

Antimicrobial activity and phytochemicals compound profile of ethanol extract moringa leaf (*Moringa oleifera*) compounds by FTIR

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ABSTRACT: Moringa plants are known in various parts of the world as a type of vegetable plant that is rich in nutrients and has multiple properties, one of which is as an antimicrobial. This research was conducted to test the antimicrobial inhibition of Moringa leaf decoction and ethanol extract against the growth of *Escherichia coli* and *Aspergillus niger*. The antimicrobial test in this study used the Disc diffusion method using various concentrations, including 25%, 50%, 75%, and 100%. There were two test samples, Moringa leaf decoction and ethanol extract using the maceration method with 96% ethanol solvent. In addition to the test group, there was also a control group, namely a positive control using chloramphenicol and ketoconazole antibiotics, negative control using aquadest as a decoction solvent, and DMSO as an extraction solvent. The ethanol extract was identified by the FTIR method. The results showed that Moringa leaf decoction and Moringa leaf ethanol extract could be used as antibacterial. Inhibition zones on *Escherichia coli* bacteria produced at concentrations of 25%, 50%, and 75% had a weak zone of inhibition category for both decoction and ethanol extract of Moringa leaves. Meanwhile, at a concentration of 100%, the decoction has a weak zone of inhibition and 100% ethanol extract of Moringa leaves can inhibit 8.25 mm which is included in the medium category. The inhibition zones of the fungus *Aspergillus niger* produced at concentrations of 25%, 50%, 75%, and 100% had no inhibition on both the decoction and ethanol extract of Moringa leaves. From the analysis of functional groups using the FTIR method, it can be predicted that in the Moringa leaf extract with methanol as solvent there are groups of phenolic compounds or flavonoids.

KEYWORDS: Antimicrobial, *Moringa oleifera*; screening FTIR.

INTRODUCTION

In health cases, diseases caused by microbes are ranked at the top of the triggers of pain and death in developing countries, including Indonesia. High infectious diseases have a devastating impact on health, lower costs for treatment, and reduced productivity. The spread of the source of this infection can be through various intermediaries or vectors, namely air, weather, animals, inanimate objects, and humans themselves [1]. The disease is often caused by *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* [2]. For the World Health Organization, infectious diseases are the leading cause of death in children. From World Health Organization (WHO) data in 2012, it was reported that infectious diseases caused the mortality rate of children under 5 years old in Indonesia with a percentage of 1-20% [3].

Escherichia coli belongs to Gram-negative bacteria. *Escherichia coli* is a typical bacterium in the intestines, but it becomes a pathogenic bacterium in abnormal conditions. *Escherichia coli* of a pathogenic nature can cause diarrhoea, urinary tract infections, pneumonia, wound infections, especially in the abdomen, and meningitis [4]. According to Rikesdas (Basic Health Research, 2007), diarrhoea disease is a health problem in Indonesia. The morbidity survey conducted by the Sub-Directorate of Diarrhea in the health sector from 2000 to 2010 tended to increase incidence. In 2008, there were extraordinary events in 69 districts with a total of 8133 cases and 239 deaths (CFR (Case Fatality Rate) 2.94%). In 2009, in 24 districts, the number of cases was 5,756 people, with 100 deaths CFR (Case Fatality Rate) 1.74%. Meanwhile, in 2010, there were outbreaks of diarrhoea in 33 districts, with a total of 4204 patients and 73 deaths (CFR) (Case Fatality Rate) of 1.74%. In East Java alone in

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2010, diarrhoea cases occupied the second position after Sulawesi Tengah, with 21 times the frequency of diarrhoea.

Fungus quickly grow in areas that have a tropical climate, such as Indonesia. In recent years, there has been an increase in the incidence caused by fungal infections. *Aspergillus niger* is a multicellular fungus that makes threads. The hyphae that are formed are insulated, and some are not partitioned. Hyphae located above the surface of the medium are called aerial hyphae that play a role in breeding. In contrast, hyphae in the medium are called vegetative hyphae, which absorb nutrients [5]. Aspergillosis is an exogenous infection because microbes enter from the outside into the body through the skin or mucous membranes [6].

Indonesia is a country rich in biodiversity, and many people use the surrounding plants as daily necessities or as traditional medicine. One of the plants around the community that can be used as an antibacterial medicinal ingredient is *Moringa oleifera*, often referred to as Moringa. The Miracle Tree or Moringa plant has been proven by previous research to be a source of nutrition with medicinal properties and a complete content [7]. Moringa leaves are easily found in the community, where people manage Moringa as steeping water has health benefits, including antioxidants and antibacterials because of the content of the Moringa leaves. According to Krisnadi's research, in 2015, Moringa leaves contain active compounds of saponins, flavonoids, alkaloids, and tannins. These compounds can be used as antimicrobials by damaging microbial cell membranes. Based on the description above, researchers are interested in conducting comparative research on antibacterial and antifungal inhibitory tests of infusion and extract of Moringa leaves where people often consume this steeping water as a health drink. In addition, researchers are interested in identifying phytochemical compounds present in Moringa leaves according to certain solvents. This research is expected to be helpful for the world of health, namely reducing the use of antibiotics, which can result in resistance, and using Moringa leaf steeping water is relatively more accessible, safer, and more manageable in its application [8] [9].

▪ MATERIALS AND METHODS

Materials

The equipment used in this study included measuring cups, Erlenmeyer, test tubes, beaker glass, ovens, Petri dishes, blenders, sieve tools, glass jars, rotary evaporators, glass bottles, spiritus or bunsen lamps, autoclaves, incubators, spectrophotometers, drip pipettes, analytical scales, tweezers, ose needles, spatulas, thermometers, callipers, and stirrers. The materials used in this study include Moringa leaves (*Moringa oleifera* L.), *Staphylococcus aureus* bacteria, pure aquadest, 96% ethanol, Nutrient Broth media (NB), filter paper, label paper, Mueller Hinton Agar media (MHA).

Simplicia making

Moringa leaf collection from Puri Village, Puri District, Mojokerto Regency, East Java Province. Wet Disorientation to separate fresh Moringa leaves from yellowed Moringa leaves. The process of washing Moringa leaves is carried out using running water. In drying, Moringa leaves in the oven at a temperature of 40°C, up to simplicia levels of less than 10%. Moringa leaves that have been dried are then ground into powder to facilitate extraction.

Infusion

A total of 10 grams of simplicia powder is put in a 250 mL glass beaker filled with 100 mL of water. After that, it is heated in a water bath at a temperature of 90°C for 15 minutes while stirring once. After filtering with flannel using a funnel, the filter results are stored in a 100 mL measuring pumpkin flask. The result of the sieve is a sufficient volume of hot water poured on a sediment [10].

Extract

The maceration procedure is done by soaking the simplicia powder with a suitable grinding solution for three days at room temperature and safe from rays. During the maceration process, the grinding solution is stirred and exchanged every day. The sediment obtained is separated, and the filtrate is concentrated using a rotary evaporator [11].

Phytochemical screening

Alkaloid test

Weigh 0.1 g of simplicia, put 5 ml of 10% hydrochloric acid, mix well and then add 5 mL of 10% ammonia solution. The extract uses 10 mL of chloroform. For evaporation residue, add 1.5mL of 2% hydrochloric acid, then add three drops of Dragendroff reagent to the second tube until a brick-red precipitate is formed, indicating the presence of alkaloids [12].

Steroid test

Weigh 0.1 g of extracted simplicia into 10 mL of ether. A total of 0.5 ml of solution was tested with the Lieberman Burchard reagent. The formation of a blue or green indicates the presence of steroids [12].

Flavonoid test

Weigh 0.1 g of simplicia dissolved in 2.5 mL of water, put in a water bath, then put in a test tube and add 100mg of magnesium powder, 1mL of concentrated hydrochloric acid, and 3mL of amyl alcohol, shake vigorously, and let separate, red, yellow, and orange colours on the amyl alcohol layer indicate the presence of flavonoid compounds [12].

Saponin test

Weigh 0.1 g of simplicia, dissolve in 2.5 mL water, put in a water bath, transfer to a tube, and beat vertically for 10 seconds until stable foam forms. Let stand for 10 minutes, and add one drop of hydrochloric acid; if the foam does not disappear, there are saponins [12].

Tanin test

Weigh 1g of simplisia plus 10% NaCl by five drops, strain, and then add 1% gelatin and 10% NaCl. The formation of a white precipitate indicates the presence of tannins contained in simplisia [13].

Polifenol test

Weigh 0.1 g of simplisia dissolved in 2.5 ml of water, put in a water bath, and add a few drops of 1% ferrous (III) chloride solution to the test tube. The formation of a dark blue or green-black filtrate indicates the presence of polyphenols [12].

Antifungal activity test

Suspension of Fungus in a thorough swab on all surfaces of PDA media, then allowed to stand for approximately 5 minutes, disc paper that has been soaked for 15 minutes in maceration extract, and infusion of Moringa leaves that have been prepared concentrations of 100%, 75%, 50%, 25% and control (positive & negative). Then using pure tweezers, the disc paper is placed on a pure Petri cup for 1 minute until there is no dripping solution. After that, the disc paper is placed on the surface of the PDA media and pressed slightly so that it sticks. PDA incubated at 37 °C for 24 hours.

Antibacterial activity test of disc diffusion method

Bacterial suspension in a thorough swab on the surface of the Mueller Hinton Agar (MHA) medium, then allowed to stand for approximately 5 minutes. The disc paper that has been soaked for approximately 15 minutes in maceration extract and moringa leaf infusion has been prepared with a concentration of 100%, 75%, 50%, 25%, and control (negative and positive). Using sterile tweezers, the disc paper is placed on a sterile petri dish for approximately 1 minute until there is no dripping solution. After that, the disc paper is placed on the surface of the MHA media and pressed slightly so that it sticks. MHA media was incubated at 37 °C for 24 hours [14].

FTIR analysis

The extract is weighed as much as 0.0020 g, placed on parchment paper and then labelled and set aside. We weighed 0.1980 g KBr on parchment paper and then set aside. Then, each sample is smoothed and moulded to form a thin (transparent) plate. The sample is read using the FTIR-Bruker alpha tool. Furthermore, the resulting chromatogram is compared with the IR table [15].

Data analysis

The inhibition zone diameter data is expressed as the average \pm SD. The data were analyzed using the Kruskal-Wallis method with a confidence level of 5%.

RESULTS

Characteristics of ethanol extract and decoction

The sample used in this study was fresh Moringa leaves obtained from Randugenengan Village, Dlanggu District, Mojokerto Regency, which had previously been determined at the Purwodadi Botanical Garden Plant Conservation Center, which showed that the sample of this study was *Moringa oleifera*. Moringa leaf simplivia (*Moringa oleifera*) is in the form of dried simplicia, fresh green, tasteless, and has a characteristic aroma. Moringa leaf simplicia shown in Table 1.

Table 1. Extract characteristics

| No. | Parameter | Moringa Leaf Ethanol Extract | Moringa Leaf Decoction |
|-----|-----------|------------------------------|------------------------|
| 1. | Shape | Extract viscous | Extract liquid |
| 2. | Colour | Blackish Green | Brown |
| 3. | Aroma | Aromatic | Aromatic |

The results of Moringa leaf simplicia (*Moringa oleifera*) were then extracted using two methods: extraction by maceration method and extraction by boiling Moringa leaf simplicia (*Moringa oleifera*). Extraction of maceration method on moringa leaf simplicia sample (*Moringa oleifera*) as much as 250 g using 96% ethanol solvent as much as 1875 ml. Obtained a thick extract of 32.19 g with % a marinade of 12.876%.

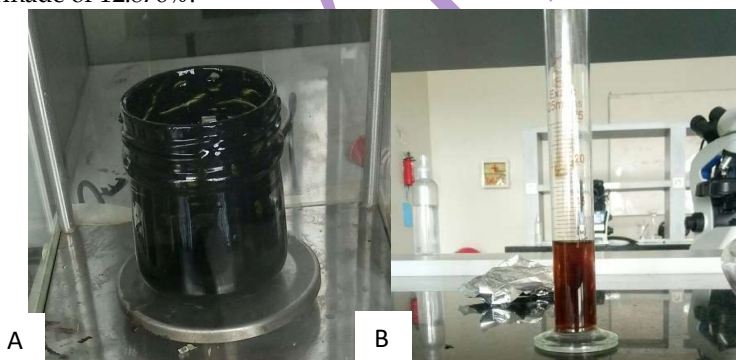


Figure 1. The result of maceration extraction and decoction of Moringa leaves
A = Moringa leaf ethanol thick extract; B = Liquid extract of Moringa leaf decoction

The viscous extract of Moringa leaves has a characteristic blackish-green colour and a distinctive aroma. The extraction results by the decoction method have the characteristics of a liquid extract, brown in color, and a distinct smell. It is shown in Figure 1.

Phytochemical screening

The results obtained in the phytochemical screening test of Moringa leaf decoction (*Moringa oleifera*) can be seen in Table 2.

Table 2. Phytochemical test of moringa leaf decoction

| No. | Screening test | Observation | Result |
|-----|---|--|--------|
| 1. | Alkaloid Filtrat C + reagen dragendorf Filtrate D + reagen Wagner | The brown precipitate formed Redness. | + + |
| 2. | Flavonoid Filtrate B + HCL Filtrat → Heat | Dark red Orange | + + |

| | | | |
|----|--|--|---|
| 3. | Filtrate C + HCL + powder Mg Saponin Ekstark + water → strong shake | Froth can last more than 10 minutes | + |
| 4. | Tanin Filtrat + gelatin salt | There is a white residue. | + |
| 5. | Steroid Filtrat B + asam asetat anhidrat + H ₂ SO ₄ pekat Filtrat C + H ₂ SO ₄ | There is a change in color to yellow There is a red ring | + |
| 6. | Polyphenol Filtrate + FeCl ₃ | Discoloration occurs to greenish-black | + |

Description: (+) = contains the compound in question

The results obtained in the phytochemical screening test of Moringa leaf extract (*Moringa oleifera*) can be seen in Table 3.

Table 3. Phytochemical test of moringa leaf ethanol extract

| No. | Testing | Observation | Result |
|-----|--|---|--------|
| 1. | Alkaloid Filtrat C + reagen dragendorf Filtrat D + reagen Wagner | The brown precipitate formed Brown precipitate formed Redness | + + |
| 2. | Flavonoid Filtrat B + HCL Filtrat → Heat Filtrate C + HCL + powder Mg | Dark red Orange | + + |
| 3. | Saponin Ekstark + water → strong shake | Froth can last more than 10 minutes | + |
| 4. | Tanin Filtrat + gelatin salt | There is a white residue. | + |
| 5. | Steroid Filtrat B + asam asetat anhidrat + H ₂ SO ₄ pekat Filtrat C + H ₂ SO ₄ | There is a change in color to yellow There is a red ring | + + |
| 6. | Polyphenol Filtrate + FeCl ₃ | Discolouration occurs to greenish-black | + |

Description: (+) = contains the compound

The results of the phytochemical test on Moringa leaf ethanol extract are the same as the phytochemical test of Moringa leaf decoction. In the phytochemical test of Moringa leaves, the results were obtained that the decoction of Moringa leaves and ethanol extract of Moringa leaves contained alkaloids when added dragendorf reagents and Wagner reagents were characterized by the formation of reddish-brown deposits. Contains flavonoid compounds, characterized by a change in colour to dark red and orange after treatment. It contains tannins, forming foam that can last more than 10 minutes. It contains steroids, characterized by the occurrence of a change in colour to yellow, and there is a red ring. It contains polyphenols that are characterized by a change in colour to greenish-black.

Antifungal activity test

The results obtained in the inhibition zone test of Moringa leaf decoction (*Moringa oleifera*) against *Aspergillus niger* can be seen in Table 4.

Table 4. *Aspergillus niger* inhibitory zone against Moringa Leaf Decoction and Ethanol Extract

| No | Concentration | Decoction Inhibition Zone (mm) | Category | Extract Inhibition Zone (mm) | Category |
|----|---------------|--------------------------------|-------------|------------------------------|-------------|
| 1 | Control (+) | 5,25 | Weak | 5,5 | Weak |
| 2 | Control (-) | 0 | No activity | 0 | No activity |

| | | | | | |
|---|------|---|-------------|---|-------------|
| 3 | 25% | 0 | No activity | 0 | No activity |
| 4 | 50% | 0 | No activity | 0 | No activity |
| 5 | 75% | 0 | No activity | 0 | No activity |
| 6 | 100% | 0 | No activity | 0 | No activity |

The result of measuring the diameter of the inhibition zone was reduced by the diameter of the disc paper, which was 6 mm. In the sample, there is no diameter of the inhibition zone on *Aspergillus niger*.

Antibacterial activity of disc diffusion method

The results obtained in the inhibition zone test of Moringa leaf decoction (*Moringa oleifera*) against *Aspergillus niger* can be seen in Table 5.

Table 4. *Escherichia coli* inhibition zone against moringa leaf decoction and ethanol extract

| No | Concentration | Decoction Inhibition Zone (mm) | Category | Extract Inhibition Zone (mm) | Category |
|----|---------------|--------------------------------------|-------------|------------------------------------|--------------|
| 1 | Control (+) | 11,25 | Strong | 10,25 | Strong |
| 2 | Control (-) | 0 | No activity | 0 | No activity |
| 3 | 25% | 2,75 | Weak | 2,75 | Weak |
| 4 | 50% | 3,75 | Weak | 4,75 | Weak |
| 5 | 75% | 4,25 | Weak | 5,5 | Weak |
| 6 | 100% | 5,75 | Weak | 8,25 | Intermediate |

The result of measuring the diameter of the inhibition zone was reduced by the diameter of the disc paper, which was 6 mm. In the sample, the diameter of the inhibitory zone is in the weak and medium categories of *Escherichia coli*.

Bioactive components in plants are generally present in relatively small amounts. An extraction technique is a technique that can obtain an extract with a high yield without altering most of the functional properties of the extract. The withdrawal of bioactive compounds extracted from Moringa leaves is the first step in using phytochemicals to develop dietary supplement products, foodstuffs, pharmaceutical products, antimicrobial agents, and antifungal agents.

The study's results have examined the antimicrobial activity of aqueous extracts and ethanol extracts from Moringa leaf simplicia. Moringa water extracts showed different levels of activity against the growth of *Aspergillus niger*, and *Escherichia coli* showed low antimicrobial activity. Antifungal and antibacterial activity in aqueous extracts is less than optimal compared to ethanol extracts. Water solvents are less than optimal in carrying out the withdrawal process of bioactive compounds compared to ethanol solvents [16].

The yield of the extraction process is influenced by one of them, which is the type of solvent. In research by Rahmi et al. (2021), variations of the extract method and the type of solvent used to obtain the extract with good biological activity were done. The study's results obtained the highest total phenolic obtained from ethanol extract. Selecting practical and efficient solvents is one of the ultimate targets of extracting natural materials.

The test results showed that extracts with ethanol solvents were 96% more optimal in antimicrobial activity than extracts with water solvents. Phenolic components are generally more easily soluble in polar organic solvents according to the principle of "like dissolve like". The high solubility of phenolics in ethanol solvents leads to a high concentration of this compound in the extract obtained using ethanol solvents. Ethanol solvents can also damage the structure of cell compartments and efficiently penetrate cell membranes, thereby allowing the extraction of high amounts of endocellular components.

Table 5. Mann-Whitney *Escherichia coli* test against moringa leaf decoction

| | K+ | K- | 25% | 50% | 75% | 100% |
|------|-----------------------|-----------------------|-----------------------|------------------------|------------------------|-----------------------|
| K+ | - | 0,011 ^(BS) | 0,015 ^(BS) | 0,015 ^(BS) | 0,015 ^(BS) | 0,015 ^(BS) |
| K- | 0,011 ^(BS) | - | 0,011 ^(BS) | 0,011 ^(BS) | 0,011 ^(BS) | 0,011 ^(BS) |
| 25% | 0,015 ^(BS) | 0,011 ^(BS) | - | 0,040 ^(BS) | 0,015 ^(BS) | 0,015 ^(BS) |
| 50% | 0,015 ^(BS) | 0,011 ^(BS) | 0,040 ^(BS) | - | 0,186 ^(BTS) | 0,015 ^(BS) |
| 75% | 0,015 ^(BS) | 0,011 ^(BS) | 0,015 ^(BS) | 0,186 ^(BTS) | - | 0,022 ^(BS) |
| 100% | 0,015 ^(BS) | 0,011 ^(BS) | 0,015 ^(BS) | 0,015 ^(BS) | 0,022 ^(BS) | - |

Information: BTS = Differences are not Significant ($p < 0,05$)
BS = Significant Differences ($p > 0,05$)

Table 4.2 Mann-Whitney *Escherichia coli* test against Moringa leaf ethanol extract

| | K+ | K- | 25% | 50% | 75% | 100% |
|------|------------------------|-----------------------|-----------------------|------------------------|------------------------|------------------------|
| K+ | - | 0,011 ^(BS) | 0,015 ^(BS) | 0,015 ^(BS) | 0,017 ^(BS) | 0,082 ^(BTS) |
| K- | 0,011 ^(BS) | - | 0,011 ^(BS) | 0,011 ^(BS) | 0,013 ^(BS) | 0,011 ^(BS) |
| 25% | 0,015 ^(BS) | 0,011 ^(BS) | - | 0,015 ^(BS) | 0,017 ^(BS) | 0,015 ^(BS) |
| 50% | 0,015 ^(BS) | 0,011 ^(BS) | 0,015 ^(BS) | - | 0,231 ^(BTS) | 0,015 ^(BS) |
| 75% | 0,017 ^(BS) | 0,013 ^(BS) | 0,017 ^(BS) | 0,231 ^(BTS) | - | 0,017 ^(BS) |
| 100% | 0,082 ^(BTS) | 0,011 ^(BS) | 0,015 ^(BS) | 0,015 ^(BS) | 0,017 ^(BS) | - |

Information: BTS = Differences are not Significant ($p < 0,05$)
BS = Significant Differences ($p > 0,05$)

From the results of the analysis of Mann-Whitney decoction of Moringa leaves shown in the table, there were insignificant or meaningless differences in the control (+) with a concentration of 100% and a concentration of 25%, 50%, and 75%. In the Moringa leaf ethanol extract shown in the table, there was a significant difference in the control (+) with a concentration of 100%, and there was an insignificant or meaningless difference in concentrations of 25%, 50%, and 75%.

FTIR analysis

The FTIR analysis showed that the fraction of Moringa leaf extract in ethanol, ethyl acetate, and n-hexane solvents absorbed several functional groups. The IR spectrum is shown in Table 5.

Table 4.4. Peak Spectra FTIR Moringa leaf extract with solvent ethyl acetate, ethanol, n-hexane

| Ethanol Solvents | | | |
|-----------------------------|-------------------------------------|--------------------------------------|------------------|
| No. | Gugus Fungsi | Wavenumbers (cm⁻¹) | Intensity |
| 1 | Gugus halogen | 669,06 | Sharp |
| 2 | Gugus halogen | 706,33 | Sharp |
| 3 | Gugus halogen | 779,01 | Sharp |
| 4 | Gugus halogen | 877,78 | Sharp |
| 5 | Gugus halogen | 916,93 | Sharp |
| 6 | Gugus halogen | 982,15 | Sharp |
| 7 | Gugus halogen | 1026,88 | Sharp |
| 8 | Eter | 1058,56 | Sharp |
| 9 | Eter | 1339,98 | Sharp |
| 10 | Nitro aromatic (C-NO ₂) | 1405,21 | Sharp |
| 11 | Nitro aromatic (C-NO ₂) | 1507,71 | Sharp |
| 12 | Nitro aromatic (C-NO ₂) | 1541,25 | Sharp |
| 13 | Nitro aromatic (C-NO ₂) | 1559,89 | Sharp |
| 14 | Amina (N-H) | 1653,07 | Sharp |
| 15 | Alil nitril (C=N) | 2359,40 | Sharp |
| 16 | Alkohol (O-H) | 3259,56 | Widened |
| Etil Asetat Solvents | | | |
| No. | Gugus Fungsi | Wavenumbers (cm⁻¹) | Intensity |
| 1 | Asilhalida (C=O) | 1749,98 | Sharp |
| 2 | Double bond 3 | 2359,4 | Sharp |
| 3 | Aldehyd | 2847,68 | Sharp |
| 4 | Alkana (C-H) | 2922,23 | Sharp |
| n-hexane Solvents | | | |
| No. | Gugus Fungsi | Wavenumbers (cm⁻¹) | Intensity |
| 1 | Asilhalida (C=O) | 1749,98 | Sharp |
| 2 | Aldehyd | 2851,43 | Sharp |
| 3 | Alkana (C-H) | 2920,36 | Sharp |

DISCUSSION

The amount of the inhibitory zone obtained in the sample decoction or ethanol extract of Moringa leaves with the largest concentration proves the result of the largest inhibitory zone size in inhibiting bacterial growth. This condition is influenced by the fact that the content of active compounds in the greatest concentration is more significant than in low concentrations. The wider the inhibitory area formed around the disc paper, the greater the antibacterial power in the Moringa leaves.

The antibacterial force produced by decoction or ethanol extract of Moringa leaves is linear with the amount of concentration. This means that the greater the moringa leaf decoction or extract concentration, the greater the diameter of the resulting inhibitory zone. Antibacterial activity is caused by the presence of phytochemical compounds or antibacterial compounds that can inhibit the development of bacteria or cause bacterial cell death by inhibiting the mechanism of inhibition of bacterial cell wall formation, inhibiting the function of their respective cell components, inhibiting protein formation needed by bacterial cells or inhibition in forming nucleic acid compounds.

The phytochemical tests that have been carried out show that Moringa leaves have secondary metabolites in the form of alkaloids, flavonoids, saponins, tannins, steroids, and phenols. According to Haryoto (2021) and Retnowati et al. (2011), chemical compounds have antibacterial effects due to their phenol content and derivatives, such as flavonoids, saponins, tannins, and alkaloids. Bacterial inhibition occurs due to the reaction of a chemical compound as an antibacterial [18], [19].

Flavonoid compounds provide a mechanism for bacterial inhibition because flavonoids can create complex compounds with proteins, denature bacterial cell proteins, and interfere with the formation of cytoplasmic membranes with the destruction of proteins in bacterial cells, resulting in bacterial metabolic activities being inhibited, causing bacterial cell death. [20]. Other studies state that the mechanism of flavonoids inhibits cell membranes' function by disrupting cell membrane permeability and inhibiting the bonding of enzymes such as ATPase and phospholipase [21].

Alkaloids have antibacterial activity. The method predicted the mechanism of activity by disturbing the stability of the constituent units of peptidoglycan in bacterial cells so that the arrangement of cell walls is not optimal in their formation so that cell death occurs [22].

Saponins with antibacterial activity can cause leakage of proteins and enzymes from the cell. Saponins can inhibit the growth of bacteria because the surface active substance is closely similar to detergents, and the mechanism uses saponins to lower the surface tension of the bacterial cell wall. Destroying membrane permeability results in increased permeability or cell leakage and the emergence of intracellular compounds [23]. The destruction of bacterial cell membranes can interfere with the survival of bacteria. Saponins diffuse through the outer membrane and cell wall, which afterwards binds to the cytoplasmic membrane, impairing cell membrane stability. This can cause the cytoplasm to leak and cause cell death [24].

Tannins are a group of polyphenol compounds that have antibacterial activity. Tannin activity as an antibacterial is predicted to damage cell walls or cell tissues and damage the permeability of the cell itself. Due to the disruption of permeability, cells cannot carry out cell life activities, so their growth is inhibited, or cell death occurs [25]. The mechanism of action of steroids in antibacterial is related to lipid membranes and sensitivity to steroid components that cause liposome leakage. Steroids can interact with cell phospholipid membranes, causing membrane integrity to decrease and causing the morphology of cell membranes to change so that cells will be brittle and lysis [26].

The inhibitory zone produced in the decoction is relatively tiny compared to the inhibitory zone produced by Moringa leaf ethanol extract. This is because, in the decoction method, *simplicia* is extracted by a heating process with a water solvent. Heating during the extraction process can cause damage to the secondary metabolites of the plant [27]. Bioactive compounds such as flavonoids, tannins, and phenols can be damaged at temperatures above 50°C because there can be changes or damage to chemical structures and produce secondary metabolite compounds in small amounts [28] so that the inhibition zone created by the decoction is smaller than that of the extract.

The weakness of this study is that the resulting inhibitory zone is minimal. Previous studies that have been carried out by [4] regarding the test of moringa leaf extract activity against *Escherichia coli* and *Staphylococcus aureus* bacteria using the suitable method at a concentration of 80% have an inhibitory zone of 20.50 mm which is included in the strong category of *Staphylococcus aureus*. In comparison, *Escherichia coli* has an inhibitory zone of 22.66 mm, which is a powerful inhibitory zone. The resulting difference in the inhibitory zone is due to differences in testing methods.

The research (Dima et al., 2016) used the excellent method, while the study used the Disc diffusion method. This is because, in the excellent method, the extract can be directly inserted into each hole so that the effect aims to limit bacteria to be stronger. On the other hand, in the Disc diffusion method, the disc must be soaked in a drip plate containing Moringa leaf ethanol extract and Moringa leaf decoction, and then the disc is placed on an agar medium. The excellent method can create a more significant inhibition zone diameter, and the antibacterial mechanism in the excellent method is utilizing the osmolarity of a more significant extract concentration. In the excellent method, each hole is filled with extract concentrations until osmolarity occurs more widely and homogeneously, and the concentration of extracts obtained is larger and more robust, limiting the development of germs [29]. In addition, isolates move not only on the upper surface of nutrients in order but also to the bottom [30]. The weakness of this disc diffusion test is that the clear zone parameters created are related to the incubation situation, inoculum, diffusion, preincubation, and medium thickness. Also, this disc method cannot be applied to a microorganism that experiences growth lag [31].

Moringa leaf extract results using different solvents were then analyzed with FTIR to see functional groups based on the intensity of infrared rays absorbed by the extract compounds. FTIR spectra of extracted compounds prove typical uptake in some functional groups. Moringa leaf extract with ethanol solvent indicates a vibration of bending molecules. At wave values of 3259.56 cm⁻¹ with widened peaks proving the vibration of the hydroxy functional group (-OH), wave numbers 1653.07 cm⁻¹ demonstrating the typical uptake of the amine functional group (N-H), wave numbers 1405, 21; 1507, 71; 1541, 25; 1559.89 cm⁻¹ proved the absorption of the functional group Nitro aromatic (C-NO₂), wave number 1339.98; 1058,56; 982,15; indicates the absorption of the ether functional group, wave number 1026.88; 982,15; 916,93; 877,78; 779,01; 706,33; 669.06 indicates uptake of halogen group function. From the analysis of phenol groups, it can be predicted that there are phenolic compounds or flavonoids in Moringa leaf extract with methanol solvents. It is characterized by the uniqueness of phenolic compounds or flavonoids in their O-H group.

▪ CONCLUSION

The application of boiled water and ethanol extract of Moringa leaves (*Moringa oleifera*) can inhibit the growth of *Escherichia coli* bacteria characterized by forming clear zones around the disc paper but is included in the moderate category at a concentration of 100%. The application of boiled water and ethanol extract of Moringa leaves (*Moringa oleifera*) cannot inhibit the growth of the fungus *Aspergillus niger*, characterized by the non-formation of clear zones around the disc paper. The FTIR results of Moringa leaf extract in polar solvents are suspected to have phenolic compounds, namely flavonoids and tannins.

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