacterial.

Antibacterial has a way of working against Gram-Positive and Gram-Negative bacteria which consists of five categories, namely inhibiting cell walls, inhibiting protein synthesis, inhibiting nucleic acid synthesis, inhibiting folic acid synthesis, and causing disruption of the cytoplasmic membrane (Mahdiyah et al. 2021). The presence of a minimum inhibitory concentration of Ulin fruit extract (Eusideroxylon zwageri) against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria may occur because the administration of Ulin fruit extract (Eusideroxylon zwageri) has a mechanism of action by inhibiting cell wall synthesis. This is reinforced by research according to Darussalam (2017) which states that damage to bacterial cells by Ulin wood extract against Staphylococcus aureus bacteria is inhibition of the cell wall and Ulin leaf methanol extract can penetrate into the peptidoglycan and penetrate the outer membrane of the Salmonella typhi and Escherichia coli bacterial cell wall20. This is based on the presence of flavonoids and alkaloids which are secondary metabolites found in extracts of wood, leaves, and fruit of Ulin (Eusideroxylon zwageri) (Darussalam 2017; Mariani et al. 2020; Faisal et al. 2021).

This research has never been done before. Based on the results of the study, the antibacterial activity of Ulin
fruit extract (Eusideroxylon zwageri) had inhibitory power, but did not bactericidal bacteria Salmonella typhi, Escherichia coli, and Staphylococcus aureus. The ability of inhibition is due to the presence of secondary metabolites such as alkaloids, flavonoids, steroids, triterpenoids, phenols, saponins, tannins (Faisal et al. 2021). Based on the results obtained, Ulin fruit extract can be used as an alternative in the treatment of infections in humans, especially infections caused by the bacteria Salmonella typhi, Escherichia coli, and Staphylococcus aureus, because Ulin fruit extract has secondary metabolite compounds that can be used as antibacterial with bacteriostatic properties. In accordance with the results of determining the Minimum Inhibitory Concentration (MIC) of Ulin fruit extract (Eusideroxylon zwageri). Secondary metabolite compounds contained in Ulin fruit extract (Eusideroxylon zwageri) have similar mechanism of action as antibacterial, for example, Fosfomycin and Amoxicillin. However, Ulin fruit extract based on this study did not have the ability to kill so that it could not be used as a bactericidal antibacterial.

**KESIMPULAN**

Based on the results of the research on the antibacterial activity of Ulin fruit extract (Eusideroxylon zwageri) against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria, it can be concluded that Ulin fruit extract (Eusideroxylon zwageri) has the ability as an antibacterial against Salmonella typhi bacteria with the highest inhibition zone of 19.07 mm category moderate inhibition, Escherichia coli with the highest inhibition zone of 12.57 mm was in the weak inhibition category, and Staphylococcus aureus with the highest inhibition zone 12.31 mm in the weak inhibition category, which was in accordance with the results of antibacterial activity screening. Ulin fruit extract (Eusideroxylon zwageri) has the ability to inhibit or Minimum Inhibitory Concentration (MIC) against Salmonella typhi, Escherichia coli, and Staphylococcus aureus bacteria each at a concentration of 5 mg/ml with a significance value in the One Way Anova Test 1,000 (p> 0.05) which means there is no significant difference, but does not have the ability to kill or Minimum Bactericidal Concentration (MBC) against Salmonella typhi, Escherichia coli, and Staphylococcus aureus.

**UCAPAN TERIMA KASIH**

Thank you to Sari Mulia University Banjarmasin where this research to place.

**REFERENSI**


