Antibacterial Activity Of Leaves Extracts Of Jatropha Curcas, Linn Against Enterobacter Aerogenes

Beni Setha, Amran Laga, Meta Mahendradatta, Firdaus

Abstrak: This study aims to analyze the antibacterial activity of leaves extract of Jatropha curcas against Enterobacter aerogenes, and determine of Minimum Inhibitory Concentration. The extraction process is done by maceration method using hexane, chloroform, ethyl acetate and methanol. Extracts obtained test antibacterial activity against Enterobacter aerogenes. Concentration all extract of Jatropha curcas is 1%, 10%, 20%, and 30% (w / v). Positive control using synthetic antibiotic ampicillin and negative controls used dimethyl sulfoxide (DMSO). The results showed that the negative control (DMSO) and positive control (ampicillin) do not provide inhibition against test bacteria. Positive controls that do not provide inhibition against test bacteria showed that bacteria tested was resistant to antibiotics ampicillin used. All types of extracts with concentrations of 1% to 30% (w / v) gave inhibition against test bacteria. The highest inhibition diameter found in the chloroform extract with a concentration of 20% (14.83 ± 1.66 mm) and the lowest was in 1% hexane (6:53 ± 0.18mm). The diameter of the inhibition of the bacterium Enterobacter aerogenes to all types of extracts tended to increase with increasing concentration of the extract. MIC values for all existing extract at a concentration of 0.75% (w / v).

Indeks Terms: Antibacterial activity**,** Jatropha curcas, Enterobacter aerogenes

1. INTRODUCTION

In Indonesia, *J. curcas* has long been used in traditional medicine, because it contains chemical compounds that are antibacterial, fever, anti-inflammatory and inhibitor of bleeding [1]. *Jatropa curcas* leaves are often used as a medicine for skin infections, the seed is used for constipation, treating cervical cancer, and fungal infections [2]. In some countries, *J. curcas* used as a drug, such as malaria medicine in Mali [3] and in Africa as a drug haemostatik [4], skin infections, diarhea, and several other diseases caused by microorganisms [5,6]. *Jatropha curcas* oil is used as a medicine constipation, skin diseases and relieve the pain of rheumatism. In addition, the fruit of *J. curcas* is used directly as a medicine constipation and anthelmintic drugs. [4]. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in developing countries [7]. Previous studies have reported that *J. curcas* exhibits antimicrobial activity [8,9,10]. The crude stem extracts of *J. curcas* to inhibit the growth of bacteria family Enterobacteriaceae like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* [11]. The ability of the crude stem extracts of *J. curcas* to inhibit the growth of bacteria is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections. This research tested the antibacterial activity of leaves extract of Jatropha curcas against pathogenic bacteria Enterobacter aerogenes are included in the family Enterobacteriaceae.

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2. MATERIALS AND METHOD

Material

Jatropha curcas leaves were collected from Waitatiri village, Ambon, Maluku Province, Indonesia, where the plant grows under natural condition. Jatropha curcas leaves are used in this study is that older leaves (dark green). The organic solvent used is hexane, chloroform, ethyl acetate and methanol, distilled water, filter paper, nutrient agar (NA), nutrient broth (NB) and bacterial culture namely, *Enterobacter aerogenes* were obtain from the Microbiology laboratory, Faculty of Medicine, University of Indonesia. The microorganisms were maintained at 4°C on Nutrient Agar slant in the Microbiology laboratory, Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia and fresh subcultures were made before use.

Equipment

The equipment used was an analytical balance, bottles, funnel, rotary vacuum evaporator, petri dish, beaker glass, paper, cotton, needle ose, incubators, electric cooker, autoclave, Bunsen, micro pipette, calipers and a set of glassware.

Research Methods

Extraction

The extraction process is done by maceration method using hexane, chloroform, ethyl acetate and methanol. The filtrate obtained was separated from the solvent with a rotary evaporator vacuum. The yield of each extract (% w / w) was calculated with the formula = (weight of crude extract (g) / weight of powder (g)) x 100%.

Antibacterial Activity Test

Antibacterial activity test of *J. curcas* extract using the streak plate method [12] with procedures: solid medium is heated until melted, then cooled to a temperature of ± 40 ^{o}C. After that, poured in a sterile petri dish is then allowed to become solid. Take a bacterial culture with ose needle, then scratch at the surface of the agar medium until evenly.

Paper discs dripped with 20 µL of the four extracts. The fourth extract concentration was 1%, 10%, 20%, and 30% (w / v). Positive control using synthetic antibiotic ampicillin and negative controls using dimethyl sulfoxide (DMSO). Paper discs containing extracts and controls placed on the surface of an agar medium in a petri dish, then covered petri dish. Furthermore petri dishes were incubated at 37 ^{o}C for 18-24 hours. The diameter of the inhibition zone formed was measured using calipers [13].

Determination of Minimum Inhibitory Concentration (MIC) of Extract

Jatropha curcas extract that provides an inhibitory effect on the *E. aerogenes* followed by determination of minimum inhibitory concentration using the streak plate method (the same as the determination of antibacterial activity). Variations in the concentration of Jatropha curcas leaves extract ranging from 0.10% (w / v) to 0.75% (w / v). The diameter of the inhibition zone formed was measured using calipers [13].

Time and Research Location

This study was conducted from May to October 2013. Extraction process, test antibacterial activity, and determination of MIC leaves extract Jatropha curcas performed at the laboratory of Phytochemistry and Microbiology, Faculty of Pharmacy, University of Hasanuddin, Makassar, Indonesia.

3. RESULTS AND DISCUSSION

Extraction Yield

The yield of the methanol extract was highest, and the lowest yield present in the chloroform extract (Table 1). Polar extracts extracted with methanol gave the highest yield is because methanol is a universal solvent that dissolves all types of compounds capable of either polar, semi-polar and non-polar. In the extraction process, composition, color, aroma and the resulting yield will be influenced by the type, size and level of maturity of raw materials, the type of solvent, temperature, extraction time and extraction method [14]. Percentage weight secondary metabolites is strongly influenced by the type of plants and parts of plants, (fruits, seeds, stems, bark, wood, flowers, leaves) but is generally less than 10% [15].

Table 1. Extraction yield of *Jatropha curcas*

Solvent	Yield (w/w)
Hexane	3,05 %
Chloroform	1,39 %
Ethyl Acetat	1,53 %
Methanol	3,40 %

Antibacterial Activity

The test results showed that the antibacterial activity of the negative control (DMSO) and positive control (ampicillin) do not provide inhibition against the bacteria *Enterobacter aerogenes*. Positive controls that do not provide inhibition against test bacteria showed that bacteria are already resistant to the synthetic antibiotic ampicillin were used in this study. The analysis shows that all types of extracts with concentrations of 1% to 30% (w / v) was given the inhibition of the bacteria *E. aerogenes* (Table 2). Effect of the extract with various concentrations of the antibacterial activity of *E. aerogenes* is indicated by the formation of inhibition zones in the form of clear area around the paper discs after incubation for 24 h at 37 ^{o}C. The antibacterial activity due to the presence of secondary metabolites contained in the extract. Effect of antibacterial activity of the leaf extract of *Jatropha curcas* against bacteria *E. aerogenes* can be proved by looking at observational data on the negative control (DMSO), where bacteria grow around the paper disc and not formed a clear zone. While on paper discs were given extracts of leaves of Jatropha does not grow bacteria but rather formed a clear zone around the paper disc.

Table 2. Average of Inhibition diameter of extract against E. aerogenes

Crude Extract	Concentration (% w/v)			
	1	10	20	30
Heksan	6.53±0.18	7.64±0.61	8.14±0.25	8.57±0.76
Kloroform	8.08±0.11	10.50±0.49	14.83±1.66	12.17±0.73
Etil Asetat	8.14±0.23	8.18±0.64	8.42±0.67	8.44±0.45
Metanol	7.83±1.05	7.78±0.44	8.36±0.43	8.18±0.16

The highest inhibition diameter found in the chloroform extract with a concentration of 20% (14.83 ± 1.66 mm) and the lowest was in 1% hexane (6:53 ± 0.18mm). The diameter of the inhibition of the bacterium *Enterobacter aerogenes* to all types of extracts tended to increase with increasing concentration of the extract. Increasing the diameter of the inhibition of the bacterium E. aerogenes due to the higher concentration of the extract, the higher the content of secondary metabolites contained in the extract. Thus, the ability of secondary metabolites in inhibiting bacterial activity increases. However, sometimes too high concentration of extract that can interfere with the penetration of secondary metabolites penetrate the bacterial cell wall. Many factors and circumstances that may affect the operation of antibacterial, among others, the concentration of antibacterial agents, the number of bacteria, species of bacteria, the presence of organic matter, temperature and pH of the environment [16]. Inhibition by antimicrobial compounds in general can be caused by interference with components of the cell, and cytoplasmic membrane, inhibition of protein synthesis and interference with the function of the genetic material (17). Resistance of bacteria to antimicrobial compounds closely related to the structure of the cell wall. *Enterobacter aerogenes* is a Gram-negative bacterium. Cell wall of gram-negative bacteria is more complex, having inner and outer membranes. Layer of the outer membrane contains phospholipids, lipopolysaccharides and lipoproteins. This layer is impermeable to large molecules, but small molecules can pass. Lipopolysaccharide and peptidoglycan are filters for a wide range of molecular sizes, whereas the plasma membrane is impermeable to molecules whose size is much smaller [18]. All extracts that provide inhibition against the bacteria *E. aerogenes* followed by determination of the MIC. In this study, the MIC is

expressed as the lowest concentration of the extract gave inhibition against the bacteria *E. aerogenes*. MIC values for all the extracts against the bacteria *E. aerogenes* were at a concentration of 0.75% (w / v), while the MIC values of methanol extract of leaves Jatropha curcas against bacteria *S. aureus* and *E. coli* at 5 mg / ml [6].

4. CONCLUSION

Based on the results of this study, it could be concluded that all *Jatropha curcas* extract has antibacterial activity against Enterobacter aerogenes, where the highest inhibition diameter value contained in the chloroform extract with a concentration of 20%. MIC values for all existing extract at a concentration of 0.75% (w / v).

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