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# Antibacterial Activity of Subfraction of Ethanol Extract of Kebo Rubber Leaves (Ficus Elastica Roxb. ex Hornem) Escherichia Coli Bacteria

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A B S T R A C T
This study aims to determine the antibacterial activity of kebo rubber leaves (Ficus elastica Roxb ex. Hornem) extracts against E. coli and identify the chemical content of the most active subfraction. The leaves were macerated with 70% ethanol and fractionated using n-hexane, ethyl acetate, and water. Subfractions were obtained via vacuum liquid chromatography (VLC) and tested for antibacterial activity using the diffusion method. The ethyl acetate fraction showed the highest antibacterial activity (19.30 mm zone of inhibition). Subfraction 3, with an inhibition zone of 18.73 mm, was the most effective, containing flavonoid compounds with a total flavonoid value of 79.8950 mgQE/g. TLC analysis confirmed the presence of flavonoids and tannins.

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## INTRODUCTION

Antibiotics can treat bacterial infections, but widespread use of antibiotics is a major factor causing resistance. Bacteria that are resistant to antibiotics will not die from antibiotics; instead, it will multiply and spread, making it more dangerous. Limited synthetic antibiotics for treating bacterial diseases have been associated with the development of antibiotic resistance(Adeniyi, et al., 2017). *E. coli* is one of the most common types of gram-negative bacteria in the normal flora of the human colon. If its growth in the body exceeds normal limits, the bacteria have the potential to become pathogenic. The mechanism of action of *E. coli* can cause inflammation and dehydration. The majority of patients suffer from diarrhea, nausea, and stomach cramps due to *E. coli* infection (Leonard et al., 2018).

Following the emergence of increasing strains of antibiotic-resistant bacteria, this is evidenced by the increasing resistance of bacteria to antibiotics. Effective treatment to treat disease is by utilizing traditional medicine. Kebo rubber leaves (*Ficus elastica*) are the antibacterial plant used in this product. Ficus is a variety that is rich in polyphenolic compounds such as flavonoids, which have strong cell-strengthening properties so they can help ward off and treat various diseases (Iqbal, 2017a).

The kebo rubber plant is a plant originating from India, which is often found in South Asia, including Indonesia. Kebo rubber can rise as high as 8 to 40 meters. This plant only has one long leaf, which is green on the old leaves and red on the young leaves (Zukhri & Nurhaini, 2019). Kebo rubber leaves contain saponins and flavonoids (Suhaenah, et.al., 2021). *Ficus elastica* has antimicrobial activity and extracts from its leaves are used for dysentery, skin allergies, skin infections and other (Iqbal, 2017b). Karet rubber leaves are used as a medicine for skin diseases, diarrhea. These leaves have antioxidant and antimicrobial properties (El-Hawary, 2012).

According to several studies, rubber tree leaf extract showed antibacterial activity against *E. coli* with an inhibitory power diameter (DDH) of 3.0 mm and a minimum inhibitory concentration (MIC) of 20 mg/mL, and an antibacterial activity concentration of 40 mg/mL. Rubber tree leaves have an inhibitory effect on gram-positive and negative antibacterials. The content of chemical compounds tannins, saponins, flavonoids, and phenolics (Bhawana *et al*., 2018). The bioactivity of metabolites from endophytic fungi associated with rubber tree leaves against *E. coli* bacteria has activity with an inhibition zone diameter of >15 mm (Ding *et al*., 2019). Antibacterial agents, which are substances that can kill bacteria and prevent their growth by disrupting unfavorable metabolic systems, are found in many medicinal plants. Microorganisms can cause disease in other living animals because they can change from mildillness to serious infections or even death (Radji, 2011).

One group of compounds can be differentiated from another group, the chemical content of a sample can only be dissolved in a solvent of the same polarity. Therefore, further research is needed using the subfraction method. Using two solvents with different polarities, fractionation is a method of

separating mixtures, because there is a difference in polarity, fractionation is needed to separate the main group of contents from one other main group. Subfractions were carried out on the selected fractions and then sub fractionated using vacuum liquid chromatography (VLC) to simplify the fractions that had been obtained (Suhaenah *et al*., 2021). Based on the description above, previous research on karet kebo leaves only reached the extraction stage, so researchers are interested in continuing research to the subfraction stage on the ethanol extract of kebo rubber leaves against *E. coli* bacteria and looking at the content of the most active chemical compounds.This research aims to determine whether subfractions of kebo rubber leaves extract can inhibit *E. coli* bacteria, the most active subfraction which shows antibacterial properties against *E. coli* and determine the chemical content of the most active subfractions in kebo rubber leaves.

## THEORETICAL REVIEW

### *Extract*

An extract is a concentrated preparation obtained by separating active compounds from plant or animal simplicia using an appropriate solvent. The dissolved substances are then dispersed, and the larger mass of powder is processed according to established regulations.

### *Fractionation*

The initial extract, derived from a mixture of various compounds, must be separated into fractions with similar polarity and molecular size because a single separation technique is employed for this purpose. Several liquid-liquid extraction techniques that can be used for fractionation include vacuum liquid chromatography (VLC), column chromatography (CC), size exclusion chromatography (SEC), and solid-phase extraction (SPE) (Sticher, 2008). Sub-fractionation is then performed on selected fractions using column chromatography to simplify the obtained fractions (Putri *et al*., 2013).

### *Antibacterial Activity*

The antibacterial activity of *E. coli* against endophytic fungi was evaluated based on the MIC values of isolated compounds against the tested microorganisms. The results showed growth inhibition activity against *E. coli* with an MIC value of 32 µg/ml (Ding *et al*., 2019). Rubber leaf (*Ficus elastica*) was effective in inhibiting *E. coli* growth with an inhibition zone of 3.0 mm at a concentration of 40 mg/ml and an MIC of 20 mg/ml (Bhawana *et al*., 2018). Studies on the antibacterial activity of the *Ficus* genus against *E. coli* demonstrated inhibition with an MIC of 300 mg/ml (Odunbaku *et al*., 2008).

### *Chromatography*

Chromatography is a separation method for mixtures based on differences in the propagation speed of components within a specific medium. According to Gandjar and Rohman (2007), both the mobile phase and the

stationary phase interact with the mixture. Commonly used techniques include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and vacuum liquid chromatography (VLC) (Atun, 2014).

## METHODOLOGY

### *Tools*

The tools use in this study were a set og glassware, autoclave, rotary evaporator, micropipette, drop pipette, capillary tube, tube needle, incubator, UV lamp, LAF (Laminar Air Flow), GF254 silica gel plate, UV-Vis spectrophotometry (Shidmazu).

### *Materials*

The materials used in this study were kebo rubber leaves simplicia, E. coli, Nutrien Agar (NA), Mueller Hinton Agar (MHA), Simmons Citrat Agar (SIM), Lysine Iron Agar (LIA), Kligrer Iron Agar (KIA), disks antibiotics chloramphenicol, distilled water, dichloromethane, methanol, n-hexane, ethyl acetate. Fecl3 1%, Nacl, Hcl, DMSO, ethanol 70%, water, chloroform, anhydrous acetic acid, $H_2SO_4$, Lugol's iodine, alcohol, Dragendroff's reagent, Mayer's reagent, Mg powder, concentrated Hcl, Hcl 2N, TLC plate.

### *Work ProceduresExtraction*

1500 grams of kebo rubber leaves powder was macerated with 70% ethanol solvent (1:10) put in a macerator covered with aluminum foil and macerated for 24 hours, stirring occasionally for the first 6 hours. Then let it sit for 18 hours. Strain the maceration with filter paper to separate it. Maceration was repeated 2 times, the sample was filtered again using filter paper. After that, a rotary evaporator is used to evaporate the filtrate at a temperature of between 40-50°C until a thick extract is obtained. Calculate the percentage yield w/w (Kemenkes RI., 2017).

### *Fractionation*

The liquid method was used to fractionate the ethanol extract of kebo rubber leaves. Every 10 grams of kebo rubber leaves ethanol extract was dissolved in a small amount with ethanol: water (1:1) then fractionated with 75 mL of n-hexane which was dissolved in a separating funnel. Let it sit until a layer forms, the n-hexane layer on top and water on the bottom. The n-hexane fraction was carried out 3 times. The water fraction was added with 75 mL of ethyl acetate mixed with the water fraction and shaken until two layers formed. The top layer consists of the ethyl acetate fraction, and the bottom layer consists of the water fraction. To obtain the ethyl acetate fraction, the procedure was repeated three times. The remaining filtrate from the ethyl acetate solvent fractionation process is the water fraction. Evaporation was used to obtain a viscous fraction from n-hexane, water and ethyl acetate fractions.

***Identification of the Compound Content of Extracts, Fractions and Subfractions of Kebo*** rubber ***Leaves***

Phytochemical screening involves examining color and precipitation reactions to a wide variety of compounds, including:

a. Alkaloid - Divide 5 mL of HCl 2N solution into three parts: one as a blank, three drops of Dragendroff's reagent in the second tube, and three drops of Mayer's reagent in the third tube. The second tube had an orange precipitate and the third tube had a white precipitate, indicating that the sample showed a positive result for the identified alkaloid (Agustina, 2017).
b. Flavonoid - Mg (Magnesium) powder and three drops of concentrated HCl were then added to 2 mL of sample. The yellow or orange colored sediment contains flavonoids in the sample (Adjeng *et al*., 2020).
c. Tanin - 1 mL of sample was taken and 3 drops of 1% $FeCl_3$ were added. The tannin test is shown when a blackish green color form (Adjeng et al., 2020).
d. Saponin - Add 2-3 mL of sample, add 10 mL of water to the test tube, then cool and shake vigorously for 10 seconds, if there is foam formed with a stable character with a height of 1-10 cm. When 1 drop of HCl 2N was added, the foam remained unchanged and did not disappear for approximately 10 minutes.
e. Triterpenoid and steroid - 2 mL sample was obtained using the Lieberman- Bouchard method, which involves evaporating the residue in a cup, dissolving it with 0.5 mL of chloroform, transferring it to a test tube, and then adding 0.5 mL of anhydrous acetic acid and 1-2 mL of concentrated sulfuric acid. to the wall of the test tube. Steroids, on the other hand, have a green-blue color at the boundary of the two solvents, while triterpenoids have a reddish or brownish purple or violet color (Habibi *et al*., 2018).

***Antibacterial Activity Test of Extracts and Fractions***

The Kirby and Bauer diffusion method, which uses paper discs, is used in this procedure. Extract samples, n-hexane fraction, ethyl acetate fraction, and water fraction. The media tested was *Muller Hinton Agar* (MHA). The petri dish was filled with 30 mL of MHA medium. To make a stock solution, extract, n-hexane fraction, ethyl acetate fraction, and water fraction were made at a concentration of 400 mg/mL. 10% DMSO is soluble in concentrate and water, while the n-hexane fraction uses n-hexane solvent and the ethyl acetate fraction uses a soluble ethyl acetate fraction. The compacted petri dish is aseptically etched with microorganisms. Next, 50 µL of the extract solution, n-hexane fraction, ethyl acetate fraction, and water fraction were pipetted. Next, put the sterilized paper disc into a petri dish and let it sit at temperature for 10-20 minutes. After the paper disc is cleaned of all concentration, the solidified media is placed on top. Stored at 37°C for 24 hours. In this experiment DMSO was used as a negative control, while chloramphenicol was used as a positive

control. The zone of inhibition was measured with a caliper (Bhawana *et al*., 2018).Data were analyzed using SPSS with a P value > 0.05.

***Separation of Subfractions by Vacuum Liquid Chromatography (VLC)***

Carefully weighed 3 grams of the selected viscous fraction (the fraction that gives the largest inhibition zone) based on the antibacterial activity test. The stationary phase of the silica gel column and the mobile phase used were a combination ratio of dichloromethane (DCM) and methanol (MeOH) solvents, as shown in Table. The powder was then added to 3 grams of the stationary phase, stirred until it became a powder, and sub fractionated using vacuum column chromatography as shown in Table 1.

Table 1**.** Comparison of solvent combinations

| No | Eluen | Eluen Composition | Total volume (mL) |
|---|---|---|---|
| 1 | DCM:MeOH | 100:0 | 100 |
| 2 | DCM:MeOH | 90:10 | 100 |
| 3 | DCM:MeOH | 80:20 | 100 |
| 4 | DCM:MeOH | 70:30 | 100 |
| 4 | DCM:MeOH | 50:50 | 100 |
| 6 | DCM:MeOH | 0:100 | 100 |

Using vacuum liquid chromatography, the 6 subfractions obtained were thenevaporated to form a thick subfraction, which was then tested for its antibacterial activity. The subfraction that produced the greatest inhibitory power was then subjected to phytochemical screening, TLC test, and total flavonoid content test.

The subfraction that produced the greatest inhibitory power was then subjected to phytochemical screening, TLC test, and total flavonoid content test.

***Subfraction Antibacterial Activity Test***

The method used is the Kirby and Bauer diffusion method using disc paper. Disc diffusion test is carried out to determine the antibacterial activity of plants. Samples of subfractions 1 to 6. The media used is *Muller Hinton Agar* (MHA). Petri dishes that have been filled with 30 mL of MHA media. Subfraction solutions 1 to 6 are made with a concentration of 400 mg/mL. Microorganisms are aseptically scratched into the solidified petri dish. After that, a volume of 50 µL of solution from subfractions 1 to 6 is carefully transferred and dropped onto sterilized disc paper, the solution is left at a certain temperature for 10-20 minutes. After all the disc paper is saturated with all concentrations, the disc paper is placed on the surface of the solidified media. Chloramphenicol serves as a positive control in this test, while DMSO serves as a negative control. Then incubated for 24 hours at 37 ° C, with three repetitions. Furthermore, the inhibition zone was measured using a caliper (Bhawana et al., 2018). Data were analyzed using SPSS with a P value> 0.05.

***Separation of Compounds by Thin Layer Chromatography (TLC)***

The samples used were ethanol extract, n-hexane fraction, ethyl acetate fraction, water fraction and subfraction 3 (the subfraction that inhibits the largest zone).

Flavonoid-Identification of flavonoids using mobile phase chloroform:acetone:formic acid (7:2:1) with quercetin as a comparator was used to identify flavonoid compounds. Wavelengths of 254 nm and 366 nm were observed in ultraviolet. After using ammonia to make spots, put them in the oven for five minutes at a temperature of 100°

The presence of flavonoid compounds is indicated by spots in visible light showing a yellow color that glows.

Tanin - Identification of tannins using the mobile phase ethyl acetate: methanol: water (6:3:1) with gallic acid as a comparator. seen between UV wavelengths 254 and 366 nm. By spraying $FeCl_3$, the spots were observed in UV light with a wavelength of 366 nm. Tannin compounds are indicated by brown or black spots.

***Determination of Total Flavonoid Content of the Most Active Subfraction***

Weigh 0.01 grams of subfraction dissolved in 10 mL of ethanol, then stir until homogeneous. Filter and put 10 mL into a measuring flask. Add 1.5 milliliters of ethanol, 0.5 milliliters of test solution, 0.1 milliliters of 10% aluminum chloride, 0.1 milliliters of 1M sodium acetate, and 2.8 milliliters of distilled water. Allow 30–32 minutes for incubation. The maximum wavelength is used to calculate the absorbance. Blank measurements should be carried out in the same way but without aluminum chloride and test solution.

## RESULTS AND DISCUSITION

***Extraction***

Researchers used 1500 g of kebo rubber leaves powder to obtain 172 g of extract and obtained a yield percentage of 11.95%. The amount of bioactive compounds in plants has an impact on the yield value. Moreover, examining the results is expected to determine the capacity of the solute to absorb dynamic substances from the sample and the concentration level resulting from the underlying sample weight.

***Fractionation***

Using a separating funnel, fractionation of ethanol extract produced a water fraction yield of 41.60% higher than the ethyl acetate fraction, which was 14.34% higher than the nhexane fraction which was 12.56%. There was a difference in the yield capability of compounds extracted from rubber tree leaves using different solvents - for each solvent. Because water is a polar solvent, the ethanol extract of rubber tree leaves contains more polar compounds. Differences in chemical composition and dissolved compound content cause variations in the yield of extracts and their fractions. The yield of extracts fractionated with n-hexane and ethyl acetate solvents was relatively

low. The small amount of non-polar content in the 70% ethanol extract that could be absorbed by the ethyl acetate and n-hexane fractions made this possible (Pratiwi *et.al*., 2016).

***Phytochemical Screening Ektract and Fractionation***

The chemical content of kebo rubber leaves extract, n-hexane fraction, ethyl acetate fraction, and water fraction are known to contain flavonoid, tannin, and saponin compounds. Chemical compounds belonging to the flavonoid, tannin, and saponin groups are found in the ethanol extract. The findings show that the ethanol extract of kebo rubber leaves positively contains flavonoids, tannins and saponins. This finding is in line with research by Ginting et al., (2020) which found that kebo rubber leaves extract contains phenolics, flavonoids, protein and tannins. Based on research by Preeti, et.al, (2015), tests showed chemical compounds such as tannins, carbohydrates, flavonoids and phenolics contained in kebo rubber leaves. Research by Bhawana *et al*., (2018) shows that karet kebo leaves contain chemical compounds, namely saponins, flavonoids and phenolics. The structure of flavonoids contains a benzopyrone core which can be reduced to produce red or orange flavonoid salts. Flavonoids are chemicals with two aromatic rings and several hydroxyl groups. The saponin test shows that glycosides can be hydrolyzed into foam in the air to become glucose and other mixtures (Agustina, 2017). The result of the phytochemical screening can be seen in Table 2.

Table 2. The Results of Phytochemical Screening Identification of Ethanol Extract, N-Hexane Fraction, Ethyl Acetate Fraction, and Water Fraction of Kebo Rubber Leaves

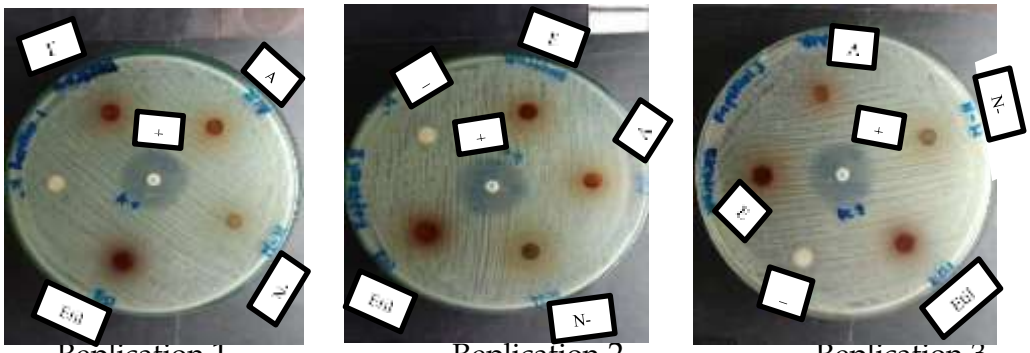
| No | Phytochemical Test | Ethanol extract | n-hexane fraction | Ethyl acetate fraction | Water fraction |
|---|---|---|---|---|---|
| 1. | Alkaloid | - | - | - | - |
| 2. | Flavonoid | + | - | + | - |
| 3. | Saponin | + | - | - | - |
| 4. | Tanin | + | + | + | + |
| 5. | Triterpenid/steroid | - | - | - | - |

Based on the research results in Table 2, the identification of chemical compound content is flavonoid compounds in the ethyl acetate fraction samples and tannin compounds in the ethyl acetate fraction and water fraction samples. According to the results of phytochemical screening research by Asriani Suhaenah *et al*., (2021), it shows that the compounds in the ethyl acetate fraction contain flavonoid compounds. The flavonoid group will be identified as a mixed group because the expansion of magnesium metal and HCl reduces the benzopyrone core in the flavonoid structure and results in a color change from yellow to orange. The oxidation reaction between flavonoid compounds and magnesium metal as a reducing compound decreases with increasing HCl levels. The group of tannin compounds detected in the ethyl acetate fraction and water fraction tests were positive for containing tannins because generally tannins are polar in nature so that in the concentrate cycle

there are more tannins in the water and ethyl acetate parts. The large dielectric constant of water which is able to extract tannin compounds is believed to be the cause of the water solvent being able to extract tannin compounds optimally

***Antibacterial Activity Test of Extracts and Fractions***

The greatest activity against *E.coli* showed an inhibitory zone diameter of 19.30 mm. Differences in the antibacterial inhibitory activity of ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction were analyzed with a P value (>0.05). The results of statistical analysis on negative control samples all had significant differences in antibacterial activity. Extract samples, n-hexane fraction, water fraction are in 1 subset which shows significantly with the ethyl acetate fraction. The ethyl acetate fraction was significantly different from the positive control so that it was not equal to the positive control. The results of the inhibitory power can be seen in Table 3. The impact of extracts and fractions on bacterial inhibition depends on the concentration of the extract and fraction given.The active fraction has the highest antibacterial activity. Based on the Tukey test statistics for all sample groups, only the ethyl acetate fraction was significantly different, it can be concluded that the activity of the ethyl acetate fraction was the best, the suspected chemical compounds in ethyl acetate were flavonoid compounds. According to Markham (1998), ethyl acetate solvent can be used to extract compounds with medium polarity, such as tannins and flavonoids, in the form of O-glycosides. Free flavonoids, such as flavones, isoflavones and flavanols, can dissolve in semi-polar solvents because they are semi-polar (De Las Llagas *et al*., 2014). As an antibacterial agent, flavonoid compounds stop the synthesis of bacterial cell walls by interfering with the components that make up peptidoglycan. As a result, the cell wall layer becomes incomplete and causes cell death (Suhaenah et al., 2021). This is what causes the antibacterial activity of the ethyl acetate fraction to tend to be stronger. The selection of the active fraction was carried out using the method of testing antibacterial activity against *E. coli* bacteria, then the subfraction process was carried out using liquid vacuum column chromatography. The results of the thick subfraction were then tested for the antibacterial activity of 6 subfractions which were then evaporated to form a thick subfraction, then tested for antibacterial activity.



Replication 1 Replication 2 Replication 3

**Figure 1. Antibacterial activity of extract and fraction test**

Information: E: Extract, A: Water, N-H: n-heksan, E: Etil asetat, -: kontrol negatif, +:kontrol positif.

Table 3. Antibacterial Activity Test Results of Extract and Fraction Disc Diffusion Method

| Sample | Diameter of inhibisi zone (mm) replication | | | Mean ± SD |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Ekstract | 17,3 | 16,6 | 17,6 | 17,16±0,51(a) |
| n-hexane fraction | 17 | 17,3 | 16,6 | 16,96±0,35(a) |
| Ethyl acetate fraction | 19,3 | 19,6 | 19 | 19,30±0,30(b) |
| Water fraction | 17,3 | 17,6 | 16,3 | 17,06±0,68(a) |
| Positive control | 31,6 | 31 | 30 | 30,86±0,80(c) |
| Negative control | 0 | 0 | 0 | 0 |

Information:
+ = chloramphenicol
- = DMSO 10%
a = one subset is significantly different from K- and K+
b = one subset (b) is significantly different from K-, and K+ c= significantly different from a and b

***Separation of Subfractions by Vacuum Liquid Chromatography (VLC)***

Non-polar solvents will destroy the non-polar parts, while polar solvents will destroy the polar parts. This is in accordance with the principle of dissolution of substances called "like dissolves like". The chemical properties of the solvent, especially its dielectric constant, can be used to determine its polarity. Dielectric stability is the proportion of the extremity of a substance that can dissolve. Solvents with high dielectric consistency will break down additional polar mixtures, while solvents with low dielectric consistency will

break down non-polar mixtures. The results achieved can be influenced by the use of solvents with different polarities.

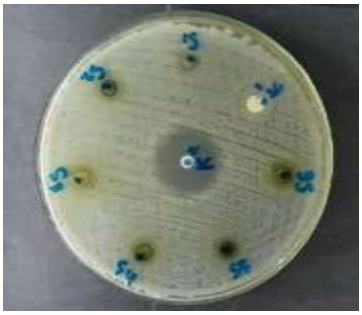
Table 4. Yield Results of Subfractions of kebo rubber leaves

| No | Solvent | Ethyl acetate fraction weight (g) | Subfraction weight (g) | Yield % |
|---|---|---|---|---|
| 1. | DCM:MeOH (100:0) | 3 | 0,4285 | 14,28 |
| 2. | DCM:MeOH (90:10) | 3 | 0,3994 | 13,31 |
| 3. | DCM:MeOH (80:20) | 3 | 0,6157 | 20,52 |
| 4. | DCM:MeOH (70:30) | 3 | 0,3481 | 11,60 |
| 5. | DCM:MeOH (50:50) | 3 | 0,4921 | 16,40 |
| 6. | DCM:MeOH (0:100) | 3 | 0,3303 | 11,01 |

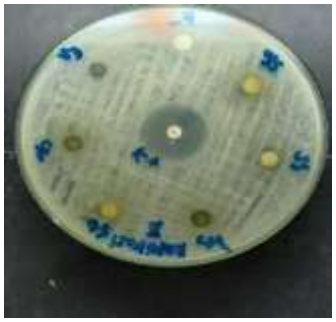
There are two types of organic solvents based on their dielectric constant: polar solvents and neutral solvents. The force that pushes two electrically charged molecules in a molecule is called the dielectric constant. The more polar the solvent, the higher its dielectric constant. Methanol and dichloromethane have dielectric constants of 9.1 and 33.60, respectively. Because the yield of compounds is based on the similarity of polarity with the solvent, the high yield of the subfraction of the ethanol extract of kebo rubber leaves with methanol solvent indicates that the methanol solvent of karet rubber leaves is more capable of extracting compounds (Verdiana *et al*., 2018).

***Subfraction Antibacterial Activity Test***

Table 5 shows the differences in antibacterial inhibitory activity of subfractions against *E.coli* bacteria analyzed using SPSS with a p value>0.05 showing the greatest activity in subfraction 3 with an inhibitory zone diameter of 18.73 mm. All statistical results were significantly different from the negative control which had activity against *E. coli* bacteria. subfractions 1 and 5 are in one subset, while subfractions 2, 4 and 6 are in one subset which shows significant differences with subfraction 3. Subfraction 3 is significantly different from the positive control but not yet the same as the positive control. The subfraction with the highest antibacterial activity is known as the active subfraction, which is measured based on the resulting zone of inhibition. The subfraction that produced the greatest inhibitory power out of the 6 subfractions was subfraction 3, then phytochemical screening and TLC testing were carried out.



Replication 1



Replication 2


**Replication 3**

**Figure 2. Antibacterial Activity Subfraction Test**

Information :

S1: DCM : MeOH (100:0) S2: DCM : MeOH (90:10) S3: DCM : MeOH (80:20) S4: DCM : MeOH (70:30) S5: DCM : MeOH (50:50) S6: DCM : MeOH (0:100)

Positive control (chloramphenicol) Negative control (DMSO 10%)

Table 5. Activity Test Results for Kebo Rubber Leaves Subfractions

| **Sample** | **Diameter of inhibisi zone (mm) replication** | | | **Mean ± SD** |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Subfraction 1 DCM:MeOH (100:0) | 14,3 | 13,6 | 13,3 | 13,73±0,51 (a) |

| | | | | |
|---|---|---|---|---|
| Subfraction 2 DCM:MeOH (90:10) | 15,6 | 16,3 | 15,3 | 15,73±0,51(b) |
| Subfraction 3 DCM:MeOH (80:20) | 19,3 | 18,3 | 18,6 | 18,73±0,51(b) |
| Subfraction 4 DCM:MeOH (70:30) | 15,3 | 14,3 | 14,6 | 14,73±0,51 (c) |
| Subfraction 5 DCM:MeOH (50:50) | 13,3 | 14,3 | 14,6 | 14,06±0,68 (b) |
| Subfraction 6 DCM:MeOH (0:100) | 14 | 15,3 | 14,6 | 14,63±0,65 (b) |
| Positive control | 31,67 | 30 | 31,3 | 30,99±0,87 (a) |
| Negative control | 0 | 0 | 0 | 0 |

Information:
+ = chloramphenicol
- = DMSO 10%
a = one subset (a) is significantly different from K-, K+ b = one subset (b) is significantly different from K-, K+ c = significant difference from a and b

***Phytochemical Screening subfraction 3***

This flavonoid compound is said to have properties as a cell strengthener, against asthma, pain reliever, antibacterial, against diabetes and other activities. Apart from being known to contain flavonoids, the kebo rubber leaf subfraction also contains tannin compounds. Tannin is known to form a blackish green color when FeCl3 reagent is added. Tannins are able to react with proteins to form water-insoluble polymers. The color change is caused by the presence of a complex compound between tannins and FeCl3. Tannins have antidiarrheal, antibacterial and antioxidant properties. According to research results by Ginting *et al*., 2020). Kebo rubber leaves in terms of phytochemical tests (secondary metabolites) contain phenolic chemical compounds, flavonoids, proteins, tannins.

Table 6. Results of Phytochemical Screening for Subfarctions of 3 kebo rubber leaves

| Compound content group | Sample (Subfraction 3) | Results | Conclusion |
|---|---|---|---|
| Alkaloid | DCM: MeOH (80:20) | color, no sediment | - |
| Flavonoid | DCM:MeOH (80:20) | Orange yellow | + |
| Tanin | DCM:MeOH (80:20) | Blackish green | + |
| Saponin | DCM :MeOH (80:20) | No foam | - |
| Triterpenoid | DCM :MeOH (80:20) | Blackish brown | - |
| Steroid | DCM :MeOH (80:20) | Jingga | - |

Information :
+ = Positive according to the literature
- = Negative, does not match the library

***Separation of Compounds by Thin Layer Chromatography (TLC)***

Table 7 shows that the extract, ethyl fraction, subfraction 3 samples showed yellow stains showing ammonia spots, meaning the results were positive for containing flavonoid compounds, whereas the n-hexane fraction and water fraction samples did not show any color (negative). Comparative standard results for quercetin with an Rf value of 0.69, ethyl acetate fraction with an Rf value of 0.69, and subfraction 3 0.69. The comparison standard used is quercetin because one of the best flavonols is generally found in glycosides. The TLC results that have been carried out act as antibacterials, namely flavonoid compounds. The TLC plate observed in visible light showed yellow spots, positive results containing flavonoids with ammonia vapor spray reagent due to the reaction between phenol (acid) and ammonia vapor (base) resulting in a color change that was easily detected in the chromatogram or solution.

Table 7. TLC Test Results for Ethanol Extract, N-hexane Fraction, Ethyl Acetate Fraction, Water Fraction, and Subfraction 3

| Chemical content (spray agent) | Sample | | | | Subfraction 3 | Information |
|---|---|---|---|---|---|---|
| | Extract | n-heksan fraction | etil asetat fraction | Water fraction | | |
| Flavonoid (ammoniak) | + | - | + | - | + | Fluorescent yellow |
| Tanin (FeCl) 5% | + | - | + | - | + | Black |

Identification of tannin compounds using TLC seen in Table 7 shows that the extract, ethyl fraction, subfraction 3 samples showed black stains on the appearance of the 5% $FeCl_3$ spots, meaning the results were positive for containing tannin compounds, while the n-hexane fraction and water fraction samples did not show any color stains. black. The sample and comparison standard used gallic acid with an Rf value in the comparison standard of 0.79, in the extract with an Rf value of 0.81, in the ethyl acetate fraction 0.79 and subfraski 3 with an Rf value of 0.79. Because gallic acid is a class of phenolic compounds and is cheaper than its competitors, gallic acid is usually used as a standard solution. The phenolic content of this organic acid is pure and stable (Marfuah *et al*., 2018).

***Determination of Total Flavonoid Content of the Most Active Subfraction***

The research continued with a quantitative test of all flavonoid intensification using UV-Vis spectrophotometry to determine the total flavonoid content in subfraction 3 with a ratio of DCM:MeOH (80:20) of kebo rubber leaves. Quercetin (QE) is a standard solution used in UV-Vis spectrophotometric measurements. One of the most abundant groups of

flavonoids, quercetin, can react with $AlCl_3$ to form a complex, and the molecular formula of quercetin is almost identical to the molecular formula of flavonoids. Quercetin is a type of flavonoid that is commonly used as a standard for estimating flavonoid levels (Suhaenah et al., 2021). A UV-Vis spectrophotometry instrument with a maximum wavelength of 429.5 nm and an operating time of 30-32 minutes was used for quantitative measurements. Quercetin is a type of flavonoid with many antioxidants. Quercetin is usually used in solution. The absorbance of quercetin standard solutions at concentrations of 20, 40, 60, 80, and 100 ppm was measured. From this analysis, the absorbance value of the standard quercetin solution was obtained, y = 0.0075x, and the coefficient of determination R2 (coefficient of determination) was 0.9987. Absorbance measurements on samples were carried out using UV-Vis spectrophotometry. This measurement was carried out three times on samples of the Ethyl acetate subfraction from kebo rubber leaves to obtain accurate data.

Table 8. Determination of Total Flavonoid Content in Subfraction 3 DCM:MeOH (80:20) Kebo Rubber Leaves

| Replication | Initial total flavonoid content (mg/L) | Total flavonoid levels (mgQE/g subfraction) | Average total flavonoid levels (mgQE/g subfraction) |
|---|---|---|---|
| 1 | 40,2261 | 73,8093 | |
| 2 | 45,9366 | 89,1973 | 79,8980±8,1827 |
| 3 | 41,0229 | 76,6783 | |

Table 8 The aim of determining the total flavonoid content is to determine how much flavonoid content is contained in the kebo leaf extract subfraction. The result is a value of total flavonoid levels in the sample. To obtain accurate data, 3 repetitions were carried out on subfraction 3 of ethyl acetate. The average flavonoid level obtained was 79.8950. Research conducted by (Suhaenah *et al*., 2021). The flavonoid content of the ethyl acetate fraction of Kebo rubber leaves was tested at 74.345 so it was not significantly different from previous research.

## CONCLUSIONS AND RECOMMENDATIONS

From the research results it can be concluded that : 1. Subfractions 1 to 6 have antibacterial activity against *Escherichia coli* bacteria. 2. The most active subfraction is subfraction 3 which provides the most effective antibacterial activity in inhibiting the growth of *Escherichia coli* bacteria compared to the other subfractions. 3. The most active subfraction from subfraction 3 of kebo rubber leaves which has antibacterial activity and antibacterial compound content, namely with a total flavonoid of 79.8950 mgQE/g subfraction, meaning that the flavonoid content in each gram of ethyl acetate subfraction is equivalent to 79.8950 mg of quercetin.

## FURTHER STUDY

Further research can be focused on the identification and characterization of active compounds in subfraction 3 using techniques such as HPLC, FTIR, and mass spectrometry to determine the specific compounds that play a role in antibacterial activity. In addition, it is necessary to conduct activity tests on other bacteria, including resistant strains, as well as an analysis of their antibacterial mechanism of action. Toxicity and safety testing is also important to ensure the feasibility of using these compounds in pharmaceutical formulations. Optimization of extraction methods and product formulations, such as antibacterial ointments or tablets, can support further development as natural antibacterial agents.

## REFERENCES

Adeniyi, O., Olaifa, F., Emikpe, B., & Ogunbanwo, S. (2017). Phytochemical Components and Antibacterial Activity of Tamarindus indica Linn. Extracts against Some Pathogens. Biotechnology Journal International, 17(2), 1–9. https://doi.org/10.9734/bji/2017/30618

Adjeng, A. N. T., Hairah, S., Herman, S., Ruslin, R., Fitrawan, L. O. M., Sartinah, A., … Sabarudin, S. (2020). Skrining Fitokimia dan Evaluasi Sediaan Sabun Cair Ekstrak Etanol 96% Kulit Buah Salak Pondoh (Salacca zalacca (Gaertn.) Voss.) Sebagai Antioksidan. Pharmauho: Jurnal Farmasi, Sains, Dan Kesehatan, 5(2), 3–6. https://doi.org/10.33772/pharmauho.v5i2.10170.

Agustina, E. (2017). Uji Aktivitas Senyawa Antioksidan Dari Ekstrak Daun Tiin (Ficus Carica Linn) Dengan Pelarut Air, Metanol Dan Campuran Metanol-Air. Klorofil: Jurnal Ilmu Biologi Dan Terapan, 1(1), 38. https://doi.org/10.30821/kfl:jibt.v1i1.1240

Bhawana, Robin, Kaur, J., Vig, A. P., Arora, S., & Kaur, R. (2018). Evaluation of antibacterial potential of Ficus species. Journal of Pharmaceutical Sciences and Research, 10(5), 1251–1255.

De Las Llagas, M. C., Santiago, L., & Ramos, J. D. (2014). Antibacterial activity of crude ethanolic extract and solvent fractions of Ficus pseudopalma Blanco leaves. Asian Pacific Journal of Tropical Disease, 4(5), 367–371. https://doi.org/10.1016/S2222- 1808(14)60589-2.

Ding, Z., Tao, T., Wang, L., Zhao, Y., Huang, H., Zhang, D., … Han, J. (2019). Bioprospecting of novel and bioactive metabolites from endophytic fungi isolated from rubber tree ficus elastica leavess. Journal of Microbiology and Biotechnology, 29(5), 731–738. https://doi.org/10.4014/jmb.1901.01015

El-Hawary, S. s. (2012). Antitumor and antioxidant activity of Ficus elastica Roxb.and Ficus bengalensis Linn. Family moraceae 2012 World Applied Sciences Journal.pdf.

Habibi, A. I., Firmansyah, R. A., & Setyawati, S. M. (2018). Skrining Fitokimia Ekstrak nHeksan Korteks Batang Salam (Syzygium polyanthum). Indonesian Journal of Chemical Science, 7(1), 1–4.

Iqbal, Z. (2017a). Fatty Acid Profile of Aerial Roots of Ficus Elastic. World Journal of Pharmaceutical Research, (January), 54–60. https://doi.org/10.20959/wjpr20178-8948

Iqbal, Z. (2017b). Gc-Fid and Physicochemical Studies of Oil From the Leaves of Ficus Elastica Linn. World Journal of Pharmaceutical Research, (January), 47–53. https://doi.org/10.20959/wjpr20178-8946

Kemenkes RI. (2017). Farmakope Herbal Indonesia EDISI II. Kementerian Kesehatan RI. Jakarta. https://doi.org/10.1201/b12934-13

Leonard, A. F. C., Zhang, L., Balfour, A. J., Garside, R., Hawkey, P. M., Murray, A. K., … Gaze, W. H. (2018). Exposure to and colonisation by antibiotic-resistant E. coli in UK coastal water users: Environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey). Environment International, 114(November 2017), 326–333. https://doi.org/10.1016/j.envint.2017.11.003.

Marfuah, I., Dewi, E. N., & Rianingsih, L. (2018). Kajian Potensi Ekstrak Anggur Laut (Caulerpa racemosa) Sebagai Antibakteri Terhadap Bakteri Escherichia coli Dan Staphylococcus aureus. J. Peng. & Biotek. Hasil Pi., 7(2), 1–3.

Pratiwi, L., Fudholi, A., Martien, R., & Pramono, S. (2016). Ethanol Extract, Ethyl Acetate Extract, Ethyl Acetate Fraction, and n-Heksan Fraction Mangosteen Peels (Garcinia mangostana L.) As Source of Bioactive Substance Free-Radical Scavengers. JPSCR : Journal of Pharmaceutical Science and Clinical Research, 1(2), 71. https://doi.org/10.20961/jpscr.v1i2.1936

Radji, M. (2011). Buku Ajar Mikrobiologi. Jakarta: Panduan Mahasiswa Farmasi dan Kedokteran. Suhaenah, A., Pratama, M., & Amir, A. H. W. (2021). Penetapan Kadar Flavonoid Fraksi Etil Asetat Daun Karet Kebo (Ficus elastica) Dengan Metode Spektrofotometri Uv-Vis. AsSyifaa Jurnal Farmasi, 13(1), 48–54.

Verdiana, M., Widarta, I. W. R., & Permana, I. D. G. M. (2018). Pengaruh Jenis Pelarut Pada Ekstraksi Menggunakan Gelombang Ultrasonik Terhadap Aktivitas Antioksidan Ekstrak Kulit Buah Lemon (Citrus limon (Linn.) Burm F.). Jurnal Ilmu Dan Teknologi Pangan (ITEPA), 7(4), 213. https://doi.org/10.24843/itepa.2018.v07.i04.p08

Zukhri, S., & Nurhaini, R. (2019). Antibacterial Effectiveness Test of Ethanol Extract of Kebo Rubber Leaves (Ficus elastica Roxb. ex Hornem.) Against Bacteria. Journal of Health Sciences, 14(01), 93–112