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ANTIBACTERIAL EFFECTIVENESS OF EXTRACTS OF LIME (CITRUS AURANTIFOLIA SWINGLE) AND KAFFIR LIME (CITRUS HYSTRIX DC) LEAVES AGAINST ESCHERICHIA COLI

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ABSTRACT

The use of traditional medicine as alternatives has been used many times in Indonesia. The usage of lime and kaffir lime are several examples of traditional medicine. The researcher is interested in knowing the antibacterial effects between lime leaves extract, and kaffir lime leaves extract towards *Escherichia coli* ATCC 25922. This research is experimental research with a descriptive design. Lime leaves and kaffir lime leaves were extracted by maceration, and its sensitivity towards the growth of Escherichia coli ATCC 25922 was tested via *the Kirby Bauer* method with 10%, 30%, 50%, 70%, and 100% extracts. Test results show that lime leaves extract and kaffir lime leaves can inhibit the growth of *Escherichia coli.* The most excellent suspension was lime leaves extract at 100% concentration (13,25 mm). There was no significant difference between the inhibitory zone of lime leaves extract and kaffir lime leaves extract towards *Escherichia Coli.*

KEYWORDS: *kaffir lime leaves (Citrus hystrix DC), lime leaves (Citrus aurantifolia), Escherichia coli, Kirby Bauer method.*

INTRODUCTION

For a long time, herbs have been recognized and used for their pain relief and healing abilities, and today we still rely on the curative properties of plants for about 75% of our medicines. Over the centuries, people worldwide have developed their traditions to understand medicinal plants and their uses. Some of these traditional medicinal traditions and practices may seem strange and miraculous, and others seem rational and reasonable. It was done to overcome the disease and improve the quality of life.[1] However, despite the advancements and advantages of conventional medicine, it is clear that traditional medicine has a lot to offer. We tend to forget that for over 70 years, humans have relied almost entirely on plants to treat all sorts of ailments, from coughs and colds to life-threatening diseases like tuberculosis and malaria.[1]

Indonesia is a country with a rich biodiversity of medicinal plants. In Indonesia, traditional medicine is part of the nation's culture and has been widely used by the community for centuries ago. The natural wealth of plants in Indonesia includes 30,000 plant species from 40,000 plant species in the world. There are 940 species of medicinal plants, and this number is the number of medicinal plants in Asia.[2; 3] Traditional medicine treatment is one of the essential health programs and an alternative to meet the population's basic needs in the health sector. Besides being cheap and easy to obtain, traditional medicine has low side effects compared to conventional medicine. Thus, the body is more accessible to accept drugs from plants than chemical drugs. However, research on the application of medicinal plants in Indonesia is still very few and limited compared to other countries. It hampers the development of plants into medicine because of the lack of literature on chemical content and research on the medicinal plants' effects on humans. In order to increase the role of traditional medicine, especially medicinal plants, it is necessary to encourage efforts to identify, research, test and develop the efficacy and safety of a medicinal plant.[3; 4]

The use of traditional medicines led to the development of the Herbal Medicine Industry. The Indonesian herbal medicine industry is reported to have risen, marked by the increasing number of herbs used as essential ingredients for treatment, and has also been recommended by the Indonesian Doctors Association (IDI) and the Ministry of Health (DepKes) as medicine for patients. The Indonesian herbal medicine industry in 2018 is expected to grow by 10 per cent with a turnover of IDR 17 trillion. The herbal and traditional medicine industry nationally increases by an average of 5 per cent per year. In the previous year, it generated a turnover of around Rp. 15 trillion. Overall, the national drug industry can absorb as many as 15 million people. About 3 million people are absorbed in the herbal medicine

industry, which functions as medicine, around 12 million are absorbed in the herbal food, beverage, cosmetic, spa, and aromatherapy industries.[5; 6]

Some of the plants that have medicinal benefits are lime leaves and kaffir lime leaves. People usually use lime and kaffir lime fruit instead of the leaves themselves in their daily life. One of the benefits of the leaf itself is as an antibacterial. Antibacterial is a drug that can inhibit to kill pathogenic bacteria. The use of synthetic antibacterials can cause allergic reactions for unsuitable users. Thus, it is necessary to manufacture natural antibacterials derived from plants to be investigated.[7]

Lime and kaffir lime are shrubs that are widely grown and developed in Indonesia. The part used as a medicine other than the fruit and the leaves can also be used for high blood pressure (hypertension). The chemical constituents of lime leaves are alkaloids, terpenoids, tannins, saponins, steroids, flavonoids, and essential oils. The nutritional content in 50 grams of lime contains 44 grams of water, 24 mg of vitamin C, 11 mg of calcium, 9 mg of phosphorus, 0.2 mg of iron, 0.6 grams of carbohydrates, 0.1 grams of fat, 0.4 grams of protein, fibre, 1 g, and water 44 g. In addition to lime leaves, kaffir lime leaves are also antibacterial because they contain flavonoids, tannins, alkaloids, and essential oils.[8; 9; 10]

Essential oils are commonly used in the food, beverage, pharmaceutical, cosmetic, flavour and aroma industries. One of the bioactivities of essential oils is antibacterial. The activity of essential oils in inhibiting the growth or killing of bacteria is by interfering with the process of forming membranes or cell walls so that they are not formed or formed imperfectly.[11;12] Escherichia coli (E. coli) bacteria are used because they are enteric Gram-negative bacteria (Enterobacteriaceae) which are normal flora bacteria found in the human large intestine. These bacteria are pathogenic if they are outside the intestine because they rarely inhabit the usual location. E. coli often causes diarrhoea, urinary tract infections, bile ducts, and other places in the abdominal cavity.[13]

The presence of E. coli in the oral cavity can be caused by objects entering the oral cavity and contaminated with faeces. According to data from hospitals in Jakarta, E. coli is the cause of infections in the digestive tract up to 19%. This condition is exacerbated by the increasing difficulty of treating these bacteria by hospitals worldwide, even in countries with well-known treatments such as Singapore.[14; 15; 16]

Diseases caused by bacteria are generally treated with the use of antibacterial agents. However, the high price of antibacterials is the main obstacle for people with low incomes to treat this infectious disease. In addition, improper use of antibacterials can cause resistance. Various efforts to find alternative treatments continue to be improved by developing traditional medicines from plants into phytopharmaca preparations.[17]

There is a contradiction between using broad-spectrum antibacterials to treat infectious bacteria by E. coli with concerns about high economic losses and the emergence of drug resistance, necessitating finding complementary therapies by utilizing materials from nature such as lime leaves and kaffir lime leaves which are local wisdom in tropical countries.[18]

Based on the above, the authors to compare is because there has been no comparative study of the antibacterial effectiveness of the two types of plants in inhibiting E. coli bacteria. Thus, researchers are interested in experimentally testing the effectiveness of the two types of plants above against E. coli bacteria. Due to a large number of citrus species in Indonesia. The author only focuses on lime leaves and kaffir lime leaves.

Based on this background, the formulation of the problem in this study is formulated as follows: a) Can lime leaf extract and kaffir lime leaf extract inhibits the growth of E. coli bacteriostatically and bacterially?, and b) What is the level of effectiveness between lime leaf and kaffir lime leaf? kaffir lime leaf against E. coli bacteria?. To know the antibacterial effectiveness of extracts between lime leaves and kaffir lime leaves against E. coli.

Literature Review

Scientifically, lime is named Citrus aurantifolia. The term "lime" in English comes from Arabic, namely lemonade and Persian, namely limou. In Indonesia, to be precise, Java is known as tamarind oranges, tamarind limes (Sundanese), Durga oranges (Madura) and some are better known as "baby oranges". That said, this lime comes from the East Indies. The lime plant that grows in Indonesia comes from North Burma, South China, northern India, precisely in the Himalayas, and Malaysia, which the Dutch brought. In the 19th century, limes became a mainstay for sailors and soldiers when their fruit set sail. The declining body condition caused British sailors to suffer from scurvy characterized by rough, scaly, dry skin, cracked and bleeding gums and gums. The way to prevent this disease is to use lime to intake vitamin C.[9]

Compound leaf plants with a smooth (laevis) and shiny (nitidus) surface. The upper leaf surface is shiny dark green, and the lower surface is light green. The edge of the leaf is ringed (crenatus). The leaf blade is oval, the tip is slightly blunt, and the leaf legs fail to be rounded. The leaves are 2.5-9 cm long and 2.5 cm wide. The petioles are pinnate with green-winged stalks and 5-25 mm wide.[9; 19] The flowers are compound/single-sized, growing at the end of the stalk with a 1.5-2.5 cm diameter. The flower shape is relatively small, and the stem is very short. The petals are like a bowl, four to five

in number, oval or oval in shape and yellowish-white.[9; 19]

Lime fruit is oval, the size of a ping pong ball with a diameter of 3.5-5 cm, has a thin surface and has thin skin. When young, the fruit's skin is green, but when it is old and ripe, the skin colour turns yellow. Seeds ovoid, flat, greenish-white. The flesh of the fruit is greenish-yellow, and the seeds are numerous.[9; 19]

Lime is usually used for drinks and flavouring dishes. Lime is also used for beauty treatments and as a household cleaner. In addition, lime leaves are also used as an ingredient in herbs and traditional medicine because they treat influenza and malaria. Several studies suggest that this plant has antioxidant, antituberculosis, antifungal, anthelmintic, larvicidal and antibacterial activities.[20; 21]

Lime also contains elements of beneficial chemical compounds such as 7% citric acid, citric acid, amino acids, 24 mg vitamin C, 11 mg calcium, 9 mg phosphorus, 0.2 mg iron, 0.6 g carbohydrates, 0.1 g fat, 0.4 g protein, 1 g fibre, 44 g water. The leaves also contain bioactive compounds such as flavonoids, alkaloids, terpenoids, saponins, tannins, steroids, and essential oils. These compounds have the ability to an antibacterial. It is well known that the dominant compounds in the lime leaf are tannins and saponins are important plant metabolites that are responsible for the activity of microorganisms.[9; 21; 22; 23]

Kaffir lime is a herbaceous plant mainly used for its fruit and leaves as a seasoning for cooking. In international trade, kaffir lime is known as kaffir lime. Because the shape is different from other types of citrus in the market, the appearance of kaffir limes is easy to recognize. The plant is in the form of a small tree (shrub).[24] It is a pinnate compound leaf that gives birth to one leaf. The leaf stalks are partially widened to resemble leaflets. Leaflets are ovate to oblong, base rounded or blunt, tip blunt to tapered, edge ridged, 8-15 cm long, 2-6 cm wide, both upper surfaces slightly glossy dark green, lower surface light green or green yellowish, opaque, speckled when crushed fragrant.[24; 25]

The flowers are five strands of star-shaped, reddish-white or yellowish-white. Most flowers are compound in 1 stalk, smell good because they contain a lot of nectar (honey). The shape of the fruit is oval. The skin is green, wrinkled and thick, but thin, so it is easy to peel, has bumps, tastes sour, slightly bitter. The colour of the skin is green to yellow. Seeds are oval and white. The pulp of the kaffir lime is green and whitish, with clear fruit juice like lime.[24; 25]

Kaffir lime leaves have a function as an antihistamine, anti-dandruff, antispasmodic, and hypotensive activity. The leaves are usually used as a shampoo, especially the treatment of dandruff. It is generally used as an insecticide in shampooing the head and treating the feet to kill leeches. The fruit is also beneficial for gum health, can be used to brush teeth, and is usually used for flavouring dishes and drinks. Essential oil extracts from kaffir lime leaves function as aromatherapy and include cosmetic ingredients such as soaps and lotions.[26]

Kaffir lime belongs to the Rutaceae tribe, which has the potential as a producer of essential oils. Kaffir lime leaves contain sabinene and limonene, useful for cosmetics, aromatherapy hair wash, anthelmintic, headache medicine, stomach pain, and biopesticide. The leaves are also often used as a spice that serves to give a distinctive aroma to dishes. Kaffir lime leaf essential oil is called kaffir lime oil which is widely used in the food, beverage, pharmaceutical, flavour, perfume and dye industries. For example, it is widely used as a flavouring agent in processed products in the food industry. Kaffir lime leaves contain alkaloids, essential oils, tannins, triterpenoid steroids, citronella, flavonoids. The dominant chemical compounds in citrus plant parts are flavonoids and essential oils. Kaffir lime is a plant with very high antioxidant activity, so it is widely used in daily needs, both in medical, industrial and household applications. Kaffir lime leaves contain secondary metabolic compounds. These compounds act actively in antioxidant and antibacterial activity, especially flavonoid content.[12]

Phytochemistry is the science of organic compounds formed and stored, namely chemical structure, biosynthesis, changes and metabolism, natural distribution and biological function, isolation, and comparison of chemical compositions of various types of plants. Phytochemical analysis was carried out to determine the characteristics of the bioactive compounds of a crude extract that have toxic effects or other pharmacological effects that are beneficial when tested with biological systems or bioassays.[27] Based on the chemical content found in lime leaves and kaffir lime leaves, the chemical content of lime leaves will be discussed in four groups of compounds: flavonoids, essential oils, tannins, and saponins.

Flavonoids are the largest group of phenolic compounds which have effective properties to inhibit the growth of viruses, bacteria and fungi. Flavonoid compounds are generally antioxidants. Flavonoids work as antibacterial by inhibiting the synthesis of bacterial nucleic acids and inhibiting bacterial motility. Flavonoids work by interfering with hydrogen binding to nucleic acids so that the DNA-RNA synthesis process is inhibited. In addition, flavonoids can also prevent bacterial growth by disrupting cell membrane stability and bacterial energy metabolism. This instability occurs due to changes in the hydrophilic and hydrophobic properties of the cell membrane so that macromolecules and ions leave the cell, resulting in impaired fluid exchange in the cell. It has an impact on bacterial cell death.[12; 28]

Escherichia coli (E. coli) is a bacterium found in the intestines of humans and warm-blooded animals. Most strains of E. coli are harmless. However, some strains, such as the Shiga toxin-producing E. coli (STEC), can cause severe foodborne illness. The bacteria transmit the toxin to humans mainly by consuming contaminated food such as raw or undercooked meat products, raw milk, and contaminated raw vegetables. It causes diseases, especially diseases of the gastrointestinal tract and urinary tract. STEC can be turned off thoroughly cooking food until all parts reach 70˚C or higher.[29]

E. coli has a cell size of 2.0 – 6.0 m long and 1.1 – 1.5 m wide and weighs 2 x 10-12 grams. These bacteria are rod-shaped, straight, single, in pairs or short chains, including Gram-negative bacteria that can live solitarily or in groups, generally motile, not spore-shaped, and facultative anaerobes. It typically gives positive results on indole and mannitol fermentation tests and produces gas from glucose, characteristic colony morphology with multi-coloured "sparkling" on EMB agar medium, and positive indole spot test.[14; 29]

E. coli does not have a nucleus, membrane-enclosed organelles or a cytoskeleton. E. coli has external organelles, namely villi, thin filaments for capturing specific substrates, and flagella, thin and longer filaments for swimming. E. coli is a facultative anaerobic bacterium, has a fermentative metabolism type. Good growth at an optimal temperature of 37˚C on media containing 1% peptone as a carbon and nitrogen source. E. coli is circular, convex and non-pigmented colonies on blood media. E. coli is not resistant to dry conditions or ordinary disinfectants, and these bacteria can die at 60˚C for 30 minutes.[16]

E. coli bacteria are part of the normal digestive tract flora that can be transferred from one place to another, such as hand to mouth or by passive transfer through food and drink contaminated with these bacteria. Various foods and drinks consumed by humans in daily life can not be separated from bacteria in them. In addition, the bacteria can also enter the human body through hands or tools such as bottles, pacifiers, thermometers, and eating utensils that are contaminated with faeces. However, if the food and drink are processed hygienically, its bacteria may still have a tolerance limit for consumption, especially pathogenic bacteria that cause disease. According to the Indonesian National Standard, E. coli in food and beverage ingredients is 0 (zero) in 100 ml of water.[17; 30; 31] E. coli can cause disease because of its ability to multiply and spread widely in body tissues and the presence of several substances produced by these bacteria, including toxins and toxins.[29]

Extraction is a dry, viscous or liquid preparation prepared by filtering simplicia according to a suitable method outside direct sunlight. The purpose of natural material extraction is to extract the chemical components found in natural materials. This extraction is based on the substance components mass transfer into the solvent, where the transfer begins to occur in the interfacial layer and then diffuses into the solvent.[32] Simplicia is a natural substance used as a medicine that has not undergone any processing and, unless otherwise stated, is in the form of dried material. Vegetable simplicia is simplicia in the form of whole plants, plant parts or plant exudates, cell contents that spontaneously come out of the plant or cell contents which are removed from the cell in a certain way, or other vegetable substances which are separated in a certain way from the plant and have not been pure chemical substances.[33]

The ideal technique is an extraction technique that can extract as much of the desired active ingredient as possible, quickly, efficiently, inexpensively, environmentally friendly, and the results obtained are always consistent if done repeatedly. Several methods are used to carry out the extraction: extraction using solvents (squeezing, maceration, percolation, reflux, soxhlet, digestion, infusion, and decoction) and steam distillation.[34]

Antibacterial Test Method - In this test, what will be measured is the response of the growth of the population of microorganisms to antibacterial agents. One of the benefits of antibacterial testing is the acquisition of an effective and efficient treatment system. Determination of each germ's sensitivity to a drug is to determine the minor drug level that can inhibit the growth of germs in vitro. Several methods of antibacterial testing are as follows, namely the diffusion method (paper disc method, hole or healthy method) and dilution method (agar plate thinning, serial dilution in a tube).[35; 36]

Antibacterial is a type of drug used to eradicate bacteria, especially for bacteria harmful to humans (pathogens). Substances that function as antibacterials can come from natural, synthetic or semisynthetic compounds. Antibacterial must have the highest possible selective toxicity, meaning that the drug must be highly toxic to bacteria but relatively non-toxic to the host. Based on selective toxicity, antibacterials are divided into antibacterials that inhibit growth (bacteriostatic) and antibacterials that kill bacteria (bactericidal). Antibacterial is a substance produced by microorganisms (especially fungi) that can inhibit the growth or eradicate other microorganisms such as bacteria. Meanwhile, based on the mechanism of action, antibacterials are divided into five groups.[37]

The germ cell is surrounded by a rigid cell wall, which protects the underlying protoplasmic membrane against either osmotic or mechanical trauma. Therefore, any substance capable of damaging the cell wall or preventing its synthesis will cause the formation of cells that are sensitive to osmotic pressure. Among the antibacterials that affect cell walls are penicillin, amoxicillin, fosfomycin, Ceftazidime, cycloserine, ristocetin, vancomycin and bacitracin.[36]

The cell membrane is the osmotic barrier for free diffusion between the external and internal environment of the cell. It affects the concentration of metabolites and nutrients in cells and is the site of respiration and certain biosynthetic activities. Some antibacterials can impair or weaken one or more of these functions—for example, Polymyxin and Polyene.[36] Several antibacterial drugs function primarily to disrupt or damage DNA structure and function, but only a few are available for clinical use because they are toxic. The DNA molecule structure is closely related to its two prominent roles, namely duplication and transcription. Therefore, the structure of substances that can interfere with the DNA structure will also affect all phases of the growth and metabolism of germs. Examples are Mitosin and Nalidixic Acid.

Protein synthesis results from two main processes: a) transcription or DNA-dependent ribonucleic acid synthesis and b) translation or RNA-dependent protein synthesis. Antibacterial that can inhibit one of these processes will inhibit protein synthesis. This antibacterial includes Actinomycin, Rifampicin, Streptomycin, Tetracycline, Erythromycin, and Clindamycin.[38]

Research Method

This research is a descriptive, experimental study with laboratory tests to test the antibacterial effectiveness of lime leaf extract (Citrus aurantifolia Swingle) and kaffir lime leaf (Citrus hystrix DC) against Escherichia coli in various concentrations. This research was conducted at the Microbiology Laboratory, Faculty of Medicine, Christian University of Indonesia. The process of extracting lime leaves and kaffir lime leaves was carried out at the Center for Biological Resources and Biotechnology Research (PPSHB), Institute for Research and Community Service, Bogor Agricultural University. The time needed to conduct this research is from September 2018 to December 2018. Lime leaf extract (Citrus aurantifolia Swingle) and kaffir lime leaf extract (Citrus hystrix DC). The drying process was carried out at the Herbal Medicine Laboratory, Faculty of Christian Medicine, Indonesia. The material extraction process was carried out at the PAU Laboratory (Inter-University Center) Bogor Agricultural University. The bacterial sample used was Escherichia coli ATCC 25922 obtained from the University of Indonesia, planted on EMB agar media, and incubated at 37°C for 24 hours. The samples used in this study were extracts of lime leaves and kaffir lime leaves in various concentrations of 10%, 30%, 50%, 70%, and 100%, and Ceftazidime as a positive, positive control and filter paper dipped in distilled water as a negative control. Based on the results above, the sample size used is four. This study diluted lime leaf extract and kaffir lime leaf extract to obtain various concentrations in a test tube. After forming the desired concentration, filter paper is given into it, which will later be placed on MHA media to see the zone of inhibition of the growth of E. coli. This research will be repeated four times.

RESULT AND DISCUSSION

Inhibition of lime leaf extract (Citrus aurantifolia Swingle) on the growth of Escherichia coli with concentrations of 10%, 30%, 50%, 70%, 100%, sterile distilled water as a negative control and antibacterial Ceftazidim as a positive control can be seen in the picture below.

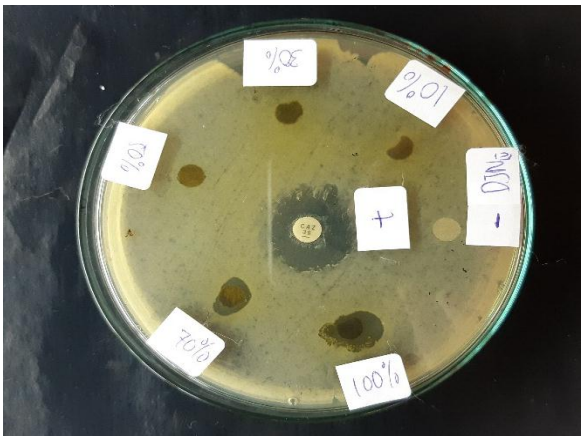

Figure 1: The results of the sensitivity test of lime leaves show there is an inhibition zone on the growth of Escherichia coli.

Source: personal documentation

Based on research that has been carried out through four treatment repetitions in a row, the results of measuring the inhibition zone diameter are shown in the table below.

Table 1: Results of Inhibitory Testing of Lime Leaf Extract on the growth of Escherichia coli ATCC 25922.

Repetition	Diameter of Inhibitory Zone of E.Coli ATCC 25922 (mm)						
	Control (-) Aquades Sterile	Control (-) Ceftadizim	Concentration of Lime Leaf Extract				
			10%	30%	50%	70%	100%
1	0	22	6.0	6.25	6.5	6.70	8.5
2	0	22	6.2	6.35	6.55	8.15	9.3
3	0	22	6.3	6.4	7.75	8.30	11.2
4	0	22	6.4	6.65	8.1	10.15	13.2
Mean	0	22	6.225	6.413	7.225	8.325	10.550

The table above shows no inhibition zone in the negative control treatment group with sterile distilled water, while the positive control is with the antibacterial Ceftazidim 30 mg. It is found that an inhibition zone of 22 mm is formed, which meets the CLSI (Clinical Laboratory and Standards Institute) criteria regarding the Ceftazidim inhibition sensitive zone to bacterial growth.[39]

The lime leaf extract treatment group with concentrations of 10%, 30%, 50%, 70%, and 100% each had an average inhibition zone diameter of 6.225 mm, 6.413 mm, 7.225 mm, 8.325 mm, mm, and 10. 55mm. The inhibitory power of kaffir lime leaf extract (Citrus hystrix DC) on the growth of Escherichia coli with concentrations of 10%, 30%, 50%, 70%, 100%, sterile distilled water as a negative control and antibacterial Ceftazidim as a positive control can be seen in the picture below.

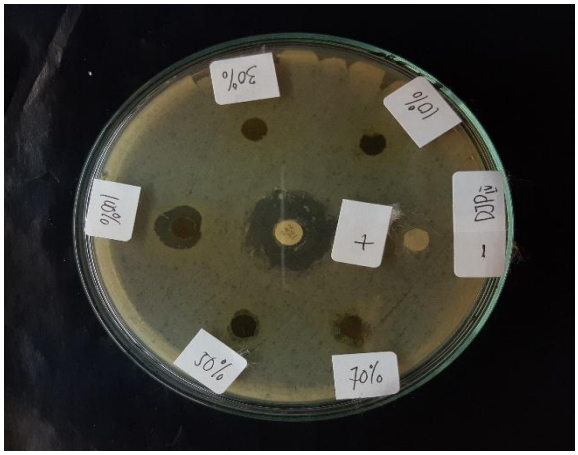

Figure 2: The results of the sensitivity test of kaffir lime leaves show that there is an inhibition zone on the growth of Escherichia coli.

(Source: personal documentation)

Based on research that has been carried out through four repetitions of treatment in a row, the results of measuring the inhibition zone diameter are shown in the table below.

Table 2: Results of Inhibitory Testing of Kaffir lime leaf extract on the growth of Escherichia coli ATCC 25922

Repetition	Diameter of Inhibitory Zone of E.Coli ATCC 25922 (mm)						
	Control (-) Aquades Sterile	Control (-) Ceftadizim	10%	30%	50%	70%	100%
1	0	22	5.7	6.15	6.3	6.9	8
2	0	22	5.8	6.45	6.55	7.15	9.35
3	0	22	6.0	6.5	7.0	8.1	10.8
4	0	22	6.1	6.55	9.0	9.15	11.35
Mean	0	22	5.90	6.41	7.338	7.825	9.875

The table above shows no inhibition zone in the negative control treatment group with sterile distilled water, while the positive control was with the antibacterial Ceftazidim 30 mg. It was found that an inhibition zone of 22 mm was formed, which met the CLSI (Clinical Laboratory and Standards Institute) criteria regarding the Ceftazidim inhibition sensitive zone to bacterial growth.[52] In the treatment group, kaffir lime leaf extract with concentrations of 10%, 30%, 50%, 70%, and 100% each had an average inhibition zone diameter of 5.9 mm, 6.4125 mm, 7.338 mm, 7.825 mm, and 9.875 mm.

This study tested the antibacterial activity of lime leaf extract and kaffir lime leaf extract against Escherichia coli by seeing whether or not the diameter of the inhibition zone was formed. The inhibition test results of lime leaves and kaffir lime leaves for each of the slightest concentrations have different inhibition zone diameters, as shown in Table 1 and Table 2. The concentration of 100% lime leaves forms an intermediate inhibition zone of 10, 55 mm, while in kaffir lime leaves, 100% formed an average zone of inhibition of 9.875 mm because seen from the test, the content of active compounds of essential oils of lime leaves and kaffir lime leaves was significant in percentage. Thus, from the tests carried out, the antibacterial inhibition of lime leaves was more significant against E. coli.

These results indicate that the greater the concentration, the greater the content of bioactive compounds with antibacterial activity. However, the formation of the inhibition zone did not match the Greenwood criteria (> 20mm) in this study. In contrast, the antibacterial Ceftazidim, used as the positive control, showed a zone of more significant inhibition against Escherichia coli. So it can be concluded that antibacterial is better in inhibiting the growth of Escherichia coli.

Several factors, among others, may influence this result, and the selected leaves are not suitable due to the growth of bacteria, fungi or the occurrence of drought during the dry season and lack of watering plants. Drying is not

good because the heating equipment does not typically work due to, among others, the temperature control of the equipment does not work correctly or drying for too long. Then at high temperatures, preparing tools and materials that are not sterile, and testing equipment with inaccurate measuring instruments (temperature) or has never been calibrated to a nationally accredited body such as the Center for Materials and Technical Goods (B4T) in Bandung.

There is no significant difference between the effectiveness of lime leaves and kaffir lime leaves against Escherichia coli.

CONCLUSION

Based on the results of the study, several conclusions can be drawn, namely: a) Based on the results of research on the effectiveness of lime leaves and kaffir lime leaves against Escherichia coli bacteria, the results showed that the largest inhibition zone was 13.20 mm for lime leaves and 11.35 mm for lime leaves. kaffir lime at each different concentration and each treatment. It illustrates that conventional medicine is still better than herbal medicine, and b) There is no significant difference in the effectiveness of lime leaves and kaffir lime leaves against Escherichia coli. Therefore, looking at the potential development of the uses and benefits of lime leaves and kaffir lime leaves, further research is needed on the inhibitory activity of lime leaf extract, and kaffir lime leaf extract, especially clinical trials, and lime leaf extract and kaffir lime leaf extract can be considered as one of the alternatives. An alternative antibacterial treatment against Escherichia coli bacterial infection and developed by the national drug industry.

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