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Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Extract of NADES Nail Henna Leaves Against *Bacillus Cereus Bacteria*

Ekstrak Konsentrasi Hambat Minimum (KHM) dan Konsentrasi Bakterisida Minimum (KBM) Daun Henna Kuku NADES Terhadap Bakteri Bacillus Cereus

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Abstract

Objective: This study aims to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) concentrations of the NADES extract of henna leaves (*Lawsonia inermis L.*) against *Bacillus cereus bacteria*.

Methods: This research is a descriptive type of research that was conducted at the Phytochemical Laboratory and Microbiology Laboratory of Sekolah Tinggi Farmasi Muhammadiyah Cirebon. Collecting data from the MIC test results by looking at the turbidity at each concentration and the MBC results by looking at the presence or absence of bacterial growth.

Results: The MIC sample could not be obtained because it looked cloudy, and bacteria were still growing on the media. In contrast, the MBC sample was only 100%, which could inhibit bacteria's growth on the media. The positive control of Ciprofloxacin at a concentration of 100 mg had a diameter of 2.34 cm, 125 mg had a diameter of 2.39 cm, 150 mg had a diameter of 2.47 cm, and the negative control NADES solution had a diameter of 0.98 cm.

Conclusion: The Minimum Inhibitory Concentration (MIC) cannot be known because the results of the NADES extract of henna leaves compared to before and after incubation still look cloudy due to factors from the sample. In the other hand, Minimum Bactericidal Concentration (MBC) can only be known at a concentration of 100%, which can kill the growth of *Bacillus cereus bacteria*.

Keywords: Bacillus cereus, minimum bactericidal concentration, minimum inhibitory concentration, nail henna leaves, natural deep autectic solvent.

Introduction

Bacillus cereus a facultatively anaerobic, toxin-producing gram-positive bacterium found in soil, vegetation, and food[1] that can cause infectious diseases[2] and poisoning with symptoms of vomiting and diarrhea. Bacillus cereus is commonly found in nature, with spores more resistant to environmental stress than its vegetative cells. Bacillus cereus can also cause other, more dangerous infections such as non-gastrointestinal infections, respiratory tract infections, central nervous system infections, urinary tract infections, and urinary tract infections—skin[3].

For many years, natural goods with therapeutic capabilities such as plants, minerals, and animal products were the primary sources of medications for the treatment of different ailments; thus, selection of Lawsonia inermis L. (Henna) to study effectivity was considered[4].

The henna leaf plant (*Lawsonia inermis L.*) or henna leaves is a pharmacologically important plant[5] that significantly can cure inflammation of the knuckles (*paniritium*) and wounds on the skin. In addition, the seeds, flowers, bark and roots have the potential to cure headaches, diarrhea, leprosy, and fever [6].

Natural Deep Autectic Solvent (NADES) is The selection of the extracting solvent is crucial to develop selective and effective methods for the extraction and isolation of target compounds in the plant matrices[7]. It is also a potential alternative to replace conventional organic solvents with non-volatile, non-flammable and toxic properties. NADES is a mixture of molecules that are Hydrogen Bonding Acceptors (HBA), which form intermolecular hydrogen bonds with one or more Hydrogen Bonding Donor (HBD) molecules, thereby reducing the melting point of the mixture to a much lower temperature than the respective components[8].

Based on the research by Silva Devi & Tuty Mulyani in 2017, the ethanol extract of henna leaves has activity against the Pseudomona aeruginosa bacteria. The results of the antibacterial activity test of the ethanol extract of henna leaves against Pseudomona aeruginosa bacteria showed an inhibition diameter of 21.6 mm at a concentration of 100% [9]. And based on Nia Murni Asih's research in 2021 that the NADES extract from henna leaves can inhibit the growth of the Pityrosporum ovale fungus. The result for the largest diameter of the inhibition zone was 2.73 at a concentration of 100% The selection of the

extracting solvent is crucial to develop selective and effective methods for the extraction and isolation of target compounds in the plant matrices[10].

In this study, the authors were interested in testing the NADES extract of henna leaves with concentrations of 0.39%, 0.78%, 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50% and 100%. Against *Bacillus cereus bacteria* using MIC and MBC testing and diffusion and dilution methods. Based on this, this study aims to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) concentrations of the NADES extract of henna leaves (*Lawsonia inermis L.*) against *Bacillus cereus bacteria*.

Methods

Research Design

This research is a descriptive type of research by looking at the turbidity of the MIC (Minimum Inhibitory Concentration) and seeing whether or not there is bacterial growth on the MBC (Minimum Bactericidal Concentration) NADES extract of henna leaves (*Lawsonia inermis L.*) against *Bacillus cereus bacteria*. This research was conducted at the Phytochemical Laboratory and Microbiology Laboratory of Muhammadiyah College of Pharmacy Cirebon. Collecting data from the MIC (Minimum Inhibitory Concentration) test results by looking at the turbidity at each concentration and the MBC (Minimum Bactericidal Concentration) results by looking at the presence or absence of bacterial growth.

Tools and materials

The tools used in this research were Microwave (Rewez multifunctional), Autoclave (Model 25 x eledric 138° max), Petri dishes (Pyrex), Ose Needles, Test Tubes, Measuring flasks, Volume pipettes, Thermometers, Stoves, Analytical balances (Ohaus), Incubator Oven (Memert), Caliper (Krisbrow), Injection Syringe (Terumo syringe), Perforator, Tipcon, Colony counter, and glassware commonly used in laboratories.

The materials used in this study were henna leaves (*Lawsonia inermis* L.), *Bacillus cereus bacterial* culture, Nutrient Broth (Oxoid), Nutrient Agar (Oxoid), NADES (citric acid + glucose), Barium chloride 1% (KGaA), 1% Sulfuric acid (KGaA), Ciprofloxacin (HJ), 0.9% NaCl (Wida NS) and Aquadest.

Research procedure

Making henna leaf simplicial -> NADES preparation -> Making henna leaf extract -> Concentration for MBC -> (positive and negative) Control -> Sterilization -> Procedure for making NB (Nutrient Broth) Media -> Preparation of NA (Nutrient Agar) Media for Petri Dishes -> Making Media so Italic -> Bacterial Rejuvenation -> Preparation of bacterial suspension -> Making comparison Mc.Farland 0.5 -> Testing MIC and MBC.

Data analysis

MIC and MBC test data were obtained from the results of turbidity and clarity, namely visual observation. Then it is arranged in the form of an observation table for discussion and conclusion.

Results

The results of this study were carried out in two stages, namely the Minimum Inhibitory Concentration (MIC) test and the Minimum Bactericidal Concentration (MBC) test using the diffusion and dilution method. In contrast, the concentrations used were 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78% and 0.39%. Here are the results:

Table 1. Results of MIC of NADES extract of henna leaves against *Bacillus cereus bacteria*

Tube Number	NADES Extract Concentration of henna nail leaves (*Lawsonia inermis* L.)	Bacterial Growth
1	100%	+ (looks cloudy)
2	50%	+ (looks cloudy)
3	25%	+ (looks cloudy)
4	12,5%	+ (looks cloudy)
5	6,25%	+ (looks cloudy)
6	3,125%	+ (looks cloudy)
7	1,56%	+ (looks cloudy)
8	0,78%	+ (looks cloudy)
9	0,39%	+ (looks cloudy)
10	Positive Control	+ (looks cloudy)
11	Negative Control	- (looks clear)

Note: (+) The liquid looks cloudy, there is still bacterial growth; (-) The liquid looks clear, there is no bacterial growth.

Table 2. Minimum Bactericidal Concentration (MBC) Test Results of NADES henna leaf extract against *Bacillus cereus bacteria*

Tube Number	NADES Extract Concentration of henna nail leaves (*Lawsonia inermis* L.)	Bacterial Growth
1	100%	- (No bacterial growth)
2	50%	+ (There is bacterial growth)
3	25%	+ (There is bacterial growth)
4	12,5%	+ (There is bacterial growth)
5	6,25%	+ (There is bacterial growth)
6	3,125%	+ (There is bacterial growth)
7	1,56%	+ (There is bacterial growth)
8	0,78%	+ (There is bacterial growth)
9	0,39%	+ (There is bacterial growth)

Note: (+) There is bacterial growth; (-) No bacterial growth

Table 3. Positive Control and Negative Control Diameter of Inhibition Power of NADES Extract of Girlfriend Leaves Against *Bacillus cereus Bacteria*

Diameter of Inhibition Area (cm)				
Times Of Measurement	Control (-) NADES solution	Control (+) Ciprofloxacin		
		100 mg/ 10 ml	125 mg/ 10 ml	150 mg/ 10 ml
1	1,26	3,56	3,10	3,56
2	1,57	2,53	3,06	2,40
3	1,75	2,56	2,75	3,20
4	1,75	3,11	3,06	3,13
Total	6,33	11,76	11,97	12,29
Average	0,98 cm	2,34 cm	2,39 cm	2,47 cm

Discussion

This study was descriptive research by conducting the Minimum Inhibitory Concentration (MIC) Test, and the Minimum Bactericidal Concentration (MBC) Test of the NADES extract of henna leaves on *Bacillus cereus bacteria*, the results of which were determined at the Minimum Inhibitory Concentration (MIC), which was seen by visual turbidity. Results are seen before and after incubation. Minimum Bactericidal Concentration (MBC), which is seen from the presence or absence of bacterial growth in the petri dish after incubation. The positive control used in this method was Ciprofloxacin and the negative control used, was NADES solution, which was carried out by means of the good method to determine the clear zone formed around the wells made on the media.

This research begins with the preparation of henna leaf Simplicia (*Lawsonia inermis L.*). The henna leaves are chopped beforehand with the aim of increasing the surface area for maximum withdrawal of the extracted substance. Chopped henna leaves (*Lawsonia inermis L.*) are dried in an oven at 40^{o}C for 24 hours. This drying process aims

to reduce the water content, prevent mould growth, to obtain simplicia that is not easily damaged, so it can be stored for a long time. The dried Simplisia leaves of henna (*Lawsonia inermis L*.) were then crushed using a blender, then weighed as much as 100 grams.

After weighing, the next step is to make a liquid extract of henna leaves using NADES solvent. NADES is considered safer for consumption (food grade), environmentally friendly, and cheaper than conventional solvents, which are more expensive and harmful to the environment. The NADES used were citric acid and glucose in a ratio (of 3:1). In the extraction process, citric acid acts as a hydrogen bond acceptor, while glucose acts as a hydrogen bond donor. The method of preparation is by mixing 450 grams of citric acid, 150 grams of glucose, and 600 ml of distilled water is added and then dissolving until it reaches a temperature of 50^{o}C until it dissolves.

NADES solvent that has been prepared is mixed with henna leaf Simplicia (*Lawsonia inermis L*) and then extracted using an efficient extraction tool, namely Microwave Assisted Extraction (MAE) at 40^{o}C for 5 minutes. The resulting extraction was then filtered and counted how much extract was obtained. The yield obtained was 202.467%. Because the results obtained are liquid extracts. Then extracts of henna leaves (*Lawsonia inermis L.*) were made with concentrations of 0.39%, 0.78%, 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50% and 100%. %.

The sterilization process uses an autoclave at 121^{o}C for 15 minutes. This sterilization aims to prevent contamination of outside organisms and guarantee the sterilization of tools and materials used.

This study could not know the results of the Minimum Inhibitory Concentration (MIC) of NADES extract of henna leaves against *Bacillus cereus bacteria*. This can be seen from Table 1; all samples at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, and 0.39% can be overgrown with bacteria. The difference that was seen before and after incubation, before incubation, all concentrations were in turbid conditions, and there were still particles present at these concentrations, so after incubation, all concentrations still looked cloudy from these concentrations there was a factor from the sample given henna nails it already looked cloudy. In contrast, in the positive control before incubation, it already looked cloudy. After incubation, it still looked cloudy. This was due to the influence of the source of the antibiotic compared to

the negative control containing NADES solution where the NADES solution contained citric acid and glucose; the citric acid had activity as an antibacterial.

Table 2: Minimum Bactericidal Concentration (MBC) test results for NADES henna leaf extract on the growth of *Bacillus cereus bacteria*, showing that bacteria can still grow as demonstrated by concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, and 0.39%. A 100% concentration shows clarity that bacteria do not grow and can inhibit bacterial growth. So it can be concluded that henna leaf extract can kill the growth of *Bacillus cereus bacte*ria.

The results of the inhibition test on the positive control ciprofloxacin with concentrations of 100 mg, 125 mg, and 150 mg showed a clear zone with an average diameter at a concentration of 100 mg of 2.34 cm, a concentration of 125 mg of 2.39 cm, and a concentration of 150 mg of 2.47 cm. In the NADES negative control, it was found that there was an area of inhibition zone around the hole of 0.98 cm, and the concentration of ciprofloxacin which had the highest inhibition zone, was 100 mg because this concentration was categorized as vital. With the constraints in the laboratory, namely the damage to the tool. So this research was done visually.

Conclusion

Based on the results of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Tests of the NADES extract of henna leaves against *Bacillus cereus bacteria*, it can be concluded that the Minimum Inhibitory Concentration (MIC) cannot be known because the results of the NADES extract of henna leaves compared to before and after incubation still look cloudy due to factors from the sample. In the other hand, Minimum Bactericidal Concentration (MBC) can only be known at a concentration of 100%, which can kill the growth of *Bacillus cereus bacteria*.

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