COMPARISON OF ANTIBACTERIAL ACTIVITIES LEAVES EXTRACTS OF AZADIRACHTA INDICA AGAINST KLEBSIELLA PNEUMONIAE

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ABSTRACT

Objective: This study aims to determine the potential of neem (Azadirachta indica, Juss) leaves and sea mango (Cerbera manghas, Linn.) leaves against Klebsiella pneumonia.

Methods: Hexane and methanol extracts of neem leaves (A. indica, Juss) and hexane and butanol extracts of sea mango leaves (C. manghas, Linn.) were tested antibacterial against K. pneumonia using paper disc and dilution methods, measured the inhibition zone diameter, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC), as a comparison used ampicillin.

Results: The MIC values for the butanol extract and hexane extract of sea mango leaves against K. pneumoniae were 1.124276 and 1.45958 mg/mL, respectively. While the MBC value of both of them had the same value, i.e. 2 mg/mL. The hexane and methanol extracts of neem leaves did not have potential against the growth of K. pneumonia.

Conclusion: Based on the results of this study, it has been obtained that extracts butanol and hexane extract of leaves of sea mango (C. manghas, Linn.) have antibacterial activity strong enough against K. pneumonia, where extracts butanol leaves of sea mango has antibacterial activity that is more powerful than the extract hexane leaf sea mango, while the hexane and methanol extract and hexane extract of neem (A. indica, Juss) leaves have weak activity against K. pneumonia.

Keywords: Antibacterial, Cerbera manghas, Azadirachta indica, Klebsiella pneumonia.

INTRODUCTION

Mastitis is an inflammation of the mammary gland characterized by physical, chemical, bacteriological, and cytological changes in milk. Pathological changes in glandular tissues of the udder and effects on the quality and quantity of milk have been observed [1]. This disease is mainly caused by microorganisms usually bacteria, including Gram-negative and Gram-positive bacteria, mycoplasmas, yeasts, and algae [2]. Bacterium Klebsiella pneumoniae is a major cause of illness nipple mastitis in dairy cows and other mammals in Indonesia [3]. Mastitis disease in dairy cattle is very detrimental because it can reduce milk dairy cows production up to 30% [4,5].

On the other hand, milk and milk products have the potential to transmit pathogenic organisms to humans. All the nutritional components that make milk and milk products an important part of the human diet also support the growth of pathogenic organisms. Early in this century, it was discovered that milk can transmit tuberculosis, brucellosis, diphtheria, scarlet fever, and Q fever to humans. Fortunately, over the decades, the threat of these diseases and the incidence of outbreaks involving milk and milk products have been greatly reduced due to improved sanitary of milk production practices and pasteurization technique. However, a variety of microorganisms still contribute to illnesses and disease outbreaks [6,7].

One of the bacteria that cause mastitis that is already resistant to methicillin is Staphylococcus aureus. Strain of S. aureus resistant to β-lactam antibiotics is known as methicillin-resistant S. aureus (MRSA). The MRSA strains have been observed to be multidrug-resistant, such as aminoglycosides, macrolides, lincosamides, streptogramins, and tetracyclines which are often used in the treatment of mastitis [8,9].

Materials

Isolate of bacteria K. pneumoniae, obtained from the Laboratory of Microbiology, Indonesian Institute of Sciences, Cibinong Bogor, Indonesia. Medium Tryptic Soy Broth (Pronadisa), bacteriological agar (Oxoid), Sea mango leaves, and neem leaves were obtained from Research Institute for Spices and Medicinal Plants, Bogor, Indonesia. Ethanol, hexane, butanol, and methanol were obtained from E-Merck. Ampicillin antibiotic (Zigma), glassware, microbiological culture media, chemicals and materials that commonly used in microbiology laboratories.

Methods

Preparation of hexane and methanol extract of neem and leaves the hexane and butanol extract sea mango leaves were carried out based on harborne method [13], as shown in Scheme 1.
Phytochemical screening for hexane and methanol extract of neem and leaves the hexane and determination The growth curve of K. pneumoniae bacteria was performed in tryptic soy broth medium incubated at 37°C and agitation at 120 rpm based on absorbance value butanol extract sea mango leaves was done by the Farnsworth method.

Determination of inhibition zone diameter (IZD) of hexane and methanol extract of neem leaves and the hexane and butanol extract of sea mango leaves against K. pneumoniae was done by agar diffusion method, using the paper disc.

Determination minimum inhibitory concentration (MIC) of hexane and methanol extract of neem leaves and the hexane and butanol extract of sea mango leaves which can inhibit the growth of test bacteria was done by microdilution method using a test tube.

The minimum bactericidal concentration (MBC) test was determined using a series of steps, undertaken after a MIC test has been completed. This determination was done by dilute the MIC results and incubated at 39°C for 24 h, then counted the number of growing colonies.

RESULTS AND DISCUSSION

Simplicia leaves of sea mango (C. manghas Linn.) and leaves of neem (A. indica, Juss) in this study after being cleaned, dried, and powdered. The extraction was done gradually using the method of extraction of Harborne method, which was multilevel extraction using different solvent of polarity. The purpose of this gradual extraction was to draw and separate the organic compounds contained in the simplicia by virtue of their polarity [13]. The results of this extraction were used to determine the antibacterial activity against K. pneumoniae. Then, on each extract was examined the chemical content qualitatively.

The results of phytochemical screening show that there were some differences in the content of the 4 test preparations consisting of butanol extract and hexane extract of sea mango, methanol extract and hexane extract of neem, while the chemical content is not tested in the table above, because according to the literature, the extract does not contain any chemical compounds that are not tested [14-16].

From the results of phytochemical screening were obtained results as shown in Table 1.

In this research, the result of the growth curve of K. pneumoniae showed that there was only two growth phases, namely the logarithmic phase and the stationary phase. In the growing curve there is no phase of adaptation but directly enter the stationary phase. The growth of this bacteria can be seen from the change of absorbance value obtained after the measurement at different minutes. Based on the curve, then determined mid-log phase. Mid-log phase is shown in 180 min with the absorbance of 0.942 and speed of 0.0066 per min (Tables 2 and 3).

To determine the concentration hexane extract of sea mango leaves which is the equivalence to antibiotic, based on IZD extract hexane of sea mango determined by the equation: y=9.110x-71.26 (Fig. 1) [17].

Based on the above curve, it can be seen that 250000 μg/ml of sea mango hexane extract is equivalent to 88,165 μg/ml ampicillin antibiotic, 500000 μg/ml sea mango hexane extract equivalent to 92.72 μg/ml and 1,000,000 μg/ml sea mango hexane extract equivalent to 101,83 μg/ml as shown in Table 4.

Related to IZD was found equivalence between antibiotic ampicillin with extract butanol of sea mango against K. pneumonia on as shown in Fig. 2.

Based on IZD was found equivalence between antibiotic ampicillin with extract hexane of neem against K. pneumonia on as shown in Fig. 2.
Table 1: Results of phytochemical screening leaf extract sea mango and neem

| No | Chemical content | Butanol extract sea mango | Hexane extract sea mango | Methanol extract neem | Hexane extract neem |
|----|------------------|---------------------------|-------------------------|----------------------|---------------------|
| 1  | Alkaloids        | -                         | -                       | +                    | +                   |
| 2  | Flavonoids       | +                         | +                       | +                    | +                   |
| 3  | Saponin          | +                         | -                       |                      |                     |
| 4  | Tannin           | +                         | -                       |                      |                     |
| 5  | Quinone          | +                         | Not tested              | Not tested           | Not tested          |
| 6  | Triterpenoids    | -                         | +                       |                      |                     |
| 7  | Coumarin         | -                         | Not tested              | Not tested           | Not tested          |
| 8  | Essential oil    | -                         | Not tested              | Not tested           | Not tested          |
| 9  | Glycoside        | Not tested                | Not tested              | +                    | +                   |
| 10 | Steroids         | Not tested                | Not tested              | +                    | +                   |
| 11 | Phenolic         | Not tested                | Not tested              | +                    | +                   |

Table 2: The average IZD value of hexane and butanol extract of sea mango leaves against bacteria *K. pneumoniae* at several concentrations (µg/ml)

| Petri dish concentration | M1 30 | M2 40 | M3 55 | H1 250 | H2 500 | H3 1000 | B1 250 | B2 500 | B3 1000 |
|--------------------------|-------|-------|-------|--------|--------|---------|--------|--------|---------|
| Average of diameter      | 11    | 12.5  | 13.5  | 17.5   | 18     | 19      | 21     | 23.5   | 25      |

M: Methicillin, H: Hexane extract of neem, B: Butanol extract of neem, IZD: Inhibition zone diameter, *K. pneumoniae: Klebsiella pneumonia*

Table 3: The average IZD value of methanol and butanol extract of neem leaves against bacteria *K. pneumoniae* at several concentrations (µg/ml)

| Petri dish concentration | M1 30 | M2 40 | M3 55 | MOH1 250 | MOH2 500 | MOH3 1000 | B1 250 | B2 500 | B3 1000 |
|--------------------------|-------|-------|-------|----------|----------|-----------|--------|--------|---------|
| Average of diameter      | 10    | 12    | 13.5  | 6        | 6        | 6         | 6      | 6      | 6       |

M: Methicillin, MOH: Methanol extract of neem, IZD: Inhibition zone diameter, *K. pneumoniae: Klebsiella pneumonia*

Table 4: Table concentration equivalence between antibiotic ampicillin with extract hexane of sea mango against *K. pneumonia on IZD*

| Extract concentration (µg/ml) | 250000 | 500000 | 1000000 |
|-------------------------------|--------|--------|---------|
| IZD of extract (mm)           | 17.5   | 18     | 19      |
| Equivalent concentrations of antibiotics (µg/ml) | 88.165 | 92.72  | 101.83  |

IZD: Inhibition zone diameter, *K. pneumonia: Klebsiella pneumonia*

Table 5: Table concentration equivalence between antibiotic ampicillin with extract butanol of sea mango against *K. pneumonia on IZD*

| Extract concentration (µg/ml) | 250000 | 500000 | 1000000 |
|-------------------------------|--------|--------|---------|
| Inhibition zone diameter (mm) | 21     | 23.5   | 25      |
| Equivalent antibiotic mg/ml   | 87.7   | 102.3  | 111     |

IZD: Inhibition zone diameter, *K. pneumonia: Klebsiella pneumonia*

Table 6: Table concentration equivalence between antibiotic ampicillin with extract hexane of neem against *K. pneumonia on IZD*

| Extract concentration (µg/ml) | 250000 | 500000 | 1000000 |
|-------------------------------|--------|--------|---------|
| Diameter of zone inhibition (mm) | 6     | 6      | 6       |
| Equivalence antibiotic          | 13.74  | 13.74  | 13.74   |

IZD: Inhibition zone diameter, *K. pneumonia: Klebsiella pneumonia*

Table 7: Concentration equivalence between antibiotic ampicillin with extract methanol of neem against *K. pneumonia on IZD*

| Extract concentration (µg/ml) | 250000 | 500000 | 1000000 |
|-------------------------------|--------|--------|---------|
| Diameter of zone inhibition (mm) | 6     | 6      | 6       |
| Equivalence antibiotic (mg/ml) | 2.792  | 2.792  | 2.792   |

IZD: Inhibition zone diameter, *K. pneumonia: Klebsiella pneumonia*

Based on the equation, y=5.129x-17.03 contained in Fig. 3, obtained the results as shown in Table 6.

From Table 6, the hexane extract of the neem leaves shows its action against *K. pneumonia* from low, medium, and high doses having a partial IZD.

To determine the concentration methanol extract of neem leaves which is the equivalence to antibiotic, based on IZD extract methanol of neem against *K. pneumoniae* determined by the equation: y=6.567x–36.61

Based on the equation, y=6.567x–36.61 contained in Fig. 4, obtained the results as shown in Table 7.

In the determination IZD of hexane extract and methanol leaves neem. The neem leaves have provided partial resistance zones to the test bacteria; this is because the antibacterial concentration that diffuses to the area decreases, which is insufficient to inhibit all bacterial growth [18]. The obstacle zone formed in the antibacterial power test is divided into two, i.e., there is a total and partial. The total obstacle zone when the area around the disc is clear, meaning the bacteria is really sensitive to the concentration of the extract given. Partial obstacle zone when there is a zone of resistance formed around the disc there were still some colonies of germs. Therefore, the hexane and methanol extracts of the neem leaves provide a partial obstruction zone, so it was not followed in the measurement of MIC and MBC. While that was continued only for hexane extract and butanol of Bintaro leaves, because it has given IZD and has MIC and MBC [18,19].

The result of MIC and MBC of hexane extract and butanol extract of sea mango leaves against *K. pneumoniae* was found as shown in Table 8.

The MIC values for the butanol extract of sea mango leaves to *K. pneumoniae* were 1.124276 mg/ml whereas in the hexane extract of the mango leaves to *K. pneumoniae* bacteria was shown at concentrations of 1.345958 mg/ml which at the concentrations were the lowest concentrations that could inhibit the growth bacteria.
Butanol extract of sea mango leaves and hexane extract of sea mango leaves have the same MBC value that was 2 mg/ml.

CONCLUSION

Based on the results of this study have been obtained, that extracts butanol and hexane extract of leaves of sea mango (C. manghas Linn.) have antibacterial activity strong enough against K. pneumonia, where extracts butanol leaves of sea mango have antibacterial activity that is more powerful than the extract hexane leaf sea mango, while the methanol extract and hexane extracts of neem (A. indica, Juss) leaves have weak activity against K. pneumonia.

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