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Sansevieria trifasciata Var. Laurentii ethanol extract can inhibit the growth of *Klebsiella pneumoniae*

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Abstract
Background: Pneumonia is an acute infection of the lower respiratory tract characterized by coughing and shortness of breath caused by Klebsiella pneumoniae bacteria. Sansevieria contains flavonoids, saponins, alkaloids, and steroids known to have antibacterial properties that can destroy the bacterial cell walls. *Objectives*: This study aimed to determine the antibacterial activity of Sansevieria leaf extract against the growth of K. pneumoniae. *Materials and Methods*: Detection of antibacterial activity of Sansevieria was conducted with disc-diffusion test. The presence of antibacterial activity is indicated by the appearance of inhibition (clear) zone after applying the extract at a concentration of 60-100% to the culture medium. As a positive control, the antibiotic chloramphenicol was used. *Results*: The higher the extract concentration, the higher the inhibition zone formed. The inhibition zone formed from extract concentrations of 60%, 70%, 80% is classified as medium (d = 8; 8.5; 9 mm). Meanwhile, the inhibition zones at concentrations of 90% and 100% are 10.5 and 13.5 mm and are classified as strong. The clear zone of the positive control was 29 mm (very strong). *Conclusions*: Sansevieria leaf extract has the potential to inhibit the growth of K. pneumoniae bacteria.

Keywords
Antibacterial, *Klebsiella pneumoniae*, Sansevieria.

1. Introduction

Pneumonia is an acute infection of the lower respiratory tract characterized by coughing and shortness of breath caused by pulmonary (alveolar) inflammation (Nurarif and Kusuma, 2015). According to reports from some special lung health services in Indonesia (Medan, Jakarta, Surabaya, Malang, and Makasar), K. pneumoniae contributes 45.18% as the most common cause of respiratory infections (Makalew, et al., 2016). Klebsiella pneumoniae typically attacks people with immunological disorders, diabetes and chronic lung disease, and alcoholics (Kuswiyanto, 2016).

Antibiotics are commonly used to treat infections caused by microorganisms like bacteria. Unfortunately, antibiotic resistance has grown due to improper use, posing a global health threat. Several studies have found that K. pneumoniae is already resistant to particular antibiotics, such as amoxicillin, sulfamethoxazole, ceftriaxone, ceftazidime, and ampicillin (Sonita and Masri, 2014; Hidayat and Tandari, 2016). Natural plant-derived ingredients are needed to inhibit bacterial growth.

Indonesia has a high biodiversity of traditional medicinal plants. Although these plants have the potential to be used as ingredients in alternative medicine, they have not been studied scientifically. Traditional plant medicines are currently popular among the larger public since they are relatively cheaper than synthetic medicines (Kusmana and Hikmat, 2015). One of the traditional medicinal plants that is widely used by the community is Sansevieria trifasciata leaf. It contains secondary metabolite compounds such as flavonoids, saponins, alkaloids, and steroids (Dewatisari, et al., 2017; Siregar, et al., 2020; Rihanah and Jura, 2020). This chemical compound is recognized to have antibacterial properties and can break the cell walls of microorganisms (Mardiana, et al., 2015; Lombogia, et al., 2016). This study aims to determine the antibacterial activity of Sansevieria leaf against Klebsiella pneumoniae growth using the inhibitory zone.

2. Materials and Methods

This research follows a true experimental design using a one-shot case study design. The extraction process took place in the Pharmacy Laboratory of Muhammadiyah University of Yogyakarta, while antibacterial testing was conducted at the STIKES Guna Bangsa Yogyakarta Microbiology Laboratory in August-September 2023.

2.1. Tools and materials

The research utilized several tools including petri dishes, test tubes, tube racks, glass beakers, Erlenmeyer flasks, measuring pipettes, tubes, burners, weighing bottles, scales, incubators, and ovens. The research used Sansevieria leaf extract, K. pnemuniae (ATCC 33495) bacterial culture, 0.85% physiological NaCl, sterile distilled water, Muller Hinton Agar media and standard solution (Mc Farland 108 CFU/mL).

2.2. Extraction process

Eight kilograms of Sansevieria leaves were sliced into small pieces and dried in an oven at 40-50°C for 2-3 days (until completely dry) using the freeze-drying technique.

Subsequently, they were grounded into a powder form known as simplicia. Simplicia of Sansevieria leaf was soaked in a 70% ethanol solvent at a ratio of 1:10 (1 part of simplicia to 10 parts of solvent) for 5 days, with stirring every 24 hours. The material is filtered using flannel fabric to obtain a green filtrate (macerate), then evaporated using a water bath at 70°C for several hours until a dense extract is obtained.

2.3. Media and reagents

Nineteen grams of Mueller-Hinton media (Merck) was dissolved in 500 mL of distilled water and sterilized using an autoclave for 15 minutes at 121°C. The media was poured into petri dishes (~20 mL), and then refrigerated in the refrigerator. The Mc Farland standard was made by mixing 9.9 mL of 1% sulfuric acid with 0.1 mL of 1% barium chloride for turbidity comparison.

2.4. The Making of bacterial suspensions

Klebsiella pneumoniae colonies were transferred from Nutrient Agar culture to 5 mL of 0.85% physiological NaCl solution. The suspension solution was compared to the Mc Farland standard solution (108/CFU) until they reached the same turbidity.

2.5. Blank discs preparation

Blank paper discs were soaked in leaf extract with concentrations of 60%; 70%; 80%; 90%; 100%, negative control (distilled water), and chloramphenicil (Oxoid) 30 µg as positive control for 10-15 minutes (until fully saturated).

2.6. Anti-bacterial test

A sterile cotton swab is dipped in the bacterial suspension and then streaked evenly on the surface of the Muller Hinton Agar medium until evenly distributed. The same procedure was likewise carried out on medium containing negative and positive controls. Paper discs of various concentrations of leaf extract were placed on the medium surface and incubated at 37°C for 24 hours. The diameter of the inhibitory zone formed (clear area) around the disc paper was measured and classified according to bacterial inhibitory response power (Table 1). The treatments, consisting of different extract concentrations, were repeated twice.

Table 1. Classification of clear zones response

Diameter (mm)	Inhibiting response
< 5	No response
5-10	Medium
10-20	Strong
> 20	Very Strong

(Source: Davis and Stout, 1971)

2.7. Ethical Clearance

Research ethical approval was issued by the STIKES Guna Bangsa Yogyakarta Health Research Ethics Commission Number 016/KEPK/VIII/2023 on August 7, 2023.

3. Results and Discussion

3.1. Extraction results

Eight kilograms sansevieria leaves produced 2.1 kg of dry material and finally 700 grams of simplicia. The purpose of material refinement is to increase the surface area of the particles so that the contact area with the solvent will be greater, thus facilitating the penetration of the solvent and increasing the extraction of compounds with the solvent used. The solvent used is 70% ethanol, according to research conducted by Rasyid et al. (2018). The advantage of ethanol solvent is its universal nature so that it can dissolve almost all secondary metabolites contained in the simplicia. Extraction using 70% ethanol solvent with a ratio of 1:10 resulted in 108.1 grams of ethanol extract.

3.2. Antibacterial test

Sansevieria leaf extract at concentrations of 60%; 70%; and 80% showed medium inhibitory activity against the growth of K. pneumoniae (Table 2), while concentrations of 90% and 100% are strong according to Amelia (2022). The positive control (chloramphenicol) showed an average inhibition zone of 29 mm (very strong), while the negative control had no inhibition zone. The study shows that Sansevieria leaf extract has inhibitory effects on the growth of K. pneumoniae bacteria, but is not as strong. The absence of an inhibition zone in the negative control indicates the antibacterial activity is not due to distilled water as a solvent, but rather from the extract.

Table 2. Diameter of the inhibition zone and category of inhibition power of Sansevieria leaf extract against the growth of K. pneumoniae

Extract Concentration Treatments Groups	Diameter of inhibition zone (mm)	Inhibiting response
60%	8,0 ± 0,0	Medium
70%	8,5 ± 0,7	Medium
80%	9,0 ± 0,0	Medium
90%	10,5 ± 0,7	Strong
100%	13,5 ± 2,5	Strong
Positive Control	29 ± 0,0	Very Strong
Negative Control	0 ± 0,0	No response

The solvent selection can influence the number of compounds contained in plant extracts. Ethanol 70% is used as the solvent due to its ability to attract more active compounds than other types of organic solvents. It possesses lower boiling point of 79°C and requires less heat to concentrate the extract. Ethanol solution is considered less toxic than the other solvents, making it safe to consume (Hasanah and Novian, 2020).

Similar research using Sansevieria extract has been conducted by Fatimah et al. (2020). In their research, Fatimah et al. used 96% ethanol as solvent and produced a clear zone of 7-13 mm (considered as weak-very strong) for the concentration 60-100%. Meanwhile, our research used 70% ethanol and produced clear zones of 8,0 ± 0,0 - 13,5 ± 2,5 mm and categorized medium-strong inhibitory response according to Davis and Stout (1971). Our research results show that 70% ethanol extract produces a larger clear zone compared to 96% ethanol extract.

Sansevieria leaf has been identified to contain flavonoid, saponin, and alkaloid compounds by phytochemical studies (Siregar, et al., 2020; Rihanah and Jura, 2020). Another study confirmed that Sansevieria leaf extract contains steroids (Dewatisari, et al., 2017). These chemical compounds possess antibacterial capabilities that can damage the cell walls of microorganisms (Mardiana, et al., 2015; Lombogia, et al., 2016).

Klebsiella pneumoniae is a gram-negative bacterium with thin peptidoplastic cell walls so that flavonoids, steroids, saponins, tannins and alkaloids can damage the bacterial cells. The cell wall functions to maintain the shape and to protect the bacteria. An extract with antibacterial properties can inhibit the formation of new cell walls in Klebsiella pneumoniae by disrupting the incorporation of glycan chains that are not cross-linked into the peptidoplasty of the cell wall, leading to bacterial death (Lien, et al., 2020). Any compound that inhibits the process of peptidoglycan synthesis would weaken the bacterial cell wall, potentially leading to cell lysis (Jawetz, et al., 2001).

Flavonoid compounds act as antibacterial by interacting with several essential enzymes, affecting nucleic acid synthesis, cytoplasmic membrane function, and aerobic metabolism, leading to cell damage due to energy deprivation (Adamczak, et al., 2019; Pragita, et al., 2020). Steroids are sensitive to lipid membranes causing leakage in lysosomes. On the other hand, saponin can induce leakage of proteins and enzymes in cells, resulting in damage to bacterial cell walls (Mardiana, et al., 2015). Alkaloid

compounds inhibit the formation of peptidolic acid in bacterial cells, preventing the formation of the cell wall layer and leading to cell death (Mardiana, et al., 2015; Apriliana, et al., 2018).

4. Conclusions

The Sansevieria leaf extract may inhibit the growth of K. pneumoniae bacteria. Sansevieria extract 100% and 90% both has showed strong inhibition against the growth of K. pneumoniae, while concentration of 60%, 70%, and 80% showed medium inhibition.

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Author Contributions: Audiah Fitriani: conseptualization, sample collection, writing-original draft; Nining Eka Sawitri, Syahdapuan Dara Khatrina: performed laboratory work, data analysis; Fitri Nadifah: review; Desto Arisandi: methodology, validation, supervision, review.

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