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Anti-Bacterial Effectiveness Test of Basil Leaf Extract *(Ocimum basilicum L.)* Against *Escherichia coli*

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Abstract

Testing the effectiveness of basil leaf extract against bacteria *Escherichia coli* aims at finding out the ability of basil leaf extract to kill or to inhibit *Escherichia coli*. This research is an experimental laboratory research with extraction method, which was conducted at the Biology Laboratory of Muhammadiyah University of Bulukumba. The data analysis technique used is a replication technique with different concentrations, namely concentration of 40%, 60%, and 80%. The basil leaves were extracted using maceration method with 96% of ethanol solvent. Antibacterial tests were carried out by treating bacteria with three types of concentrations, namely concentrations of 40%, 60%, 80%, positive control, and negative control. The results of the antibacterial test show that crude extract of the basil leaves has the ability to inhibit *Escherichia coli* at the concentration of 40%, 60%, and 80% with each halo zone diameter of 1.96 cm, 1.11 cm, 0.89 cm, 1.50 cm, 0.00 cm, and the extract most strongly inhibitedthe *Escherichia coli* at the concentration of 40% with halo zone diameter of 1.96 cm.

Keywords: Basil leaves, Escherichia coli, maceration, halo zone.

A. Introduction

Indonesia is a country that has abundant wealth. Almost all types of plants can be grown in the country. Most have been used by the people of Indonesia as traditional medicine, especially in rural areas which are still rich in plant diversity. One of the traditional medicinal plants that has many benefits is basil *(Ocimum basilicum L)*. Basil has long been used to treat various diseases such as flatulence or colds, fever, breast milk, rheumatism, canker sores and also as an antifungal (Gunardi, et al, 2010). Bacteria that are usually found in the digestive tract of humans and warm-blooded animals, and part of the microflora are *Escherichia coli. Escherichia coli* commonly abbreviated as *Escherichia coli* is a heterotrophic bacterium that obtains food in the

form of organic substances from its environment because the bacterium it self cannot make the organic substances it needs (Angelina, et al, 2015). *Escherichia coli* is a type of bacterium that usually lives in the intestines of humans and animals. It is also a gram negative, short rod-shaped, facultative anaerobic, and non-sporing bacterium, and commonly found around us (Gomes et al, 2011). Most types of the *Escherichia coli* are harmless and even help keep your digestive tract healthy. Even so, there are certain types of *Escherichia coli* that can cause severe stomach cramps, bloody diarrhea, and kidney failure. Also, *Escherichia coli* is the most common bacterium that causes gastrointestinal infections.

The highincidence is due to unhygienic food, drink and water consumed, and influenced by the cleanliness of the surrounding environment (Octaviani, 2007). Based on the mechanism of *Escherichia coli* infection in causing disease, Nataro et al, (2004) divides *Escherichia coli* into 5 groups, namely: *Enteropathogenic Escherichia coli* (EPEC), *Enterotoxigenic Escherichia coli* (ETEC), *Enterohemorrhagic Escherichia coli* (EHEC), *Enteroaggregative Escherichia coli* (EaggEC) and *Enteroinvasive Escherichia coli* (EIEC). *Escherichia coli* also has several antigens that play a role in pathogenesis such as somatic antigens, flagella, capsular, fimbriae, enterotoxins, and verotoxins. Verotoxigenic Escherichia coli (VTEC) is one of the strains that is capable of damaging Vero cells (African Green Monkey Kidney) because it produces verotoxin (Konowalchuk et al., 1977).

B. Literature Review

The traditional medicinal plants that can function as anti-bacterial is basil. Basil is a short-stemmed plant that grows in various parts of the world. Basil leaves come from the division of spermatophyta, dicotyledonous class, order amaranthaceae, genus ocimum and species *Ocimum basilicum L.* The shape of basil leaves is simple and cross facing each other with pointed leaf tips and petiole length reaching 2 cm. The leaves are elliptical in shape with leaf length reaching 5 cm and leaf width reaching 2.5 cm (Ardiana D, et al, 2013). Morphology basil plants have morphological features, namely erect branched stems, 0.6-0.9 m in height, with green or sometimes purple stems and branches. Basil (*Ocimum basilicum L*) leaves can reach 2.5 - 5 cm or more in length. The leaves are also ovoid and more or less toothed.

The petiole length reaches 1.3 - 2.5 cm. The leaves have many points like oil glands that secrete a very fragrant essential oil. Its supporting stalk is shorter than the petals, ovate and pointed. The petals have 5 mm long. The lower lip with two middle teeth is longer than the upper lip. Corolla reaches 8-13 mm long, with white, pink or purplish color. The upper filament of the stamens is slightly toothed (Bilal, et al., 2012).

Flavonoids are compounds that are widely found in green plants and it is the largest natural polyphenolic compounds, especially in the form of glycosides both as C- and O glycosides. Polyphenols are polar compounds with more than one benzene core. Alcolloids are a group of secondary compounds found in higher plant groups that have a basic structural arrangement in the form of a nitrogenous base which is one or two nitrogen atoms, usually in combination, as part of a cyclic system. Alcolloids are usually colorless and mostly crystalline but a few of them are liquid at room temperature (Hariana, 2008).

Based on some of the previous related researches that have been done on basil, it was found out that basil was efficacious as Analgesic, Anti-Amnesiac, and Nootropic, Anthelmintic, Anti-Bacterial, Anti-Cataract, Anti-Fertility, Anti-Hyperlipidemia, Anti-Inflammation, Anti-Malaria, Anti-Lipidperoxidative, Anti-Oxidant, Anti-Stress, Anti-thyroid, Anti-ulcer, Chemoprotective, Skin Disease, Diabetes, Immunomodulator, Radioprotective, Hypoglycemic Activity, Hypotensive Activity, and Anti-Cancer (Singh, et al. 2012). In addition, Maryati (2007) states that basil leaf oil *(Ocimum basilicum L.)* has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with a minimum killing concentration of 0.5 and 0.25% v/v. From the KBM price obtained in this study, it can be assumed that the essential oil of basil leaf is potent against *Escherichia coli.*

Escherichia coli is an opportunistic bacterium that is commonly found in the large intestine (colon) of humans and is a normal flora of the large intestine. *Escherichia coli* can cause primary infection in the large intestine so that it can cause diarrheal disease (Gillespie, SH and KB Bamford, 2000). The bacteria that most often causes infection through food is *Escherichia coli* (Febria et al, 2009). Infectious disease is one of the diseases that has been suffered by many Indonesian people for a long time. Currently infectious diseases can be treated using modern medicines such as antibiotics. The most common infectious disease suffered by the community is intestinal infections caused by S. aureus, *Escherichia coli* , *Salmonella typhi*, and *vibrio*

cholerae bacteria, while the cause of skin infection is *Staphylococcus aureus, Pseudomonas aeruginosa* and so on (Oktalia, 2009).

A previous research resultshows that using the flavonoid content of basil leaves (*Ocimum basilicum L.)*) can provide antibacterial effects against *Escherichia coli* , *Staphylococcus aureus*, and K. *pneumonia*. The research also shows that the combination of two flavonoid compounds in basil leaves, namely orientin and visenin, provides a synergistic (mutually reinforcing) antibacterial effect compared to the use of one of the two flavonoid compounds (Ali and Savita, 2012). Another research result also shows that there are indications showing that the combination of flavonoid compounds from basil leaves (*Ocimum sanctum*) has antibacterial activity, and it is necessary to test the antibacterial activity of ethanol extract of basil leaves against the growth of *Escherichia coli* and *Staphylococcus. aureus*. (Ali, H & Savita, D, 2012).

Escherichia coli is a type of bacteria that normally lives in the digestive tract of humans and healthy animals. *Escherichia coli* is a normal part of the human gastrointestinal flora. Under certain conditions, *Escherichia coli* causes diarrhea, urinary tract infections, pneumonia and meningitis in newborns and internal wound infections (Ariyanti et al, 2012). It is one of the inhabitants of the body. Transmission of *Escherichia coli* can occur through direct contact (contact, shaking hands and so on) and then through the mouth, but *Escherichia coli* can also be found scattered around us. Passive spread can occur through food or drink. Although E. coli is an indicator microorganism used in water analysis to test for contamination by faeces, its spread is not always through water, but is transmitted through hand-to-mouth activity or through passive transfer through food or drink.

Escherichia coli is a parasite in the digestive tract of humans and warm-blooded animals. In humans it sometimes causes enteritis, peritonitis, cystitis and so on. Bacterial life is not only influenced by external factors but on the other hand bacteria are able to affect environmental conditions, for example, can cause fever (heat) because it is infected by *Escherichia coli* bacteria in the gastrointestinal tract and causes prolonged diarrhea (Melliawati, 2009).

Certain strains of *Escherichia coli* are Gram-negative bacteria that cause many gastrointestinal infections in addition to Vibrio cholera and rotavirus. This kind of bacterium is transmitted through the fecal-oral route due to the low quality of individual (consumer) hygiene. In addition to Gram negative bacteria, Gram positive bacterial toxin such as *Staphylococcus aureus* which is thermostatic can also cause infectious diseases. *Staphylococcus. aureus* toxin plays a major role in increasing outbreaks of gastrointestinal infections due to food poisoning or food poisoning diseases. The toxin is produced by *Staphylococcus aureus* bacteria that enter and develop in food as a result of improper processing by food handlers. (Fazlisia et al, 2014).

The antibacterial effectiveness of basil leaf extract as a natural antibacterial can help people in inhibiting synthetic antibacterials that can cause side effects if used in the long term. This study used a sample of basil leaves using treatment concentrations, namely concentration of 40%, 60% and 80% which were carried out for three times of experiments. This research was conducted at the Biology Laboratory of the Muhammadiyah University of Bulukumba. This study used maceration method because it is suitable for the tools and materials available at the Biology Laboratory of the Muhammadiyah University of Bulukumba.

The purpose of this study was to determine the anti bacterial effectiveness of basil leaf extract *(Ocimum basilicum L.)* against *Escherichia coli* bacteria and to find out the most effective concentration in killing or in inhibiting *Escherichia coli* bacteria. Maceration is a simple presentation because it is done by soaking the simplicia powder in a liquid. The penetrating fluid penetrates the cell wall and enter the cell cavity which contains the active substance. The active substance dissolves and because of the difference in the concentration between the solution and the active substance inside the cell and outside the cell, the solution is pushed out.

This event repeatedly occurred 15 times, the contingency balance between the solution outside and inside the cell (Lukman, 2016).Percolation is the process of passing an organic solvent through the sample so that the solvent will unite the organic compound with the solvent. But the effectiveness of this process will only be greater for organic compounds that are highly soluble in the solvent used (Lukman, 2016). A previous relevant study wasconducted by Novia Ariani, et al (2020) with the research title'*Ethanol Activity Test of Basil Leaf Extract (Ocimum sanctum L.) Against Staphylococcus aureus in Vitro*'. The research results showed that basil leaf ethanol extract had activity in inhibiting the growth of *Staphylococcus aureus* bacteria in that the average diameter obtained from each treatment was 100% (10.08 mm), 80% (8.10 mm), 60% (6.49 mm), 40% (4, 29 mm), 20% (2.26 mm), and as a classification of antibacterial strength, it was found that the activity was strong at the concentration of 100%, it became

moderate at the concentration level of 80%-60% and weak at the concentration level of 40%-20%.

Another relevant research was also conducted by Maria Angelina, at al (2015) with the research title '*Antibacterial Activity Test of Basil Leaf Ethanol Extract (Ocimum sanctum L.) Against the Growth of Escherichia coli and Staphylococcus aureus bacteria*'to observe the inhibition zone thatwas carried out with the disc diffusion method. Based on the research results, it can beconcluded that basil leaf extract was bacteriostatic. The highest inhibition zone occurred atthe concentration of 100%, while the optimum treatment for growth inhibitors of *Escherichia coli* and *Staphylococcus* aureus was at a concentration of 80%. Based on the results of phytochemical tests, the ethanolic extract of *Ocimum* sanctum *L* has secondary metabolites, namely flavonoids, tannins, and essential oils that function as antibacterial. Also, the statistical results of Anova also showed a significant difference in the diameter of the inhibition zone of the extract treatment with positive control.

Furthermore, a relevant study was also carried by Solikhah & Nanik Wijayati (2016) with the title '*Antimicrobial Activity Test of Basil (Ocimum basilicum L.) Stem and Leaf Ethanol Extract*'. The research results showed that the antimicrobial activity of ethanolic stem extract showed no inhibitory activity at all. Also, in leaves with a concentration of 100% it gave the largest clear zone against *Staphylococcus* aureus and *Escherichia coli* bacteria with the Diameter of Inhibition Zone (DDH) of 16.75 mm and 14.94 mm, respectively. The compound analysis of ethanol extract of basil stems and leaves was carried out with FT-IR and GC-MS. The ethanol leaf extract compounds suspected of being antimicrobial are 2,6-octadiene-1,8-diol, exomethyl camphenilol, camphor, phytol, linalool oxide, cis geraniol and cis carveol.

C. Methodology

1. Research Design

This research is an experimental laboratory research with extraction method. The extraction method used in this research is meceration method with 96% of ethanol solvent. The crude extract of basil leaves dissolved in sterile distilled water was tested using three concentrations, namely at concentrations of 40%, 60%, and 80% into NA(Nutrient Agar) media treated with *Escherichia coli* bacteria. Then, the bacterial colonies were placed evenly on the NA (Nutrient Agar) media.

Then the paper disk that had been soaked for several hours in basil leaf extract was placed in a petri dish containing the test bacteria. After the incubation process for 1 x 24 hours till2 x 24 hours, the halo zone was observed during the incubation period. Further observations of basil leaf extract at a certain concentration can be done to know the effectiveness of basil leaf extract to kill or to inhibit the growth of *Escherichia coli* bacteria.

2. Instruments

The research instruments used in this study consist of tools and ingredients. The tools used are oven, incubator, autoclave, enkas, petri dishes, porcelain dishes, simpilisa bottles, extraction bottles, baker glass, measuring cups, tubes, reactions, Erlemeyer flasks, funnels, stirring rods, tweezers, scales, tray, sieve, tube rack, round loop, bunsen, horn spoon, caliper, blender, paper disk, stationery, fan, spray bottle, basket, refrigerator, stove, pot, scissors. The ingredients are basil leaves, *Escherichia coli* bacteria, sterile distilled water, distilled water, paper disk, detergent, water, 70% alcohol, NA media, NB media, 96% ethanol, silica gel, sterile syringe, label, food plastic, aluminum foil, tissue, cotton, ksteril, amoxilin, filter paper, spiritus.

In addition, the researcher also used observation sheet, namely observation of wet and dry sample collection, the process of sample extraction, the evaporation process, the process of making NA media, the process of preparing bacteria and the process of antibacterial test.

3. Technique of Data Analysis

The data analysis techniqueusedin this study is replication technique with different concentrations, namely concentration of 40%, 60%, and 80%. The plucked basil leaves were cleaned of adhering dirt and then washed with water, and then aerated in a place without sunlight. When dry, the basil leaves became samplesand were ready to be extracted. After that, the dried basil leaves were mashed with a blender. 20 grams of themashed basil leaves were dissolved in 100 ml of 96% ethanol solvent, then left for 24 hours in a place protected from the sun.

The data obtained from the observed parameters are data regarding the ability to kill or to inhibit bacterial growth from the extraction of the basil leaves against *Escherichia coli* by

measuring the halo zone formed during the incubation period for 1x24 hours to 2x24 hours. Then, 20 grams of NA was dissolved in 1 liter of sterile water with the help of heating until all ingredients were completely dissolved, then sterilized using an autoclave at 121°C for 15 minutes.

One bacterial colony was taken from the culture stock using a sterile ose needle which was planted on NA medium by scrapingobliquely. Then the bacterial colonies were incubated in a bacterial incubator at a temperature of 36-37°C for 24 hours. This rejuvenation was carried out for each of the test bacteria (*Escherichia coli).* NA medium was poured into a petri dish. While waiting for the media to harden, a crude extract concentration was made from the basil leaves with three types of concentrations, namely concentrations of 40%, 60%, and 80%. Bacterial inoculum was poured into 0.1 ml of NA medium and then flattened with a hockey stick. After that, the paper disk was inserted into three types of concentration 40%, 60%, 80% and soaked for 15 minutes. The paper disk was removed and placed on NA medium and then incubated for 1x24 hours to 2x24 hours to see the ability of the extract in killing or inhibiting bacterial growth.

D. Findings and Discussion

1. Findings

There are several methods that can be used in the extract of basil leaves, namely the maceration method, the percolation method, and the soxhletation method. This study used the maceration method because it is adjusted to the tools and materials available in the Biology Laboratory of Muhammadiyah University of Bulukumba.

This section discusses the research findings obtained from the research results on basil leaf extract which will be supported by the existing and relevant theories that correspond to the focus of the problem namely (1) the effectiveness of antibacterial basil leaf extract against (*Escherichia coli)* (2) what is the most effective concentration in killing or inhibiting (*Escherichia coli)*. Based on the results of extraction using 96% of ethanol solvent for 24 hours towards basil leaf symplisia, rough extract results are obtained as shown in Table 1;

Table 1. Results of basil extract (Ocimum basilicum L.)

Number of symplisia (grams)	Petry dish	Weight of extract (grams)			
		H0	H1	H2	H3
20 gr	I	0,64 gr	0,72 gr	0,69 gr	0,69 gr
	II	33 gr	0,84 gr	0,46 gr	0,46 gr
	III	74 gr	2 gr	1,26 gr	1,26 gr

The table 1 above shows the results of basil extraxt with 20 grams of simplisa and 70% alcohol, which were obtained from the direct observation. Based on the table, the extract's weightat the day when the simplisia was dissolved for the first time is clearly seen and day by day the weight decreases because the alcohol is volatile, so it produces extracts, and the treatment sat day 1, 2 and 3 each produces different extract weights.

Antibacterial test of coarse basil leaf extract (Ocimum basilicum L.)

Based on the antibacterial effectiveness test results, it is found that the basil leaf extract actively inhibited the growth of *Escherichia coli* obtained from the halo zone measurements as shown in Table 2 and Table 3.

Table 2. Measurement results of the 24-hour and 48-hour incubation halo zones

Concentration	Repetition						Average	
	1		2		3			
	24-jam	48-jam	24-jam	48--jam	24--jam	48--jam	24--jam	48--jam
Concentration 40%	1,26 cm	1,23cm	0,83 cm	0,88 cm	1,26 cm	1,12 cm	1,11 cm	1,07 cm
Concentration 60%	2,1 cm	1,37 cm	1,72 cm	1,5 cm	2,07cm	1,88 cm	1,96 cm	1,58 cm
Concentration 80%	1,02 cm	0,93 cm	0,95 cm	0,85 cm	0,72 cm	0,63 cm	0,89 cm	0,80 cm
K+			1,50 cm	0,84 cm			0,00 cm	0,84 cm
K -			0,00 cm	0,00 cm			0,00 cm	0,00 cm

Based on the measurement results of the 24-hour and 48-hour incubation halo zones, it is very clear that there are differences in the inhibitors of *Escherichia coli* growth. The results were obtained from the halo zone measurements by measuring the halo zone formed during the incubation period for 1x24 hours to 2x24 hours.

At the 40% concentration with 24 hours, it is seen that the inhibitor's size is 1.26 cm in the first repetition. In the second repetition, its size is 0.83 cm, and in the third repetitionit becomes 1.26 cm with an average value of 1.11 cm. While in 48 hours, the inhibitor's size becomes 1.23 cm in the first repetition. In the second repetition, its size is 0.88 cm, and in the third repetition it becomes 1.12 cm with an average value of 1.07 cm.

At the concentration of 60% with 24 hours,the inhibitor becomes 2.1 cm in replication 1. Inreplication 2 it becomes 1.72 cm, while in replication 3 its size becomes 2.07 cm with an average value of 1.96 cm. Otherwise,at the concentration of 60% with 48 hours, in the first repetition the inhibitor is 1.37 cm in size, in the second repetition it becomes 1.5 cm, and in the third repetition its size is 1.88 cm with an average value of 1.58 cm.

The most effective or deadly concentration in inhibiting Escherichia coli.

After the incubation process for 1x24 hours, the halo zone was measured on a medium. The measurements after the incubation of 2x24 hours were carried out in order to know the ability of the basil leaf extractto kill bacteria or simplyto inhibit the *Escherichia coli* growth.

Based on the halo zone measurement results with 24-hour incubation of basil leaf extraction,at the concentration of 60% with an average value of 1.96 cm, it is found out that the most effective concentration of inhibitory bacteria during 24-hour incubation was at the concentration of 60% and the lowest mean value at 24 hours incubation was 0.89 cm at 80% concentration.

Based on the halo zone measurement results during 48-hour incubation, from basil leaf extraction at a concentration of 60% with an average value of 1.58 cm, it is concluded that the most effective inhibitory bacteria concentration during 24 hours incubation is the concentration of 60% and the lowest mean value at 48 hours incubation is 0.80 cm on average at 80% concentration.

2. Discussion

This section discusses the research findings obtained from the test results of basil leaf extract *(Ocimum basilicum L.)* which is supported by the existing and relevant theories according to the focus of the problem, namely (1) The antibacterial effectiveness of basil leaf extract *(Ocimum basilicum L*.) against *Eschericia coli* (2) what is the most effective concentration in killing or inhibiting *Eschericia coli*. A research was carried out by Ali and Savita (2012). The reserach used the flavonoid content of basil leaves (*Ocimum* sanctum) to observe the antibacterial effect against *Eschericia coli*, *S. aureus*. The research results show that Visenin provides a synergistic (mutually reinforcing) antibacterial effect compared to the use of one of the two flavonoid compounds. Theseindicate that the combination of flavonoid compounds in basil leaves *(O. sanctum*) has antibacterial activity.

Thus, it is necessary to test the antibacterial activity of the ethanol extract of basil leaves against the growth of *Eschericia coli* and *S. aureus*. While this research was to test the antibacterial effectiveness of basil leaf extract (*Ocimum basilicum L*) against *Eschericia coli* and to find out the most effective inhibition concentration of *Eschericia coli*. From the research results on the effectiveness of basil leaf extract (*Ocimum basilicum L*.) against the growth of *Eschericia coli*. it can be assumed that basil leaf extract inhibits bacterial (bacteriostatic) growth, but does not kill bacteria (bacteriocidal) and basil leaf extract (*Ocimum basilicum L*.) is more effective in inhibiting bacteria at a concentration of 60%.

The research results are relevant to the research carried outby Angelina et al., (2015) which concluded that basil leaf extract showed antibacterial ability against *Eschericia coli* bacteria. The extract concentrationtreatment of 60% was the most effective concentration in inhibiting bacterial growth because it was not significantly different from the 80% concentration treatment. The similarities of the research conducted by Angelina et al (2015) with this study is that both basil leaves inhibit the growth of *Eschericia coli* bacteria. Other wise, the difference is that this study used a concentration of 60 percent to inhibit the growth of *Eschericia coli* bacteria, while the study of Angelina et al (2015) used a concentration of 70 percent to inhibit the growth of *Eschericia coli* bacteria.

The antibacterial effectiveness of basil leaf extract as a natural antibacterial that can help people in inhibiting synthetic antibacterials and cause side effects if used for a long time. This

study used samples of basil leaves with treatment concentrations, namely 40%, 60% and 80% concentration which were carried out for three trials.

The sampling time was at 09.00 WITA in the morning before the photosynthesis process occurred and the samples were stillfresh. The sampling was carried out in the morning because the basil leaves had not photosynthesized and the substances contained in the basil leaves were still complete. First, fresh basil leaves are picked, then sorted wet. Wet sorting was done to separate the impurities attached to the basil leaves. After the wet sorting process, the leaves were then washed with clean running water. After the washing process, the basil leaves were then aerated in a room that was protected from the sun. Drying aims to obtain simplicia that is not easily damaged by other microorganisms and the water content has been reduced so that it can be stored longer. This is in line with the assumption made by Lukman (2016) which states that the remaining water is simplistic in the water content. Certain substances can still be a medium for the growth of molds and other microorganisms so that the water content must be considered.

The first step is making dried simplicia samples using a blender. Pollination was done to reduce the particle size, so that the number of compounds contained in the simplicia can be bound by the solvent. Furthermore, it was sieved using a 40 mesh sieve. The use of 40 mesh is intended to obtain smoother simplicia results.

The second stage of dry simplicia samples was extracted by maceration method. The maceration was carried out with 96% ethanol solvent in a closed room to avoid the influence of the sun towards the stability of the compound to be taken. The maceration process was carried out for 24 hours and after that it entered the filtering stage which aims to separate the simplisa dregs from the solution. The filtering process used filter paper placed on a funnel.

The third stage is evaporation. The filtered maceration results were put into a porcelain dish. The evaporation was carried out to evaporate the solvent until only a thick extract remains. The evaporation was done by storing the maceration results in a glass cabinet, and to speed up the evaporation process, silica gel was added which functions to absorb the vapor produced by the solvent and this process requires a fan to help remove solvent vapor in the air.

The fourth stage is to determine media. The media used in this study are NA (Nutrient Agar) and NB (Nutrient media Broth) because these kind of media are commonly used in antibacterial test research. In addition, these media can also be over grown by all types of bacteria, including the test bacteria determined in this study, namely *Esherichia coli*.

The fifth stage of antibacterial test is basil leaf extract (*Ocimum basilicum L.*). For the next stage, 11 petri dishes containing DNA (Nutrient Agar) were confirmed to be uncontaminated, and then 0.1 ml of bacterial inoculum was given into the container petri dish using a sterile container slowly, so that the bacteria did not grow in the areas other than the media. Thus, a hockey stick was used to spread the media over the entire surface of the plate. After that, the concentration of thick basil extract was made with a concentration of 40%: 0.40 grams of thick extract with 60 ml of sterile distilled water plus a concentration of 60%: 0.60 grams of thick extract plus 40 ml of sterile distilled water. Also, concentration 80%: 0.80 gram thick extract was added with 20 ml of sterile aquadest, then positive control using amoxilin seeds 1 plus sterile aquadest until the amoxilin powder was completely submerged.

After the concentration was made, the disk paper was immersed in the concentration for 15 minutes. Each concentration was put into 4 sheets of disc paper. After that, the soaked disk paper was inserted into the media containing *Eschericia coli* bacteria. Each plate was filled with 1 sheet of paper disk and each concentration was made 3 times. After that, each cup was given a marker or label, then closed and labeled. The glass container is tightened using food plastic, then after all the processes were complete, the cup was put into the incubator and incubated for 1 x 20 hours and 2 x 20 hours.

The next process is to observe the halo zone after the incubation process for 20 hours and 40 hours. The formed halo zone was measured with a caliper. The counting process was carried out with 4 counts. It can be seen from the table 4.2 that at day 1the halo zone in size is 1.11 cm at a concentration of 40%, and at a concentration of 60% it becomes 1.96 cm. However, the size decreases and only reaches 0.89 cm at the concentration of 80%withK+= 1.50 cm, and K- = 0 .00 cm.

At day 2, it can be seen from the table 4.3 that at a concentration of 40% the size of halo zone formed is 1.07 cm, while it increases at a concentration of 60% and reaches 1.58 cm, but it deacreases and becomes 0.80 cm at the concentration of 80%, with K+ = 0.84 cm, and K - = 0.00 cm.

From the research results that have been obtained, there is a diameter of the halo zone in some treatments that inhibits bacterial growth (bacteriostatic) and does not kill bacteria (bacteriocidal). This can be seen from the size of the halo zone which decreases after the two-day incubation. Determination of the halo zone aims to determine the minimum concentration of basil leaf extract (*Ocimum basilicum L.)* in inhibiting the growth of the test bacteria. This is reinforced by the assumption of Melisa et al., (2015) which states that the halo zone around antimicrobial substances is an inhibitor of bacterial growth. The resulted halo zone only inhibits bacterial growth (bacteriostatic) and does not kill bacteria (bacteriocidal). This is indicated by the decreasing size of the halo zone after the logarithmic phase of bacteria.

E. Conclusion

Based on the results and discussion, it can be concluded that the effectiveness of basil leaf extract (*Ocimum basilicum L*.) can inhibit the growth of *Escherichia coli* bacteria. In addition, it can also be stated that the most effective or deadly bacterial inhibition was concentration of 60%.

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