JURNAL ILMU KEFARMASIAN INDONESIA
Indonesian Journal of Pharmaceutical Sciences
ISSN p-ISSN 1693-1831 (Print) e-ISSN 2614-6495 (Online) PUBLISHER : Faculty of Pharmacy Universitas Pancasila

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

Arista Wahyu Ningsih[1*], Aulia Dinda Safira[2], Andre Giovano[2], Ivan Charles S. Klau[1]

[1]*Biology Pharmacy Department, Faculty of Health Sciences, Anwar Medika University, Sidoarjo, 61262, Indonesia*
[2]*Bachelor of Pharmacy Program, Faculty of Health Sciences, Anwar Medika University, Sidoarjo, 61262, Indonesia*

* Corresponding Author e-mail: ariessmkkes@gmail.com

Received: 12 November 2021/ Accepted: 24 April 2024

ABSTRACT: 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 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 using 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* 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.

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

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 (WHO), infectious diseases are the leading cause of death in children. From WHO data in 2012, it was reported that infectious diseases caused the mortality rate of children under five 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 diarrhea, urinary tract infections, pneumonia, wound infections, especially in the abdomen, and meningitis [4]. According to Rikesdas (Basic Health Research, 2007), diarrhea 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 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 diarrhea in 33 districts, with a total of 4204 patients and 73 deaths (CFR) (Case Fatality Rate) of 1.74%. In East Java alone in 2010, diarrhea cases occupied the second position after Central Sulawesi, with 21 times the frequency of diarrhea.

Fungus quickly grows 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

How to cite this article: Ningsih AW, Safira AD, Giovano A, S.Klau IC. Antimicrobial activity and phytochemicals compound profile of ethanol extract Moringa leaves (Moringa oleifera) compounds by FTIR. JIFI. 2024; 22(1): 111-121.

https://doi.org/10.35814/jifi.v22i1.1231
ISSN: 2614-6495

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

Preparation of sample

Moringa leaves collection from Puri Village, Puri District, Mojokerto Regency, East Java Province. Wet Disoration 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 40^0C, 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 Moringa leaves 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 90^0C 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 involves soaking the Moringa leaves powder in a suitable grinding solution for three days at room temperature and away from sunlight. During this time, the grinding solution is stirred and exchanged daily. 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 1.5 mL 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 moringa leaves powder into 10 mL of ether. A 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 moringa leaves powder dissolved in 2.5 mL of water, put in a water bath, then place 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 moringa leaves powder, dissolve it in 2.5 mL water, put it in a water bath, transfer it to a tube, and beat vertically for 10 seconds until stable foam forms. Let it stand for 10 minutes, and add one drop of hydrochloric acid; if the foam does not disappear, there are saponins [12].

Tannin test

Weigh 1g of moringa leaves powder 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 moringa leaf powder [13].

Polyphenol test

Weigh 0.1 g of moringa leaves powder 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 ^{o}C for 24 hours.

Antibacterial activity test of disc diffusion method

Bacterial suspension in a through 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 ^{0}C for 24 hours [4].

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 [14].

Data analysis

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

RESULTS

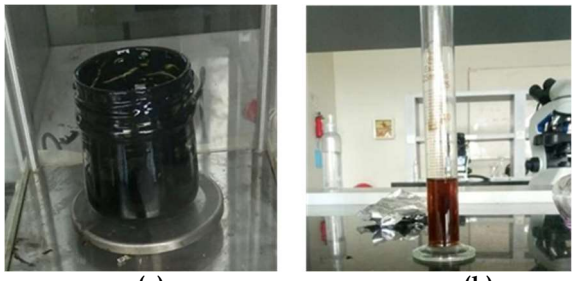
Characteristics of ethanol extract and decoction

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 simplicia is dried simplicia, fresh green, tasteless, and has a characteristic aroma (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 leaves powder were then extracted using two methods: extraction by maceration method and extraction by boiling Moringa leaf simplicia. Extraction of maceration method on moringa leaves powder 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 percent a marinade of 12.876%.



(a) (b)

Figure 1. The result of maceration extraction and decoction of Moringa leaves (a) moringa leaves powder ethanol thick extract; (b) liquid extract of Moringa leaves powder 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 of the phytochemical screening test of Moringa leaves powder decoction are shown in Table 2.

Table 2. Phytochemical test of moringa leaf decoction.

No	Screening test	Observation	Result
1	Alkaloid		
	Filtrate C + Dragendorf reagent	The brown precipitate formed	+
	Filtrate D + Wagner reagent	Redness	+
2	Flavonoid		
	Filtrate B + HCl filtrate → Heat	Dark red	+
	Filtrate C + HCl + Mg powder	Orange	+
3	Saponin		
	Extract + water → strong shake	Froth can last more than 10 minutes	+
4	Tannin		
	Filtrate + gelatin salt	A white precipitate is formed	+
5	Steroid		
	Filtrate B + anhydrous acetic acid + H_2SO_4	Color change to yellow	+
	Filtrate C + H_2SO_4	A red ring is formed	+
6	Polyphenol		
	Filtrate + $FeCl_3$	Discoloration occurs to greenish-black	+

Description: (+) = contains the compound

Table 3 shows the results obtained in the phytochemical screening test of a Moringa leaf powder extract (Moringa oleifera).

Table 3. Phytochemical test of moringa leaf ethanol extract.

No	Screening test	Observation	Result
1	Alkaloid		
	Filtrate C + Dragendorf reagent	The brown precipitate formed	+
	Filtrate D + Wagner reagent	Redness	+
2	Flavonoid		
	Filtrate B + HCl filtrate → Heat	Dark red	+
	Filtrate C + HCl + Mg powder	Orange	+
3	Saponin		
	Extract + water → strong shake	Froth can last more than 10 minutes	+
4	Tannin		
	Filtrate + gelatin salt	A white precipitate is formed	+
5	Steroid		
	Filtrate B + anhydrous acetic acid + H_2SO_4	Color change to yellow	+
	Filtrate C + H_2SO_4	A red ring is formed	+
6	Polyphenol		
	Filtrate + $FeCl_3$	Discoloration occurs to greenish-black	+

Description: (+) = contains the compound

The results of the phytochemical test on Moringa leaves powder 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 leaves decoction against *Aspergillus niger* can be seen in Table 4.

Table 4. *Aspergillus niger* inhibitory zone against Moringa leaves 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. 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 [15],[16].

Table 5. *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 yield of the extraction process is influenced by one of them, which is the type of solvent. 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 "like dissolve like" principle. 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.

From the results of the analysis of Mann-Whitney decoction of Moringa leaves shown in Table 6, 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 Table 7, 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%.

Table 6. 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)	-

Notes: BTS = Differences are not Significant (p<0.05) BS = Significant Differences (p>0.0)

Table 7. 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)	-

Notes: BTS = Differences are not Significant (p<0.05) BS = Significant Differences (p>0.0)

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 8.

Table 8. Peak Spectra FTIR Moringa leaf extract with solvent ethyl acetate, ethanol, and n-hexane.

Ethanol Solvents			
No	**Functional groups**	**Wavenumbers (cm^{-1})**	**Intensity**
1	Halogen	669.06	Sharp
2	Halogen	706.33	Sharp
3	Halogen	779.01	Sharp
4	Halogen	877.78	Sharp
5	Halogen	916.93	Sharp
6	Halogen	982.15	Sharp
7	Halogen	1026.88	Sharp
8	Ether	1058.56	Sharp
9	Ether	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	Amine (N-H)	1653.07	Sharp
15	Alyl nitrile (C=N)	2359.40	Sharp
16	Alcohol (O-H)	3259.56	Widened
Ethyl acetate solvents			
No	**Functional groups**	**Wavenumbers (cm^{-1})**	**Intensity**
1	Acylhalide (C=O)	1749.98	Sharp
2	Double bond 3	2359.4	Sharp
3	Aldehyde	2847.68	Sharp
4	Alkane (C-H)	2922.23	Sharp
n-hexane solvents			
No	**Functional groups**	**Wavenumbers (cm^{-1})**	**Intensity**
1	Acylhalide (C=O)	1749.98	Sharp
2	Aldehyde	2851.43	Sharp
3	Alkane (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[17]. 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 compound [23]. The destruction of bacterial cell membranes can interfere with the survival of bacteria. Saponins diffuse through the outer membrane and cell wall, which afterward 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 ^{o}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 utilizes 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; 1541.5; 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.

Acknowledgments: This research was carried out thanks to everyone's support. Thank you to Anwar Medika University, lecturers, and students for their support in completing this research.

Funding: Thank you to Anwar Medika University for their funding support in completing this research.

Conflict of interest statement: The authors state no conflict of interest exists.

REFERENCES

[1] A. N. Ramadhani, "Karakteristik Es Krim Berbahan Ekstrak Daun Kelor (Moringa oleifera) dengan Variasi Konsentrasi Susu Bubuk Full Cream dan Karagenan," *Skripsi*, 2020.

[2] P. I. Widiani, K. Januartha, and P. Pinatih, "Uji Daya Hambat Ekstrak Etanol Daun Kelor (Moringa oleifera) Terhadap Pertumbuhan Bakteri Methicillin-Resistant Staphylococcus aureus (MRSA)," *J. Med. Udayana*, vol. 9, no. 3, pp. 22–28, 2020, [Online]. Available: https://ojs.unud.ac.id/index.php/eum

[3] Zein U, "Diare Akut Disebabkan Bakteri," *J. Med Udayana*, vol. 9, no. 1, pp. 22–27, 2020.

[4] L. L.R.H. Dima, Fatimawali, and W. Astuty Lolo, "Uji Aktivitas Antibakteri Ekstrak Daun Kelor (Moringa oleifera L.) Terhadap Bakteri Escherichia coli dan Staphylococcus aureus," *PHARMACONJurnal Ilm. Farm.*, vol. 5, no. 2, pp. 282–289, 2016.

[5] Delina S, A. Y, and S. NMI, "Pengaruh Ekstrak Dan Fraksı Daun Afrıka (Vernonia amygdalina Delile) Terhadap Pertumbuhan Jamur Candida albicans Pendahuluan Penyakit infeksi masih menempati urutan tertinggi penyebab kesakitan dan kematian di negara-negara berkembang , termasuk Indonesia," vol. 4, no. 3, pp. 1–15, 2019.

[6] N. Ariani and A. Riski, "Aktivitas Ekstrak Etanol Kulit Buah Pisang Kepok Mentah (Musa paradisiaca forma typica) Terhadap Pertumbuhan Candida albicans Secara In Vitro," *J. Pharmascience*, vol. 5, no. 1, pp. 39–44, 2018, doi: 10.20527/jps.v5i1.5784.

[7] E. C. Purba, "KELOR (Moringa oleifera Lam.): PEMANFAATAN DAN BIOAKTIVITAS," *Pro-Life*, vol. 7, no. 1, pp. 1–12, 2020, doi: 10.33541/jpvol6iss2pp102.

[8] V. Anggi, J. Tandi, and V. Veronika, "TOTAL FLAVONOID DAN EFEKTIVITAS EKSTRAK ETANOL BIJI KELOR (Moringa oleifera L) ASAL KOTA PALU SULAWESI TENGAH TERHADAP HISTOPATOLOGI PANKREAS TIKUS PUTIH JANTAN (Rattus norvegicus) YANG DIINDUKSI STREPTOZOTOCIN," *J. Ilm. Manuntung*, vol. 6, no. 1, pp. 24–31, 2020, doi: 10.51352/jim.v6i1.294.

[9] M. S. Mursito, S., B. Lilies, and H. Mawaddah, "Ekstrak Daun Kelor (Moringa Oleifera Lamk) dalam Menurunkan Kadar Glukosa Darah pada Tikus (Rattus novergicus)," *Bionature*, vol. 21, no. 1, pp. 6–12, 2020, doi: 10.35580/bionature.v21i1.13921.

[10] M. L. Y. Purwoko, Syamsudin, and P. Simanjuntak, "Standardisasi Parameter Spesifik dan Nonspesifik Ekstrak Etanol Daun Kelor (Moringa oleifera)," *J. Ilmu Kefarmasian*, vol. 13, no. 2, pp. 124–129, 2020, [Online]. Available: http:/jurnal.unw.ac.id/index.php/ijpnp

[11] B. A. Wahyu ningsih A, Rochmanti M, "Efektifitas Antidiareal Unripewooden Pisang (Musa paradisiaca L .)," *Fol Med Indones*, vol. 56, no. 3, pp. 208–15, 2020, doi: 10.47307/GMC.2022.130.s5.38.

[12] A. Wijanarko, S. Perawati, L. Andriani, P. S. Farmasi, and P. Baru, "Standardisasi simplisia daun ciplukan," *J. Farmsetis*, vol. 9, no. 1, pp. 31–40, 2020.

[13] D. A. Setiabudi and Tukiran, "Uji Skrining Fitokimia Ekstrak Metanol Kulit Batang Tumbuhan Klampok Watu (Syzygium litorale)," *UNESA J. Chem.*, vol. 6, no. 3, p. 156, 2017.

[14] R. D. Ningsih AW, Pratama E, Komariyah S, Astuti DP, Klau ICS, "Differences in extraction methods to antidiarrheal activity in vitro and in vivo in unripe Kayu banana fruit (Musa paradisiaca L. var. Kayu," *Gac Méd Caracas*, vol. 130, no. Supl 5, pp. 1133–46, 2022.

[15] Z. Aziz, N. D. Yuliana, P. Simanjuntak, M. Rafi, and Syamsudin, "FTIR and HPLC-based metabolomics of yacon leaves extracts (Smallanthus sonchifolius [poepp & endl.] h. robinson) from two locations in Indonesia," *Indones. J. Chem.*, vol. 20, no. 3, pp. 567–578, 2020, doi: 10.22146/ijc.43453.

[16] A. A. Al-Huqail, S. I. Behiry, M. Z. M. Salem, H. M. Ali, M. H. Siddiqui, and A. Z. M. Salem, "Antifungal, antibacterial, and antioxidant activities of Acacia saligna (Labill.) H. L. Wendl. Flower extract: HPLC analysis of phenolic and flavonoid compounds," *Molecules*, vol. 24, no. 4, 2019, doi: 10.3390/molecules24040700.

[17] A. R. Hakim and R. Saputri, "PENGARUH JENIS PELARUT DAN METODE EKSTRAKSI TERHADAP AKTIVITAS ANTIBAKTERI DAN PENGHAMBATAN RADIKAL BEBAS EKSTRAK KULIT KAYU BANGKAL (Nauclea subdita) (The Effect of Solvents and Extraction Methods on Antibacterial and Free Radical Scavenging Activities f," *J. Surya Med.*, vol. 6, no. 1, pp. 177–180, 2020.

[18] Y. Retnowati, N. Bialangi, and N. Wingti Posangi, "Pertumbuhan Bakteri *Staphylococcus Aureus* pada Media yang Diekspos dengan Infus Daun Sambiloto (*Andrographis Paniculata*)," *Saintek*, vol. 6, no. 2, pp. 1–9, 2011, [Online]. Available: https://ejurnal.ung.ac.id/index.php/ST/article/view/405

[19] Haryoto and D. siwi Ardiyani, "Aktivitas Farmakologi dan Kadar Senyawa Fenolik Total dari Tanaman Andong Merah (Cordyline fruticosa L. A. Chev.)," *Uricol*, pp. 743–752, 2021.

[20] Palupi IN, "Daya Hambat Ekstrak Metanol Daun Sukun (Artocarpus altilis) Terhadap Pertumbuhan Bakteri Staphylococcus aureus dan Pseudomonas aeruginosa," *Fak. Ilmu Keperawatan dan Kesehat. Univ. Muhammadiyah Semarang*, 2016.

[21] F. D. Sulistiyono, T. Sofihidayati, and B. Lohitasari, "UJI AKTIVITAS ANTIBAKTERI DAN FITOKIMIA KULIT BAWANG MERAH (Allium cepa L.) HASIL EKSTRAKSI METODE Microwave Assisted Extraction (MAE)," *Mandala Heal.*, vol. 11, no. 2, p. 71, 2018, doi: 10.20884/1.mandala.2018.11.2.1316.

[22] Ibrahim A and K. H, "Identifikasi Metabolit Sekunder dan Aktivitas Antibakteri Ekstak Daun Sungkai (Peronema canescens JACK.) Terhadap Beberapa Bakteri Patogen," *J Trop Pharm Chem*, vol. 2, no. 1, pp. 8–18, 2012.

[23] S. Farmasi and F. Mipa, *UJI AKTIVITAS ANTIDIARE EKSTRAK ETANOL BATANG TIKUS PUTIH JANTAN WISTAR DIINDUKSI BAKTERI Salmonella typhi Oleh : NYAYU JUWITA LESTARI*. 2021.

[24] B. A. Wahyu ningsih A, Rochmanti M, "Efektifitas Antidiareal Unripewooden Pisang (Musa paradisiaca L .)," *Fol Med Indones.*, vol. 56, no. 3, pp. 208–15, 2020.

[25] A. Amalia, I. Sari, and Risa Nursanty, "Aktivitas Antibakteri Ekstrak Etil Asetat Daun Sembung (Blumea balsamifera (L.) DC.) terhadap Pertumbuhan Bakteri Methicillin Resistant Staphylococcus aureus (MRSA)," *J. UIN Ar-Raniry*, vol. 5, no. 1, pp. 387–391, 2017, [Online]. Available: https://ojs.uniska-bjm.ac.id/index.php/JST/article/download/6331/4035%0A

[26] S. Normayunita, S. Anam, and D. A. Khumaidi, "Aktivitas Antibakteri Fraksi Ekstrak Kulit Buah Mentah Pisang Ambon (Musa paradisiaca var.sapientum) Terhadap Staphylococcus aureus Antibacterial Of Unripe Fruit Banana Peel (Musa paradisiaca var.sapientum) Fraction Againts Staphylococcus aureus," *Online J. Nat. Sci.*, vol. 4, no. 3, pp. 300–309, 2015.

[27] M. Priatna, "PENGARUH REBUSAN AKAR BAYAM DURI (Amaranthus spinosus L.) TERHADAP DIARE PADA MENCIT JANTAN GALUR SWISS WEBSTER DENGAN METODE TRANSIT INTESTINAL," *J. Kesehat. Bakti Tunas Husada J. Ilmu-ilmu Keperawatan, Anal. Kesehat. dan Farm.*, vol. 16, no. 1, p. 57, 2016, doi: 10.36465/jkbth.v16i1.166.

[28] F. Handayani, H. Warnida, and S. J. Nur, "Formulasi dan Uji Aktivitas Antibakteri Streptococcus mutans dari Sediaan Mouthwash Ekstrak Daun Salam (Syzygium polyanthum (Wight) Walp.)," *Media Sains*, vol. 9, no. April, pp. 74–84, 2016.

[29] D. G. E. Prayoga, K. A. Nocianitri, and N. N. Puspawati, "IDENTIFIKASI SENYAWA FITOKIMIA DAN AKTIVITAS ANTIOKSIDAN EKSTRAK KASAR DAUN PEPE (Gymnema reticulatum Br.) PADA BERBAGAI

JENIS PELARUT," *J. Ilmu dan Teknol. Pangan*, vol. 8, no. 2, pp. 111–121, 2019.

[30] S. D. Haryati, S. Darmawati, and W. Wilson, "Perbandingan Efek Ekstrak Buah Alpukat (Persea americana Mill) Terhadap Pertumbuhan Bakteri Pseudomonas aeruginosa," *Pros. Semin. Nas. Publ. Hasil-Hasil Penelit. dan Pengabdi. Masy.*, no. September, pp. 348–352, 2017.

[31] Prayoga E, "Perbandingan Efek Ekstrak Daun sirih hijau (Piper betle L.) dengan metode difusi disk dan sumuran terhadap pertumbuhan bakteri Staphylococcus aureus," *Fak. Kedokt. dan Ilmu Kesehatan. Univ. Islam Negri Syarif Hidayatullah Jakarta; 2013*, vol. 34, 2013.