POTENCY OF MAKASAR FRUIT EXTRACT *(Brucea javanica* L. Merr*)* AS AN ANTIBACTERY OF *Escherichia coli*

Ayu Wulandari[1)], Abdul L. Mawardi[2)], Marjanah[2)]
[1,2)]Biology Education FKIP Universitas Samudra
email: ayuwuland.bio27@gmail.com

Abstract

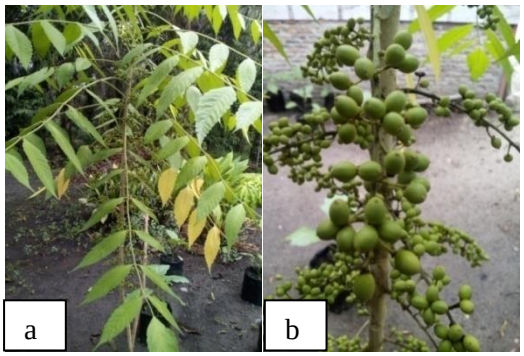
The fruit of Makasar (Brucea javanica L. Merr) is a shrub that grows wild in forest areas and is also planted as a hedge plant. This fruit contains antibacterial compounds that inhibit the growth of Escherechia coli bacteria. This study aims to determine the potential for extracts and to determine the concentration of the fruit extract of Makasar (Brucea Javanica L. Merr) which has the potential as antibacterial Escherichia coli. Makasar fruit extract (Brucea javanica L. Merr) was obtained by maceration method using 96% ethanol as solvent. This study uses the disc diffusion method. The parameter measured is the size of the estrak inhibition zone. The results analisis Oneway ANOVA of the research on the fruit extract of Makasar (Brucea javanica L. Merr) have the potential as an antibacterial agent for Escherechia coli as evidenced by the value of $F_{count} > F_{table}$ (28.370 > 2.85). The maximum zone of inhibition was at the concentration of P6 (15.33) and the zone of minimal inhibition was at the concentration of P1 (7.33). It can be concluded that the fruit extract of Makasar (Brucea javanica L. Merr) has antibacterial potential with strong criteria at P6 (15,33), P5 (14,47), P4 (14,03), P3 (13,57), and for moderate criteria at P1 (7,33) and P2 (9,17).

Key words*: Antibacterial, Escherechia coli, estract, makasar fruit (Brucea javanica L. Merr).*

1. INTRODUCTION

Indonesia is a country that has tropical forests with the second-highest biodiversity after Brazil. The wealth of natural plants is as much as 30,000 plant species from a total of 40,000 plant species in the world and 940 plant species including medicinal properties (Sunanda et al., 2020 in Masyhud 2010). The use of plants as traditional medicine has long been carried out by the people of Indonesia to deal with various health problems. One of the plants that have been used by the community is the Makasar fruit (Brucea javanica L. Merr)

The fruit of Makasar (Brucea javanica (L.) Merr) is a shrub that grows wild in forest areas and is also planted as a hedge plant. This plant grows at an altitude of 1-500 meters above sea level, has an upright, chronic stem, about 1-5 meters high, and has yellow fine hair on the trunk (Damayanti, 2008). The fruit is round and black with a thin seed coat. Makasar fruit seeds are round and white (Damayanti, 2008; Hidayat et al., 2015). The fruit contains essential oils, bruseral, breusealin, yatanosida, brusatol, brusiene, bruseosida, bitter substances, and fatty oils (Damayanti, 2008; Ifora 2019), there are also alkaloids, saponins Tammi, 2015, tannins, and steroids (Tammi, 2015; Helmi, 2015). Based on research that has been done, Makasar fruit has potential as an antibacterial.



Picture 1. a. Tree dan b. Fruit (*Brucea javanica* L. Merr) (Source: Researcher's primary data)

In daily life, humans cannot be separated from physical contact with the environment, the more the earth ages, the more health problems that attack. Health problems in humans can be caused by bacteria, one of which is *Escherichia coli* (*E. coli*). *Escherichia coli* bacteria are gram-negative bacteria that normally live in the

human intestine, but they do not rule out becoming pathogenic bacteria when they leave their habitat (Jawetz et al, 2007).

Escherichia coli bacteria is a cause of an acute diarrheal disease that can affect all ages. Diarrhea is a condition of defecating more than 3 times a day with the consistency of loose or soft stools (Fatkhur et al, 2016). Diarrhea can be caused by infection with microorganisms and non-infection. Most of the diarrhea is diarrhea caused by infection with microorganisms from viruses, bacteria, and parasites (Timpin, 2016; Abidin, 2018).

Based on this background, researchers are interested in researching the potential of Makasar fruit extract (Brucea Javanica L. Merr) as an antibacterial for *Escherichia coli*. This study aims to determine the inhibitory potential of Makasar fruit extract and the effective concentration of Makasar fruit (Brucea Javanica L. Merr) to inhibit the growth of *Escherichia coli* bacteria.

2. RESEARCH METHOD

Place and time of research

The research was conducted at the Microbiology Laboratory of FMIPA University of North Sumatra in July-August 2020

Type of research

This research is a laboratory experimental study using a completely randomized design (CRD). The study design consisted of 8 groups with 3 repetitions.

Tool

The tools used are analytical balance, incubator, petri dish, caliper, test tube, blender, autoclave, rotary evaporator, sterile cotton bud, bunsen, matches, stirrer, filter paper, Erlenmeyer, jar with lid, glass funnel, measuring cup, markers. , micropipette.

Material

The material used is 300 grams of makasar fruit taken from Bagan Toreh, Kec. Torgamba, Kab. Labusel, MHA, *Escherichia coli* bacteria from the USU FMIPA microbiology laboratory, 96% ethanol, aqua dest, aluminum oil, amoxicillin 25 μg, blank test disc.

Estract making

Makasar fruit powder (Brucea javaniva L. Merr) was weighed as much as 300 grams and put into a maceration container, the powder was then soaked in 3000 ml of 96% ethanol solvent. The maceration container is closed and stored for 5x24 hours, put in a place without exposure to sunlight while stirring occasionally, then filtered, separated between the pulp and the filtrate. Then the filtrate is evaporated or concentrated with a rotary evaporator at a temperature of 40-50^{o}C until a thick extract is obtained (Jacob et al, 2020).

Antibacterial Activity Test

1. Make a specific solution concentration test
   The test solution was made with a concentration of 15%, 30%, 45%, 60%, 75%, and 90% by adding sterile distilled water as a solvent. For negative control treatment using distilled water as a solvent and positive control using Amoxycillin.
2. Create Mueller-Hilton Agar Media
   34 grams of MHA was dissolved in 1000 mL of distilled water then heated and homogenized using a heating device and magnetic stirrer. MHA media must be completely homogeneous, visible from the clear yellow color, which indicates that MHA has been well mixed with distilled water. Before being used, the media is sterilized first using an autoclave at a temperature of 121^{o}C with a pressure of 1 atm for 15 seconds (Wahyuni, 2014: 22).

3. Antibacterial Potential Test
   Isolate bacteria aged 1 x 24 hours as much as 2-3 ose needles and immersed it in liquid nutrient then homogenized. Then the bacterial solution has been standardized and then smeared on the Mueller-Hilton Agar growth medium with an even streak technique. Then test the empty discs that have been soaked for 15 minutes in each stock of the Makassar fruit extract concentration, which is on the surface to make it hygienic. The media that had been made were incubated into an incubator at 37^{o}C for 24 hours.

Measuring the diameter of the inhibition zone for the growth of Escherichia coli bacteria at each concentration formed by using a caliper.

Data Analysis of Research Test Results

Analysis of antibacterial potency test data on zone diameter inhibits the growth of Escherichia coli bacteria using One Way ANOVA test analysis and expression with BNJ (Honest Significant Difference).

3. RESULT AND DISCUSSION

As for the potential antibacterial inhibition power of Makasar fruit extract (Brucea javanica L. Merr) against *Escherchia coli* by measuring the inhibition zone formed around the disc, it can be seen that the inhibition diameter and inhibition criteria in each treatment group (Davis, 1971; Susanto et al., 2012), in table 1.

Table 1. Antibacterial potential of Makasar Fruit Extract (Brucea javanica L. Merr)

Concentration Extract	Average Diameter Inhibition Zone (mm)	Inhibition Criteria
P0(Aquadest)	0	No drag zone
P1 (15%)	7,33	Moderate
P2 (30%)	9,17	Moderate
P3 (45%)	13,57	Strong
P4 (60%)	14,03	Strong
P5 (75%)	14,47	Strong
P6 (90%)	15,33	Strong
P7 (*Amoxicylin*)	11,5	Strong

The results of the One Way ANOVA test analysis show that there is a potential for extracting fruit of Makasar (Brucea javanica L. Merr) based on the formed inhibition zone. The results of the One Way ANOVA test were obtained with Fcount> Ftable (28.370> 2.85), then the hypothesis (Ha) was accepted and the null hypothesis (Ho) was rejected at the 5% level, so the difference in the concentration of extracts from Makasar fruit (Brucea javanica L. Merr) had potential inhibition against *Escherichia coli* bacteria. Therefore, it was continued with the Honest Real Difference Test (BNJ). The results of the Honest Real Difference Test (BNJ) can be seen in table 2.

Tabel 2. The Results Honest Real Difference Test (BNJ)

Concentration	Mean ± SD
P1 (15%)	$0,86^{a} \pm 0,009$
P2 (30%)	$0,96^{ab} \pm 0,050$
P3 (45%)	$1,13^{cd} \pm 0,044$
P4 (60%)	$1,14^{cd} \pm 0,051$
P5 (75%)	$1,15^{cd} \pm 0,029$
P6 (90%)	$1,18^{d} \pm 0,046$
P7 (*Amoxycilin*)	$1,06^{bc} \pm 0,017$

Information:

Different letter superscript shows significantly different at the significant level α0,05

Discussion

The results showed that the fruit extract of makasar (Brucea javanica L. Merr) has potential as a natural antibacterial against *Escherichia coli* bacteria. The largest zone of inhibition is at the concentration of P6 (90%), then followed by the zone of inhibition at P5 (75%), P4 (60%), and P3 (45%) with the criteria of a strong inhibition zone. P7 (Amoxycillin) positive control treatment also had an inhibition zone with strong criteria and was greater than the concentrations of P1 (15%) and P2 (30%) which had moderate inhibition zone criteria.

The ability of the fruit extract of makasar (Brucea javanica L. Merr) to inhibit bacterial growth is due to the active compounds contained in the extract. The

active compound has a polarity value where the material it contains will determine whether it is easy to absorb the compound into the cell. Antibacterial active ingredients can interfere with physiological processes and block the formation of bacterial cell components such as cell wall synthesis, cytoplasmic membranes, protein synthesis, and nucleic acid synthesis (Purwanto, 2015 in Soebandrio, 1995).

The active compounds possessed by ripe fruit extract of makasar(Brucea javanica L. Merr) from the results of phytochemical tests are saponins, tannins, flavonoids, alkaloids, and terpenoids. These compounds inhibit the growth of *Escherichia coli*, each compound has a different mechanism for inhibiting bacterial growth. Saponin compounds are antibacterial by damaging/breaking down the cell membrane, the damage to the cell membrane causes important substances to leave the cell and can also prevent the entry of important materials (Karlina et al, 2013; Monalisa, 2011). Flavonoids are antibacterial by inhibiting nucleic acid synthesis, inhibiting cytoplasmic membrane function, and inhibiting energy metabolism. Flavonoid compounds cause damage to cell wall permeability (Ilvani et al, 2019). Terpenoids have a working mechanism to react with porin (transmembrane protein) on the outer membrane of the bacterial wall, forming a strong polymer bond that causes porin damage (Supriatno, 2018).

While tannin compounds inhibit bacterial growth by coagulating bacterial protoplasm and binding to proteins so that cell wall formation will be inhibited, the tannin inhibition mechanism is using cell walls that have been lysed due to saponins, flavonoids, and terpenoids compounds, so that tannin compounds can easily enter cells. bacteria and coagulase of bacterial protoplasm (Karlina et al, 2013). Alkaloids act as antibacterials by disrupting the peptidoglycan components of bacterial cells, so that the wall layer is not formed completely and can bind to DNA causing failure of protein synthesis (Alfiana, 2016; Ilfani et al, 2019).

The antibacterial ability produced by makassar fruit extract (Brucea javanica L. Merr) is very potential to be used as an antibacterial agent. In this study, researchers used 96% ethanol solvent because it did not contain high toxicity or was not toxic. Ethanol has a low boiling point and tends to be safe (Wahyuni, 2014). The role of ethanol solvent is to dissolve almost all components, both polar, semi-polar, and non-polar (Purwanto, 2015).

The results of the One Way ANOVA test in the test group (sample extract) indicated that there were significant differences between treatments. The results of the inhibition zone measurement show that Fcount> Ftable (28.370>2.85). Based on the results of the BNJ further test, it can be seen that the makasar fruit extract with concentrations of P1, P6 shows a very significant difference. Concentrations of P1, P2, and P7 were not significantly different, while those in P3, P4, P5, and P7 also showed no significant difference. The test results showed that the makasar fruit extract (Brucea javanica L. Merr) had a different inhibition zone size.

Amoxycillin as a positive control was significantly different from the makasar fruit extract treatment (Brucea javanica L. Merr). Amoxycillin has an inhibition zone diameter of 11.47 mm with strong inhibition zone criteria because Amoxycillin is a semi-synthetic penicillin compound with a broad-spectrum antibacterial activity that is bactericidal and effective against several negative bacteria such as *Escherichia coli* (Krisna, 2013 in Mutschler, 1991).

The size of the inhibition zone is not only affected by concentration. The difference in the size of the inhibition zone for each concentration can be caused by the size of the concentration, the amount of active ingredient contained in the extracted material, the diffusion rate of antibacterials into the medium during incubation, environmental pH, media components, incubation time and metabolic activity of microorganisms (Purwanto, 2013). 2015).

4. CONCLUSION

Makasar fruit extract (Brucea Javanica L. Merr) has potential as an antibacterial agent for *Escherichia Coli* with Fcount> Fable, namely (28.370> 2.85). The

concentration of Makasar fruit extract (Brucea Javanica L. Merr) has the potential as an antibacterial agent for *Escherichia Coli* at concentrations of P1, P2, P3, P4, P5, P6 with strong bacterial inhibition criteria at P6, P5, P4, P3, while the criteria are moderate at P2 and P1. Further research is needed to test the antibacterial activity with the dilution method to determine the Minimum Inhibitory Concentration (MIC) and the Minimum Killing Level (MBC) against Escherichia coli bacteria.

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