Jurnal Pijar MIPA
https://jurnalfkip.unram.ac.id/index.php/JPM

ISSN 1907-1744 (Print)
ISSN 2460-1500 (Online)

Antibacterial Activity Test of the Combination of Methanol Extract of Arumanis Mango Leaves (*Mangifera indica* L) and Taro Leaves (*Colocasia esculanta* L) on *Escherichia coli* Bacteria Causes of Diarrhea Diseases

Siska Kumalasari, Laeli Fitriyati*, Titi Pudji Rahayu, Naelaz Zukhruf Wakhidatul Kiromah

Undergraduate Program, Faculty of Health Sciences, University of Muhammadiyah Gombong, Indonesia
*E-mail: laeli.fitriyati.lf@gmail.com

Received: October 15, 2023. Accepted: November 29, 2023. Published: January 23, 2024

Abstract: Arumanis mango leaves (*Mangifera indica* L) and taro leaves (*Colocasia esculanta* L) are plants that have the potential to be antibacterial and can be used as an alternative to treat infectious diseases caused by bacteria. Arumanis mango leaves contain secondary metabolite compounds, including flavonoids, alkaloids, steroids, polyphenols, tannins, and saponins, and taro leaves contain secondary metabolite compounds in the form of saponins, tannins, flavonoids, formic acid, glucosides, citric acid, and several minerals. Secondary metabolite compounds that have potential as antibacterials are flavonoids and tannins. This study aims to determine the antibacterial activity of a combination of methanol extracts of arumanis mango leaves (*Mangifera indica* L) and taro leaves (*Colocasia esculanta* L) against *Escherichia coli* bacteria. The research was carried out starting from the plant determination and preparation of simplisia, extraction, phytochemical screening of extracts and KLT tests, and antibacterial testing using the disc paper method. The concentration series of extract combinations used are 6.25%, 12.5%, and 25%. The antibacterial test results of the combination of *arumanis* mango leaf extract (Mangifera indica L) and taro leaf (*Colocasia esculanta L*), which showed the highest inhibition zone diameter was at a concentration of 6.25% (1:1), a concentration of 12.5% (1:2). At a concentration of 25% (2:1). From the results of this study, a single extract of arumanis mango leaves and taro leaves with a concentration of 6.25%; 12.5%; 25% and a combination of extracts in a ratio of 1:1. 1:2, 2:1, which has potential antibacterial activity against Escherichia coli bacteria, so that further research can be carried out by utilizing a combination of arumanis mango leaf extracts and taro leaves and as a source of information and insight into the combination of arumanis mango leaves and taro leaves that have antibacterial properties.

Keywords: Antibacterial; Arumanis Mango Leaves; *Escherichia coli*; Taro Leaves.

Introduction

Diarrhea is a disease caused by bacterial infection; diarrhea can also affect all age groups, especially children and toddlers [1]. The number of diarrhea patients under five years old in 2019 who were served in health facilities amounted to 179,172 (46.3%) of the estimated diarrhea in health facilities. The number of diarrhea patients of all ages in 2019 who health facilities served was 573,609 patients (61.2%) of the estimated number of diarrhea patients in health facilities. According to data from the Rapid Diarrhea Survey in 2015, the Central Java province had a diarrhea morbidity rate of all ages of 270/1,000 population [2]. Escherichia coli is the most common bacterial species found in humans. Escherichia coli bacteria are commensal, intestinal, and extraintestinal pathogens that can cause urinary tract infections, meningitis, diarrhea, and septicemia [3]. Infections caused by Escherichia coli bacteria are characterized by asymptomatic clinical manifestations until bloody or bloodless diarrhea is seen [4].

Infection prevention can be done by using antibiotics, but if the use does not follow the rules of use, it will cause resistance [5]. The use of plants for alternative medicine is increasingly being developed, and they can be utilized as antibacterials. Antibacterials are substances or drugs used to eradicate microorganisms obtained from syntheses derived from organic compounds. Antibacterials have two properties, namely bacteriostatic and bactericidal, where bacteriostatic is an antimicrobial that can only inhibit the growth of microorganisms, while bactericidal is an antimicrobial that can kill microorganisms [6]. According to research by [7], *arumanis* mango leaves contain flavonoids, alkaloids, steroids, polyphenols, tannins, and saponins. Research [8] shows that taro leaves contain alkaloids, saponins, flavonoids, and tannins. Secondary metabolites that function as antibacterials are flavonoids and tannins. Secondary metabolite compounds that have antibacterial potential can be obtained through extraction. The extraction method used is maceration using methanol solvent. The choice of solvent aims to attract active substances maximally [9].

In the research [10], arumanis mango leaves have an antibacterial activity where the n-hexane fraction of mango leaf extract has a potential antibacterial activity where the resulting inhibition diameter is 9.40 mm, and the ethyl acetate fraction is 15.20 mm against Escherichia coli ATCC25922 bacteria. Taro leaf also has the potential to be an antibacterial. As in the research by [11], taro leaf has

How to Cite:
Kumalasari, S., Fitriyati, L., Rahayu, T. P., & Kiromah, N. Z. W. (2024). Antibacterial Activity Test of the Combination of Methanol Extract of Arumanis Mango Leaves (Mangifera indica L) and Taro Leaves (Colocasia esculanta L) on Escherichia coli Bacteria Causes of Diarrhea Diseases. *Jurnal Pijar Mipa*, *19*(1), 131–135. https://doi.org/10.29303/jpm.v19i1.5850

the highest antibacterial activity at a concentration of 50mg/mL with an average inhibition zone diameter of 20.17 ± 0.05 mm against Escherichia coli bacteria. Arumanis mango leaves and taro leaves contain secondary metabolite compounds that have antibacterial potential, so they can be combined to determine how much antibacterial activity the two plants have.

Based on the above background, research on the combination of arumanis mango leaf extract and taro leaves has never been done so researchers will research the development of a combination of arumanis mango leaf extract (*Mangifera indica* L) and taro leaves (*Colocasia esculanta* L) against Escherichia coli bacteria. The combination of arumanis mango leaf extract and taro leaves to combine the two plants is expected to increase the effectiveness and antibacterial efficacy because the two plants both have potential antibacterial activity seen from the strength of the inhibition zone produced against the growth of Escherichia coli bacteria using the disc paper method.

Research Methods

Determination and Sample Preparation

Arumanis mango and taro leaves were determined at the Ahmad Dahlan University Plant Systems Laboratory, Yogyakarta. A sampling of *arumanis* mango and taro leaves from Waluyo Village, Buluspesantren District, Kebumen Regency. Arumanis mango leaves and taro leaves are made into simplisia; the dry simplisia obtained is then pulverized into powdered simplisia [12].

Extraction of Arumanis Mango Leaves and Taro Leaves

Extraction of arumanis mango leaves and taro leaves using maceration method by taking each simplisia powder of arumanis mango leaves and taro leaves and then soaking in methanol solvent for 3x24 hours while occasionally stirring. The resulting filtrate was then concentrated until a thick extract was obtained. The extract yield was calculated using the following equation [13]:

$$\text{Yield}(\%) = \frac{\text{final extract weight (g)}}{\text{amount of dried simplisia (g)}} \text{ x } 100\%$$

Phytochemical Screening Test

The phytochemical screening test was carried out with a color test using various reagents to identify secondary metabolite compounds in flavonoids and tannins in the extract [14].

Preparation of Bacterial Suspension

Escherichia coli bacteria were taken and put into 0.9% NaCl solution. Compare the turbidity of the bacterial suspension with Mac. Farland [15].

Preparation of Test Solution

Single methanol extracts of arumanis mango leaves and taro leaves were made in concentrations of 6.25%, 12.5%, and 25%, and combinations of extracts in the ratio (1:1), (1:2), and (2:1) were dissolved in DMSO solvent. The test solution of each concentration was vortexed and tested for antibacterial properties using the disc paper method.

Antibacterial Test

The bacterial suspension that has been made is then inoculated into NA media that has been poured into a Petri dish. Paper disks were dipped in a single solution of methanol extracts of arumanis mango leaves and taro leaves concentrations of 6.25%, 12.5%, and 25%. The combined concentration of 6.25%' 12.5%, and 25% extracts in the 1:1, 1:2, 2:1 ratio was placed on the surface of NA media containing bacterial suspensions aseptically. The positive control was a 2% concentration of ciprofloxacin antibiotic solution, and the negative control was DMSO (*Dimethyl sulfoxide*) solution as the extract solvent. Incubation was carried out at 37℃ for 24 hours. The diameter of the Inhibition Area (DDH) was measured based on the radius of inhibition in the form of a clear area around the disc paper using a caliper [16].

The calculation formula for the Diameter of Inhibition (DDH) is as follows:

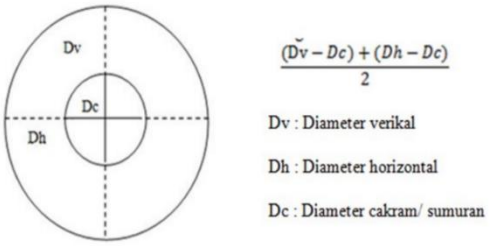

Figure 1. Calculation Formula for Bacterial Inhibition Zone Diameter [17]

Results and Discussion

Arumanis mango leaves and taro leaves before use have been determined to identify whether the leaves are true arumanis mango leaves and taro leaves. Determination was conducted at the Ahmad Dahlan University Plant System Laboratory, Yogyakarta. The determination results show that the plant used is *Mangifera indica* L. var. *arumanis* with Letter Number 050/Lab.Bio/B/I/2023 and *Colocasia esculanta* L with Letter Number 034/Lab.Bio/B/I/2023.

The leaves obtained were washed from dirt and dried under direct sunlight covered with a black cloth. The purpose of drying is to remove the water content contained in the leaves so as not to cause enzymatic processes that can damage the secondary metabolite compounds contained in the sample [18]. Arumanis mango leaves and taro leaves that have been dried, then mashed into powder and sieved using a 40 mesh sieve [12].

The dry simplisia that has been obtained is then extracted. Extraction was done using the maceration method; dry simplisia of arumanis mango leaves and taro leaves were taken 300 grams each and macerated using methanol solvent. Extraction of 300 grams of arumanis mango leaf simplisia and taro leaves each obtained a thick extract of arumanis mango leaves with an extract yield of 10.43%. In contrast, the thick extract

of taro leaves got an extract yield of 7.8%, as shown in Table 1**.**

Table 1. The yield of Mango Arumanis Leaf and Taro Leaf Extracts

Extract	Simplified Powder Weight (g)	Solvent Volume (L)	Extract Weight (g)	Yield (%)
Arumanis Mango Leaves	300	1.2	31.29	10.43
Taro Leaves	300	1.8	23.46	7.8

The thick extracts of arumanis mango leaves and taro leaves obtained are then tested for flavonoid and tannin compounds. Testing flavonoid and tannin compounds obtained results as in Table 2 and Table 3 that methanol extracts of arumanis mango leaves and taro leaves contain secondary metabolite compounds, namely flavonoids and tannins. The results of the phytochemical screening test on *arumanis* mango leaf extract are the same as the results of research conducted by [16], which states that arumanis mango leaves contain secondary metabolite compounds in the form of flavonoids, tannins, and saponins. The tube test results on taro leaf extract are similar to the research conducted by [8] in that taro leaves contain secondary metabolite compounds in the form of alkaloids, saponins, flavonoids, and tannins. Research results [16] also stated that arumanis mango leaves contain secondary metabolite compounds in flavonoids, tannins, and saponins. The results of phytochemical screening of taro leaf extract are also by the research conducted by [19] that taro leaf extract contains secondary metabolite compounds such as saponins, tannins, flavonoids, formic acid, glucosides, citric acid, and several minerals such as calcium and potassium.

Table 2. Flavonoid Compound Test Results of Arumanis Mango Leaf and Taro Leaf Extracts

Extract	Reagents	Results	Description
Arumanis Mango Leaves	Amyl Alcohol	Reddish orange	Positive [14]
Taro Leaves	Amyl Alcohol	Orange yellow	Positive [14]

Table 3. Tannin Compound Test Results of Arumanis Mango Leaf and Taro Leaf Extracts

Extract	Reagents	Results	Description
Arumanis Mango Leaves	$FeCl_3$	Green blackish	Positive [14]
Taro Leaves	$FeCl_3$	Green blackish	Positive [14]

The phytochemical screening test conducted positively contains two compounds that have antibacterial mechanisms. The mechanism of flavonoid compounds as antibacterial is by forming complex compounds on extracellular proteins that cause bacterial cell proteins to denature, thus damaging the cell membrane of the bacteria [20]. Another antibacterial activity compound is tannins; the mechanism of tannins as antibacterials is similar to the mechanism of quinones, which bind and inactivate proteins and can make substrates unavailable to microorganisms [21].

Selection in the combination of the two plants to be tested for antibacterial activity as in the research conducted by [22]. The results showed that the purified extract of arumanis mango leaves ranging from a concentration of 6.25% to 100% indicated that the results of the diameter of the inhibition zone produced the higher the concentration, the greater the diameter of the inhibition zone made successively, namely 15.69 ± 0.76 mm to 24.15 ± 0.05 mm. According to research [8], Taro leaf extract at a concentration of 35% has an average inhibition zone diameter of 14.24 mm, which is included in the strong category. Arumanis mango leaves and taro leaves contain secondary metabolite compounds that have the potential to be antibacterials, so they can be combined to determine how much antibacterial activity they have. They are expected to increase antibacterial effectiveness because both plants have good potential antibacterial activity.

The next test is the antibacterial activity test. The positive control used is ciprofloxacin 2%, with the negative control used is the extract solvent, DMSO; the purpose of using DMSO as a negative control is to ensure that the inhibition formed during the test comes from the extract, not from the methanol solvent. The results of the antibacterial testing of the disc paper method show the formation of a clear inhibition zone. The formation of the inhibition zone indicates the presence of antibacterial activity. Antibacterial test results can be seen in Table 4.

Table 4. Inhibition Zone Diameter Results of Methanol Extract of Arumanis Mango Leaves and Taro Leaves

Concentration	Average ± SD
Control (+)	33.3 ± 0.58
Control (-)	0 ± 0
ETDMA 6.25%	5.6 ± 3.85
ETDMA 12.5%	7.72 ± 2.38
ETDMA 25%	10.13 ± 0.87
ETDT 6.25%	3.72 ± 0.39
ETDT 12.5%	6.5 ± 1.32
ETDT 25%	10.18 ± 0.95
Combination 6.25% (1:1)	5.95 ± 3.60
Combination 6.25% (1:2)	5.03 ± 1.26
Combination 6.25% (2:1)	5.5 ± 2.41
Combination 12.5% (1:1)	2.92 ± 1.93
Combination 12.5% (1:2)	4.78 ± 1.95
Combination 12.5% (2:1)	4.12 ± 2.94
Combination 25% (1:1)	4.22 ± 2.48
Combination 25% (1:2)	3.92 ± 2.55
Combination 25% (2:1)	5.17 ± 2.40

Antibacterial testing shows that DMSO has no antibacterial activity, characterized by the absence of a clear zone area that formed on the disc paper. The positive control of 2% ciprofloxacin produced a clear zone with an average of 33.3 ± 0.58 mm in the powerful inhibition category. In single extracts of arumanis mango leaves and taro leaves, the largest inhibition zone diameter was obtained at a

concentration of 25% with an average inhibition zone diameter of 10.13 ± 0.87 mm and 10.18 ± 0.95 mm, respectively, which is included in the strong category. The research [23] stated that the ethanol extract of arumanis mango leaves has potential antibacterial activity by producing an inhibition zone of 14.8 mm within 1x24 hours, and at 5x24 hours of observation, the diameter of the inhibition zone increased by 16.2 mm, which is included in the strong category. The research [8] stated that the ethanol extract of taro leaves has an average diameter of the inhibition zone at a concentration of 15% of 9.35 mm, a concentration of 25% of 11.45 mm, and at a concentration of 35% of 14.24 mm which is included in the strong category. This shows that flavonoid and tannin compounds have antibacterial activity, where flavonoid compounds act as antibacterials by forming complex compounds in extracellular proteins that cause bacterial cell proteins to denature to damage the cell membrane [24]. Tannin compounds also have an antibacterial mechanism of action by disrupting cell permeability by wrinkling cell walls or cell membranes, causing cell growth to be inhibited and even death. [25].

In the combination group of arumanis mango leaf extract and taro leaf concentration of 6.25%, the largest diameter of the inhibition zone was obtained in the ratio 1:1 with an average diameter of the inhibition zone of 5.95 ± 3.60 mm, which is included in the moderate category. At a combination concentration of 12.5%, the largest inhibition zone diameter was obtained in the 1:2 ratio, 4.78 ± 1.95 mm, included in the weak category. In comparison, at a combination concentration of 25%, the largest inhibition zone diameter was obtained in the 2:1 ratio with an average inhibition zone diameter of 5.17 ± 2.40 mm, included in the moderate category. Factors that can affect the difference in the diameter of the inhibition zone resulting from the extracts used are the test organism's sensitivity level [16]. Other factors that can affect the size of the diameter of the inhibition zone produced include differences in the concentration or content of antibacterial active substances contained in the extract, the rate of diffusion of antibacterial samples into the medium, the reaction between the active ingredients and the press and incubation temperature, and the metabolic activity of microorganisms [26]. The diffusion of extract samples into the media can also affect the results where the dipping of the disc paper to each concentration is not perfectly diffused, which can cause the active substance to not diffuse properly when implanted in a medium that has been planted with test bacteria, it cannot be adequately diffused in inhibiting bacterial growth [9].

Conclusion

The combination of methanol extracts of arumanis mango leaves (Mangifera indica L) and taro leaves (Colocasia esculanta L) has not increased antibacterial activity. Still, the combination of arumanis mango leaf extracts and taro leaves has potential antibacterial activity against Escherichia coli bacteria, from single extracts to combinations of extracts from concentrations of 6.25%, 12.5%, and 25%.

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