

Comparison of *Aloe vera* leaves ethanol extract effect against *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL

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Abstrak. Aloe vera merupakan tanaman herbal yang mengandung beberapa zat aktif yang telah terbukti mempunyai efek antibakteri untuk berbagai macam bakteri. Dapat ditemukan banyak infeksi yang disebabkan oleh bakteri gram negatif. Beberapa bakteri gram negatif ini memproduksi enzim β lactamase mutan, yang diketahui sebagai *Extended Spectrum Beta Lactamase* (ESBL). Bakteri ESBL resisten terhadap beberapa macam antibiotik dalam golongan beta laktam, hal ini menjadi salah satu permasalahan utama di rumah sakit. Berdasarkan latar belakang yang ada, tujuan dari studi ini adalah untuk membandingkan efek antibakteri dari ekstrak etanol daun lidah buaya terhadap *Escherichia coli* ESBL dan *Klebsiella pneumoniae* ESBL. Studi ini merupakan eksperimental laboratorium. Konsentrasi Hambat Minimal (KHM) dan Konsentrasi Bunuh Minimal (KBM) ditentukan dengan metode dilusi. Konsentrasi yang digunakan pada penentuan KHM untuk kedua bakteri adalah 90%; 80%; 70%; 60%; 50%; 40%; 30%; dan 20%. Penentuan KBM dilakukan dengan cara penanaman suspensi dari *muller hinton broth* pada *nutrient agar plate*. Hasil yang didapatkan dianalisis menggunakan metode deskripsi. KHM untuk kedua bakteri ditemukan pada konsentrasi yang sama yaitu 80% (8 g ml⁻¹). KBM untuk kedua bakteri juga ditemukan pada konsentrasi yang sama yaitu 80% (8 g ml⁻¹). Penentuan KHM dan KBM dilakukan sebanyak 10 kali replikasi. Dapat disimpulkan bahwa ekstrak etanol daun lidah buaya tidak mempunyai efektivitas yang berbeda terhadap *Escherichia coli* ESBL dan *Klebsiella pneumoniae* ESBL.

Kata kunci: *Escherichia coli* ESBL - *Klebsiella pneumoniae* ESBL - Ekstrak etanol daun lidah buaya- antibakteri - dilusi

Abstract. Aloe vera is a herbal which has some active substances that have been proven for its antibacterial effect for some bacterias. There are many infections caused by gram negative bacterias. Some of gram negative bacterias produce mutant β lactamase enzyme, that known as *Extended Spectrum Beta Lactamase* (ESBL). ESBL bacteria are resistant to some antibiotics in beta lactam class and become one of main problem in hospital. Based on this background, the aim of this study was to compare the antibacterial activity of aloe vera leaves ethanol extract between *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL. This study was a lab experimental. Minimum Inhibitory Concentration (MIC) values and Minimum Bactericidal Concentration (MBC) values were determined by dilution method. The concentration used in MIC determination for both bacteria is 90%; 80%; 70%; 60%; 50%; 40%; 30%; and 20%. The MBC values were determined by suspension streaking from muller hinton broth on nutrient agar plate. The result is analyzed with description method. The MIC value for *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL is found in the same concentration which is 80% (8 g ml⁻¹). The MBC values for *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL also found in the same concentration 80% (8 g ml⁻¹). MIC and MBC values have been proved in the first until tenth replications. Thus, aloe vera leaves ethanol extract has no different effectiveness against *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL.

Keywords: *Escherichia coli* ESBL - *Klebsiella pneumoniae* ESBL - *Aloe vera* leaves - antibacterial - dilution method

Introduction

Extended Spectrum β -Lactamases (ESBL) is a plasmid enzyme that could hydrolyze and deactivate some of beta lactam antibiotics, such as third generation of cephalosprin, penicillin and aztreonam[1]. This enzyme is a mutation of TEM and SHV beta-lactamase enzyme that

could be found in Enterobacteriaceae. TEM enzyme is coded by antibiotic resistant gen that located in plasmid. SHV enzyme is coded by antibiotic resistant gen that located in chromosome. This mutation usually happens in enzyme active site, that turns out to be higher activation of the enzyme. This resistant

mechanism could be happen because of the improper use of antibiotics.

ESBL mostly found in Enterobacteriaceae, such as *Escherichia coli* and *Klebsiella pneumoniae*. This bacteria could be found in community infection, but it's more often happens by nosocomial infection. ESBL is known as the main cause of UTI, peritonitis and abscess [2].

Carbapenem is still being used for the main therapy of ESBL infection [3]. Although carbapenem is effective for ESBL, antibiotic therapy is still pretty expensive from the economic side. Also prevention of antibiotic resistant by the excessive use of antibiotic is needed. Thus, we need an alternative therapy for ESBL infection.

Aloe vera as one of herbal plant, could be used for ESBL alternative therapy. From the previous study, aloe vera known to has some active substances that have antibacterial effect such as anthraquinone, aminoglycoside, saponin and fenol [4]. In some previous study, aloe vera extract has been proved for its antibacterial effect for some gram positive bacteria, such as *Streptococcus mutans* [5] and *Staphylococcus spp.* [6]. In previous study, aloe vera also found to be effective in some gram negative bacteria by diffusion method, even in the ESBL bacteria like *Escherichia coli* ESBL and *Klebsiella pneumonia* ESBL [7]. The aim of this study is to compare the antibacterial activity of aloe vera leaves ethanol extract between *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL.

Materials and methods

The sample of Aloe vera leaves ethanol extract is extracted in Balai Materia Medica Batu, East Java Indonesia, and the sample of *Escherichia coli* ESBL & *Klebsiella pneumoniae* ESBL were from Microbiology Laboratory Faculty of Medicine Airlangga University.

Aloe vera leaves ethanol extract preparation

Peel the skin of aloe vera leave, and dry it. Puree the skin of aloe vera leave with blender and weigh for 2 kg and macerate with 2000ml of

ethanolproanalysis in room temperature for 72 hours. Filter the suspension and then steam it with Vacuum Rotary Evaporator in 35°C to separate the solvent and the extract [8].

Escherichia coli ESBL and *Klebsiella pneumoniae* ESBL suspension preparation

Make the *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL suspension in the Mueller Hinton broth until the turbidity is as much as the turbidity of 0,5 McFarland. Use inoculum standard 10^5 CFU/ml-1 for dilution method.

Experiment steps

This study is using macro dilution method. From dilution method we could get Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The steps are: Prepare 10 tubes for the experiment, and named it T1-T8 for bacterial suspension; and K+ & K- for control. Fill K- tube with 2 ml of aloe vera leaves ethanol extract with 100% concentration. Fill K+ tube with 2 ml of bacterial suspension. Fill T1 tube with 1,8 ml of aloe vera leaves ethanol extract, 2 µl of bacterial suspension, and 198 µl of MH broth. So the aloe vera leaves ethanol extract concentration in the tube will become 90%. Fill T2 tube with 80% aloe vera leaves ethanol extract. Fill T3 tube with 70% aloe vera leaves ethanol extract. Fill T4 tube with 60% aloe vera leaves ethanol extract. Fill T5 tube with 50% aloe vera leaves ethanol extract. Fill T6 tube with 40% aloe vera leaves ethanol extract. Fill T7 tube with 30% aloe vera leaves ethanol extract. Fill T8 tube with 20% aloe vera leaves ethanol extract. Incubate all the tubes for 24 hours in 37°C temperature. The MIC is determine by the smallest concentration of aloe vera leaves ethanol extract in the tube, which its suspension changes from turbid into clear. Take the suspension from each tube, plant it to Nutrient Agar plate and incubate for 24 hours in 37°C. The MBC is determine by the smallest concentration of aloe vera leaves ethanol extract in the tube, which doesn't show bacterial colony growth after being plant on nutrient agar plate. All these steps are treated for *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL separately, with 10 times replications for each bacteria.

Data analysis

All the data will be presented and analyzed by description method.

Ethical clearance

The study protocol was approved by the Medical Research Ethics Committee of Faculty of Medicine Airlangga University, Surabaya, Indonesia [Komite Etik Penelitian Kesehatan Fakultas Kedokteran Universitas Airlangga] (Ethical clearance approval number 403/EC/KEPK/FKUA/2016).

Result

The MIC for *Escherichia coli* ESBL is found at 80 % (8 g ml⁻¹) and the MBC for *Escherichia coli* ESBL is also found at 80 % (8 g ml⁻¹). (Picture1, picture 2, table 1)

The MIC for *Klebsiella pneumoniae* ESBL is found at 80 % (8 g ml⁻¹) and the MBC for *Klebsiella pneumoniae* ESBL is also found at 80 % (8 g ml⁻¹). (Picture3, picture 4, table 2)

Discussion

The MIC and MBC of Aloe vera leaves ethanol extract against *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL are found in the same concentration, which is 80% (8 g ml⁻¹). These concentration are found in big concentration because of the complexity of gram negative bacteria cell wall that result in high number of bacteria resistant [9]. Antibacteria effect of Aloe vera leaves ethanol extract is caused by some active substances, such as anthraquinone, aminoglycoside, saponin dan and fenol. Each of these active substances have different mechanism to cause antibacteria effect. Anthraquinone disturb bacteria protein synthesis on *Escherichia coli* [10]. But it has different mechanism in *Klebsiella pneumoniae*, anthraquinone will disturb bacteria morphology by breaking the cell wall component, which is peptidoglikan [11]. Aminoglycoside has same mechanism for both bacteria, it will disturb protein synthesis process at translation stage. Specifically amynoglycoside disturb the process in 30s ribosom, so it can't be attached to 50s ribosom [12], and the final result is aminoglycoside will break the bond of

bacteria protein cell [13]. Saponin also has same mechanism for both bacteria, it will make the bacteria plasma membrane unstable [14] and leak. This state makes the protein and enzyme of the bacteria come out [15]. Thus ATP metabolism is reduced and bacteria will die. Fenol has specific mechanism for each bacteria. In *Escherichia coli* fenol will denature cell membrane protein of the bacteria. Fenol is a lipophilic substance. So, if there are more lipid content on bacteria cell membrane, it will make the fenol effectivity higher [12]. Besides denature the cell membrane protein, fenol has additional mechanism in *Klebsiella pneumoniae*. This active substance can also coagulate the *Klebsiella pneumoniae* protein [11]. The antibacteria effect of Aloe vera leaves ethanol extract is the synergism result of all the four active substances mechanism that mentioned above.

Effectivity of Aloe vera leaves ethanol extract against the *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL can happen because the extract is resistant to ESBL enzyme. This can be possible because all the active substances of aloe vera leaves are not containing beta lactam ring, so the enzyme can't destruct it.

There is no difference effectivity of MIC and MBC between *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL because both bacteria are gram negative bacteria that have the same cell wall and plasma membrane structure, and as mentioned above all the active substances works on bacteria cell wall and plasma membrane.

This study is using crude ethanol extract of aloe vera leaves, and the effectivity is found at 8 g ml⁻¹. If the estimate of human blood volume is 5L, the dose needed for therapy effectivity is very high. So, it's better if we can isolate only the active substance of the aloe vera leaves to lower the therapy dose. The safety dose of Aloe vera leaves ethanol extract must be known. Even in previous study aloe vera is mentioned as practically non toxic substance based on mice experimental [16], aloe vera can causes allergic effect to human [17]. Besides, non decolorized extract of aloe vera has been proved to

cause colon tumor in mice [18]. This colon tumor that happen in mice has the same molecular and morphological pathway with colorectal cancer in human. Aloe vera also has laxative effect that can causes diarrhea. But, this laxative effect can lower the gut absorption, thus it will lower the other side effects [19](Qadir, 2009).

In conclusion, Aloe vera leaves ethanol extract has same effectivity against *Escherichia coli* ESBL and *Klebsiella pneumoniae* ESBL. So, it could become one of alternative therapy for ESBL infection. For further study, the safety dose of Aloe vera leaves ethanol extract must be known.

No conflicts of interest acknowledgment

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