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RESEARCH ARTICLE
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Scented Root Extract (*Vetiveria zizanioides L.*) Inhibits *Bacillus cereus* Growth In-Vitro

Bramantyas Kusuma Hapsari[1], Rosdiana Sahabuddin[2], Rizky Indah Vebrianty[3]

[1]Department of Microbiology, Faculty of Medicine and Health Sciences, University of Muhammadiyah Makassar, Makassar, Indonesia
[2]Department of Obstetrics and Gynecology, Faculty of Medicine and Health Sciences, University of Muhammadiyah Makassar, Makassar, Indonesia
[3]Undergraduate Program, Faculty of Medicine and Health Sciences, University of Muhammadiyah Makassar, Makassar, Indonesia
Coresspondence Author: bramantyas.sari@med.unismuh.ac.id

Abstract:

Bacillus cereus, a gram-positive bacterium with a high incidence of causing infection through food, is a common cause of infection. Plant extract from *Vetiveria zizanioides (L.)* contains alkaloids, flavonoids, saponins, and tannins, and has been demonstrated to exhibit antibacterial action against other bacteria such as *Staphylococcus aureus, Vibrio cholerae, Escherichia coli, Salmonella typhi, and Klebsiella pneumoniae*. The purpose of this study is determining the antibacterial activity of vetiver root extract (*Vetiveria zizanioides (L.)*) against the growth of Bacillus cereus bacteria in vitro. In this experimental study, the inhibition effect of vetiver root extract against *Bacillus cereus* growth was measured using disc diffusion method. Extract consentraion used in this tudy were 60%, 80% and 100%. The diameter of area with no bacterial growth around disc (inbition zone) was measured and the average of diamter from four independent measurement was used to decide the sensitivity of bacteria towards the extract. The average measurement results from 60 percent concentration of extract were 6.70 mm, 7.04 mm for 80 percent concentration, and 7.05 mm for 100 percent extract concentration. The positive control, Amoxicillin 500 mg, had a diameter of 14.35 mm, whereas the negative control, sterile distilled water, had no impact. Bacillus cereus bacteria growth can be inhibited by *Vetiveria zizanioides (L.)* extract.

Keywords: Bacillus cereus; Infection; Vetiveria zizanioides (L.)

Introduction

Food poisoning is a common phenomenon. Family food (46.9%), catering services (18.9%), and food stalls are all potential sources of food poisoning (18.3%). If not treated promptly, food poisoning can result in increased morbidity and mortality. The most common pathogens in food poisoning are *Escherichia coli* (20%), *Bacillus cereus* (19.4%), and *Staphylococcus sp* (18.3%).[1]

Bacillus cereus is a gram-positive bacterium that produces enterotoxin. These bacteria produce two types of toxins, namely toxins emetics and diarrhea. This toxin can cause symptoms of poisoning different food. Bacillus cereus is usually found inside milk, meat, spices, and cereals. Foodstuffs that contain starch are an optimal source for growth meat.[2]

Bacillus cereus food poisoning symptoms arise 1-6 hours after a person consumes food, particularly meat.[3] Diarrhea and vomiting are common symptoms. Treatment with antibiotics is required to overcome this. Antibiotics must be used in compliance with the dose because failure to do so can result in antibiotic resistance. Antibiotics must be used as per indications and doses to tackle drug resistance.[3]

The use of traditional plants is expected to be a reasonable alternative treatment for Bacillus cereus bacterial illness in the community. Plants such as vetiver are one of the classic plants that can be used as a natural antibacterial (*Vetiveria zizanoides L*). Vetiver (*Vetiveria zizanoides L.*) is an annual grass with morphological traits similar to grass in general, such as long stems, parallel and thin leaves, and a semi-rigid structure.[4] Vetiver plant extract contains alkaloid compounds, flavonoids, saponins and tannins, as well as proven to have activity antimicrobial against several pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Vibrio cholera, Bacillus cereus, and Klebsiella pneumoniae*.[5] Although medicinal plants have the potential to be developed further for the treatment of infectious diseases, many have yet to be scientifically validated for their bioactivity.[6] Traditional medicine, according to Article 1 Number 36 of the Health Law of 2009, is a concoction of ingredients in the form of plant, animal, mineral, or extract preparations (galenic), or a mixture of these materials that have been used for generations for treatment and can be applied in accordance with cultural norms. Natural antibacterials have become popular in recent years since they do not harm the body and may be simply derived from traditional plants or spices.

The findings of this study are likely to develop a framework for future research on root boiled water fragrances that can be utilized as herbal antibiotics in cases of communal food poisoning.

Research Method

Study Design

The focus of this research is to see how vetiver extract (*Vetiveria zizanoides L.*) affects the bacterium Bacillus cereus growth at various concentrations; 60, 80, and 100 percent in streile aquadest. This is a legitimate experimental study that employs the Completely Randomized Design approach.

Samples

The raw materials plants, vetiver, were chosen as the study's sample (*Vetiveria zizanoides L.*). Vetiver root extract (*Vetiveria zizanoides L.*) will be used to investigate the sensitivity of *Bacillus cereus* bacteria in vitro using the disc diffusion method at concentrations of 60%, 80%, and 100% in sterile saline. Bacillus cereus was employed, and it was cultured in a petri dish on medium Mueller-Hilton Agar (MHA) and incubated at

370C for 24 hours. In this study, there are five treatment groups, as shown in Table 1.

Table 1. Treatment

Group	Treatment
1	Vetiver extract with concentration 60%
2	Vetiver extract with concentration 80%
3	Vetiver extract with concentration 100%
4	Positive Control (Amoxicillin)
5	Negative Control (Sterile aquades)

The total number of samples was 20, which meant that each sample group would have four repetitions, with the final result depending on the average inhibition zone in each group.

Inhibition zone interpretations

Based on the classification or category of bacterial inhibitory power according to Davis & Stout. The findings of measuring the diameter of the clear zone are divided into three categories: diameter 20 mm has a very strong inhibitory strength, diameter 10-20 mm has a strong inhibitory power, and diameter 5-10 mm has a very strong inhibitory power. The inhibition is weak in the medium and 5 mm ranges.

Results

The diameter of the clear zone which was formed around the disc is measured with a caliper to determine inhibition zone. Results The inhibition zone is calculated as shown in Table 2.

Table 2. Average results of inhibition zone diameter from four separate experiments

Compound concentration	Inhibition Zone Diameter			
	I	II	III	IV
60%	9,09	9,50	7,85	7,03
80%	9,78	9,61	8,58	7,23
100%	8,62	10,43	8,86	7,33
Control*	11,66	11,96	15,99	14,60

*Amoxicillin

The graph for figure 1 describes the average of various treatments given a set concentration of vetiver extract and the diameter of the *B. cereus* inhibitory zone in each treatment group using the data from the several tables above.

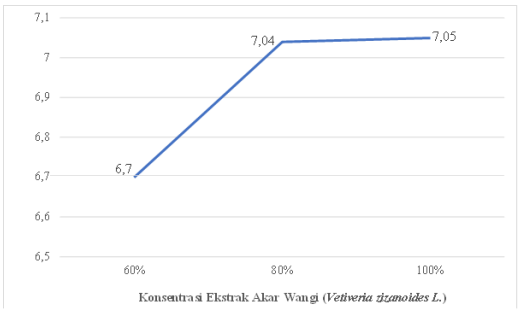

Figure 1. Average Inhibitory Zone Diameter Graph (mm)

The average diameter of the inhibitory zone differs at each concentration (60 percent, 80 percent, and 100 percent) of vetiver extract, as shown in the graph above. The diameter of the inhibitory zone grew as the concentration of vetiver extract rose, as shown in the graph above (*Vetiveria zizaniodes L.*).

Discussion

Based on the classification of bacterial inhibition according to Davis & Stout, the results of measuring the diameter of the clear zone are categorized into diameter ≥ 20 mm having a very strong inhibition, 10-20 mm having a strong inhibition, 5-10 mm having a

medium inhibition, and ≤ 5 mm has a weak inhibition.

A clear zone developed in the petri dish surrounding the paper disc based on the activity test of vetiver root extract (*Vetiveria zizanoides L*.) on the growth of *Bacillus cereus* in vitro with various concentration variables, namely 60 percent, 80 percent, and 100 percent. This suggests that vetiver root extract (*Vetiveria zizanoides L*.) is antibacterial against *Bacillus cereus* bacterium. The inhibitory zone measurements were only calculated using a caliper on the first day after being incubated for 24 hours.

The average diameter of the clear zone formed in the inhibition test of vetiver root extract (*Vetiveria zizanoides L*.) on the growth of *Bacillus cereus* in vitro (Table V.1 to table V.5) at the 60% concentration variable from the five experiments was 6.70 mm. The average diameter of the clear zone formed at 80 percent concentration was 7.04 mm, and the average diameter of the clear zone formed at 100 percent concentration was 7.05 mm. The positive control used was Amoxicillin, which is a broad-spectrum antibiotic that has a sensitive effect on the growth of *Bacillus cereus* when tested; the average diameter of the inhibition zone was 14.35mm, while the negative control, sterile distilled water, yielded negative results with no inhibition.

Vetiver extracts at 60 percent, 80 percent, and 100 percent concentrations were found to have moderate sensitivity in preventing *Bacillus cereus* bacterium growth. The positive control in the form of the antibiotic Amoxicillin with a diameter of 14.35 mm was assessed as having Intermediate or Moderate sensitivity based on the measurement results. The sterile distilled water used as a negative control was found to have no sensitivity in suppressing Bacillus cereus growth. The prepared vetiver root extract (*Vetiveria zizanoides L*.) and Bacillus cereus bacteria suspended in Mueller Hinton Agar (MHA) medium revealed no damage or issues in the classification of measurement data for positive and negative controls.

In simplicia, there is no water content adjustment utilizing the distillation concept, which is the separation of mixtures based on differences in boiling points. The toluene distillation method is used in this study, according to the Indonesian Herbal Pharmacopoeia (2017), with the goal of drying (removing water content) because a higher water content can lead to the growth of microbes/microorganisms in simplicia.

It is not suggested to utilize distilled water in the production of vetiver extracts containing more than 10% plant simplicia. To meet the standards of a good extraction simplicia, with a good water content of less than 10%, according to the Indonesian Ministry of Health (2008). It is predicted that a water content of less than 10% will avoid microbial contamination and maintain the quality of the simplicia. As a result, H0 (Null Hypothesis) is maintained, but Ha (Alternative Hypothesis) is rejected.

In this study there were limitations, in the fifth experiment, the MHA medium used was used up and it was re-inoculated in the fifth petri dish, so the incubation schedule in the fifth petri dish was late and did not coincide with the other petri dishes. This causes the growth of bacterial colonies in the fifth petri dish; no growth occurs when you want to take inhibition zone measurements.

Conclusions

The vetiver root extract (*Vetiveria zizanoides L*.) with concentrations of 60%, 80%, and 100% contained antibiotics and had moderate inhibitory power against *Bacillus cereus* bacteria.

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