



CERTIFICATE

OF APPRECIATION

THIS CERTIFICATE IS AWARDED TO:

Muhammad Yanis Musdja

as **ORAL PRESENTER [ABS-39]**

at The Second International Conference on Health Sciences (ICHS 2022)
organized by Faculty of Health Sciences UIN Syarif Hidayatullah Jakarta
Jakarta, August 24-25, 2022



Dr. apt. Zilhadia, M.Si.

ICHS 2022 CHAIRPERSON

apt. Yardi, Ph.D.



UIN SYARIF HIDAYATULLAH JAKARTA

**ANTIBACTERIAL ACTIVITY OF BANGLE RHIZOME (*Zingiber montanum*,
J.Koenig) EXTRACT AGAINST SOME GRAM POSITIVE BACTERIA USING
MACERATION TECHNIQUE WITH MICROWAVE**

M. Yanis Musdja, Ayu Haryati, Hendri Aldrat

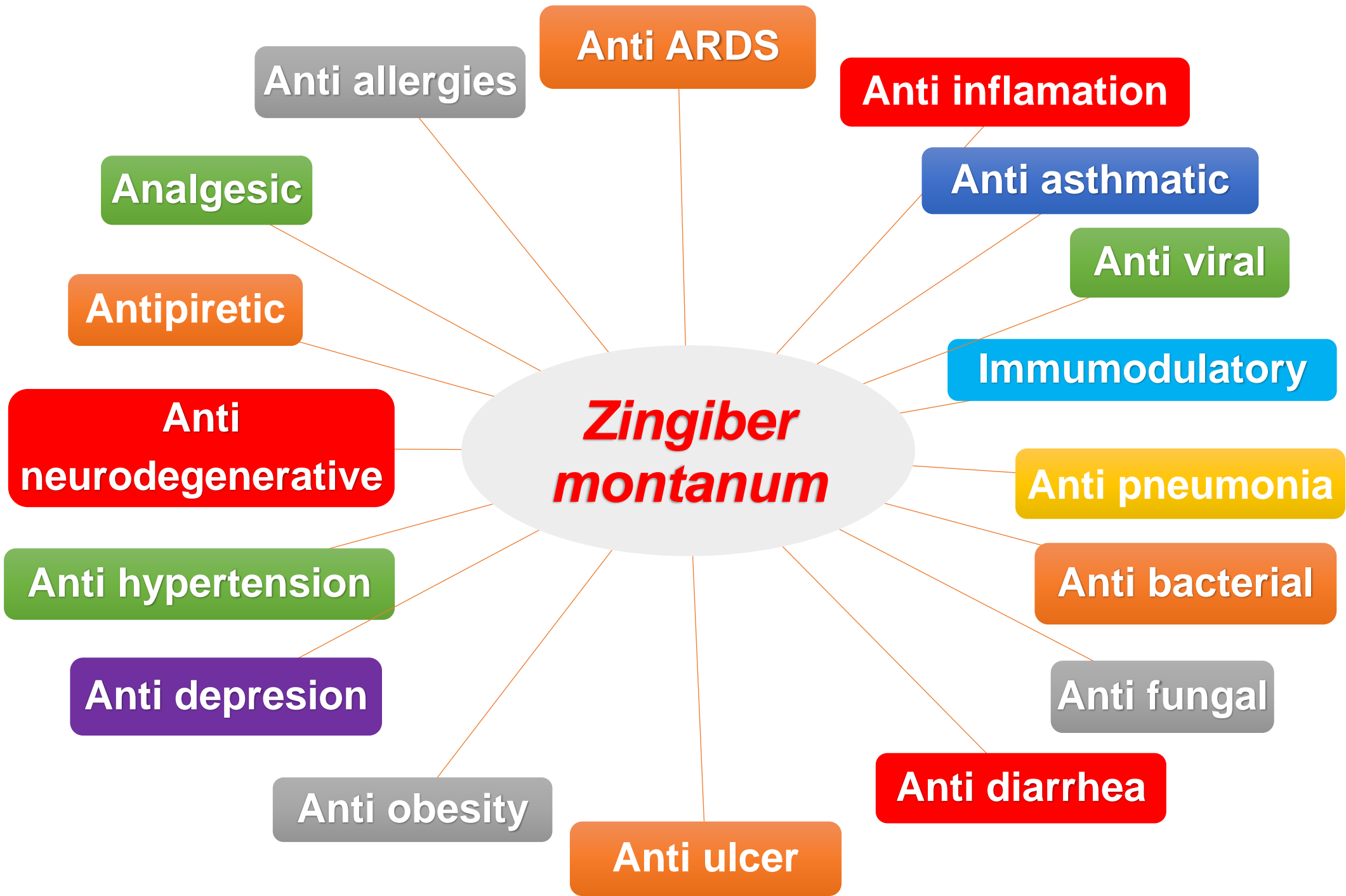
PHARMACY STUDY PROGRAM

INTRODUCTION

- Plants are still used for medicinal purposes around the world.
- 80% of the world's population still relies on plant medicines to treat illness (WHO figures)
- Infectious diseases account for about half of the death in tropical countries
- Plants remain the most common source of antimicrobial agents and is reported to have minimal side effects
- The number of antibiotics that can be used for treatment is limited, from day to day the number of antibiotics is increasingly resistant. According to WHO, if there are no new antibacterial discoveries, then in 2050 it will be more difficult to cure infectious diseases

Bangle
Zingiber montanum





OBJECTIVE

This study aimed to determine the activity of the 70% ethanol extract of Bangle rhizome macerated using a microwave against several gram-positive bacteria, namely:
B. cereus, B. subtilis, P. acnes, S. aureus, S. epidermidis

METHODOLOGY OF RESEARCH

Pengumpulan Bahan dan Penyiapan Simplisia



Dikumpulkan 4
Kg Bangle



Dicuci dan
dibersihkan



Di potong tipis-
tipis lalu
dikering
anginkan



Dihaluskan
menggunakan
blender dan
diperoleh
simplisia
serbuk

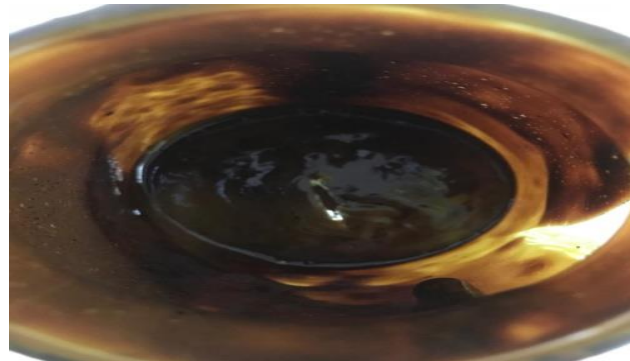
Bangle extract preparation



700 g of simplicia powder was extracted with 70% ethanol using 100 W microwave, 5 minutes



Filtered and obtained a liquid extract.



Viscous extract obtained as much as 56,62 g (Yield = $\pm 8\%$)



Evaporated with a vacuum evaporator at a temperature of $\pm 40\text{ }^{\circ}\text{C}$

Preparation of Antibacterial Activity Test with sterile materials and tools

Media Preparation and Sterilization

Weigh as much as 3.8 g MHA or 2.1 g MHB and prepare 100 mL of aquadest



The prepared MHA or MHB was mixed with aquadest in an erlenmeyer



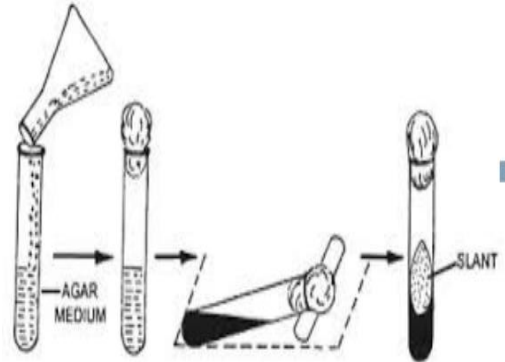
The prepared MHA or MHB was mixed with aquadest in an erlenmeyer



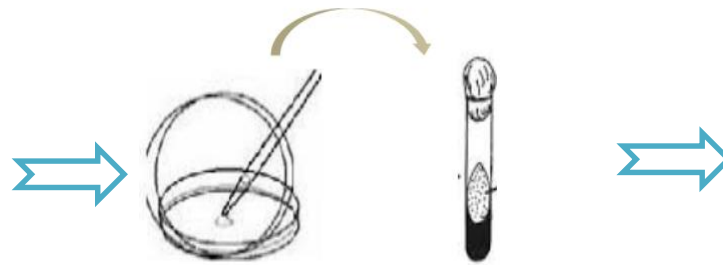
The media was homogenized using a magnetic stirrer on the plate until it boils.

Antibacterial Activity Test Preparation

Bacterial Rejuvenation



MHA which has been sterilized is poured into a test tube. Left at 45o C until hardened



One ose of bacteria is taken and then scratched in a zigzag way



Incubated at 37° C for 24 hours

Antibacterial Activity Test Preparation

Bacteria Suspension Preparation

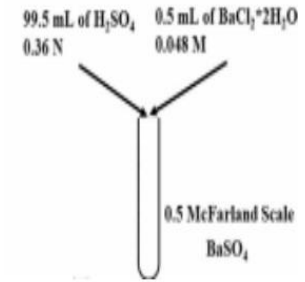


Diambil satu ose bakteri, dicampurkan dengan NaCl 0,9%

Vortex

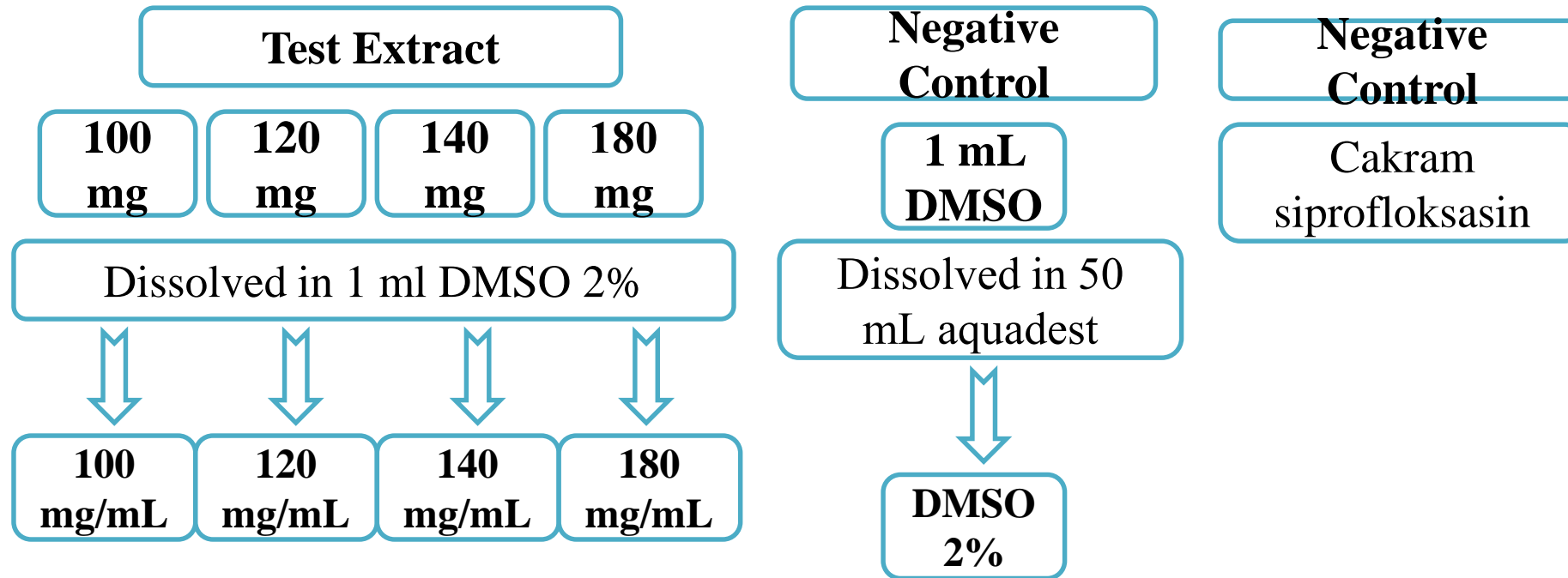
Made equivalent to 0.5 McFarland

This compound was used for inhibition zone parameters and analysis of the effect of the test extract on bacteria



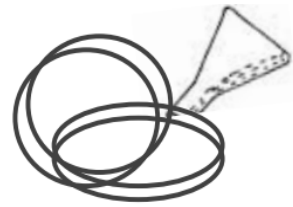
Antibacterial Activity Test Preparation

Preparation of Test Solutions and Control Inhibitory Diameter Zone

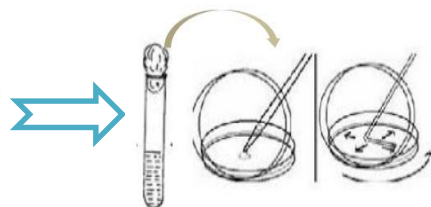


Antibacterial Activity Test Preparation

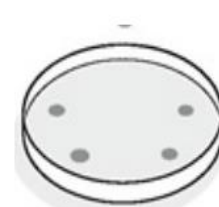
Determination of Inhibitory Zone Diameter, Disc Diffusion Method



A total of 20 mL of sterile MHA was poured into a petri dish and allowed to solidify



100 L of bacterial suspension was placed in a petri dish, then spread over the entire surface using a spreader

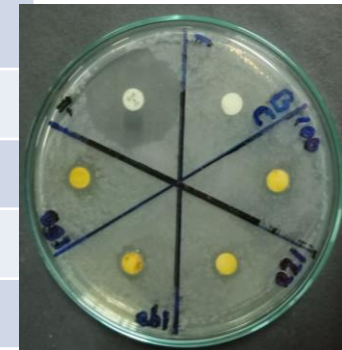


A disc containing 20 L of the test and control solution was placed. Incubated 24 hours at 37o C

The diameter of the clear zone formed around the disc was measured using a ruler and a caliper

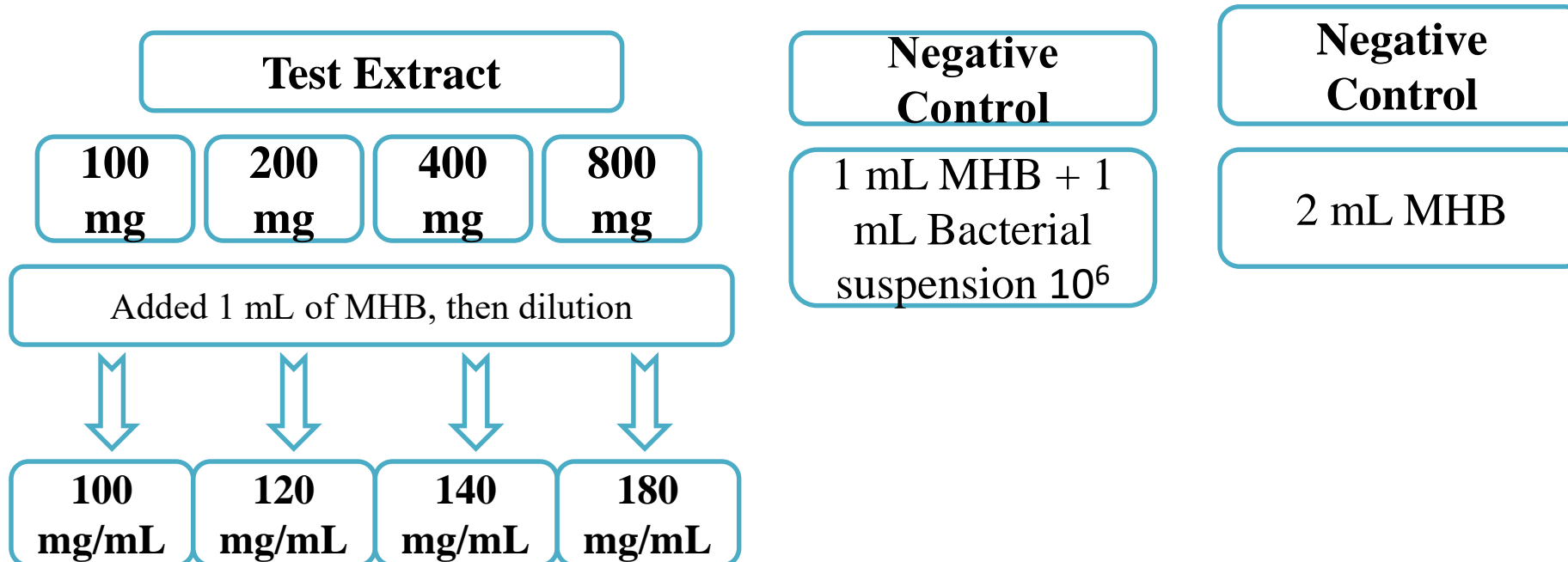
Hasil Pengamatan (Lanjutan)

No.	Name Bacteria	Positive control (mm)	Negative control (mm)	Test concentration			
				100 mg/mL (mm)	120 mg/mL (mm)	140 mg/mL (mm)	180 mg/mL (mm)
1.	<i>B. cereus</i>	28,96	-	9,26	10,66	10,73	11,73
2.	<i>B. subtilis</i>	29,35	-	8,55	8,9	9,45	9,45
3.	<i>P. acnes</i>	31,35	-	7,25	7,6	8,2	8,5
4.	<i>S. aureus</i>	26,7	-	7,55	7,8	8,65	8,95
5.	<i>S. epidermidis</i>	27,7	-	8,4	8,63	9,36	10,06



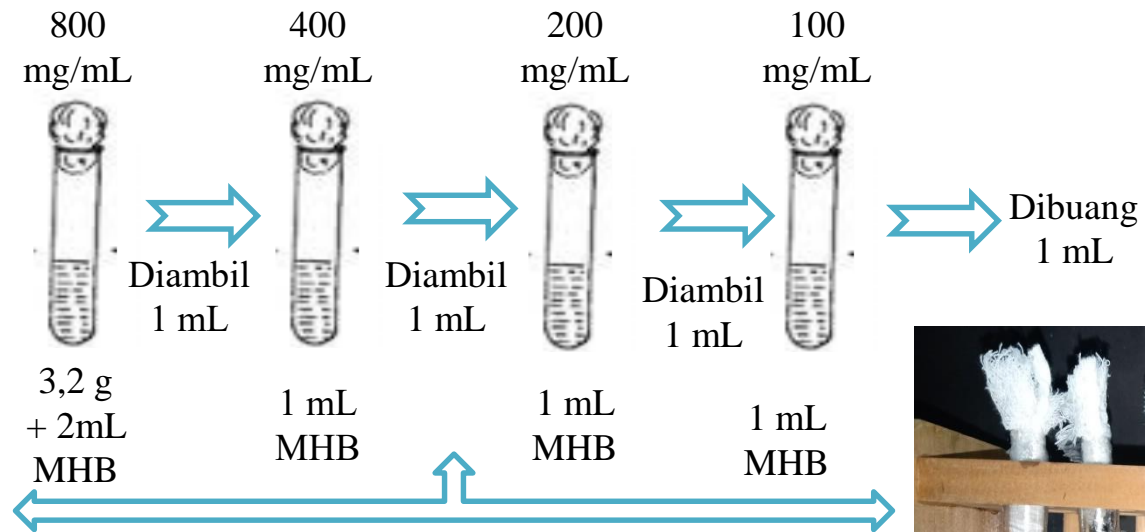
Antibacterial Activity Test Preparation

Preparation of MIC Test and Control Solutions



Antibacterial Activity Test Preparation

Determination of MIC



Add 1 mL of bacterial suspension to each tube (bacterial suspension 10^6).



Antibacterial Activity Test Preparation

Determination of MBC



Observe:
MBC = absence of
bacterial growth

The MIC
liquid is
scratched on
the MHA

Incubation for
19-24 hours
at 35 °C



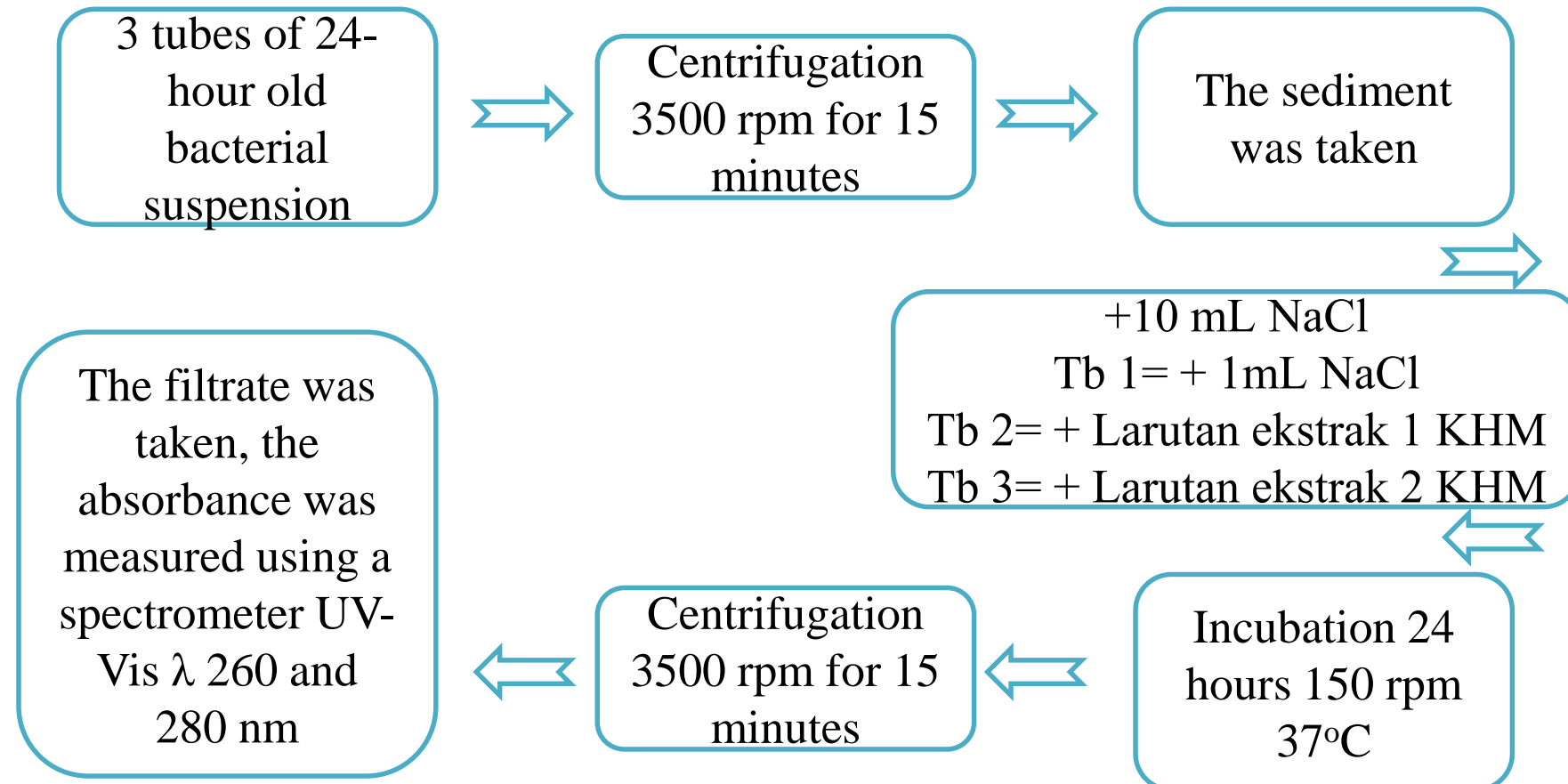
Results of Determination of MIC and MBC

No.	Name Bacteria	Positive control	Negative control	Test concentration			
				100 mg/mL	200 mg/mL	400 mg/mL	800 mg/mL
1.	<i>B. cereus</i>	+	-	+	+	+*	-**
2.	<i>B. subtilis</i>	+	-	+	+	+*	-**
3.	<i>P. acnes</i>	+	-	+	+	+*	+**
4.	<i>S. aureus</i>	+	-	+	+	+*	-**
5.	<i>S. epidermidis</i>	+	-	+	+	+*	+**

Note. (+)= there is bacterial growth, (-)= no bacterial growth,
(*)= MIC, and (**)= MBC

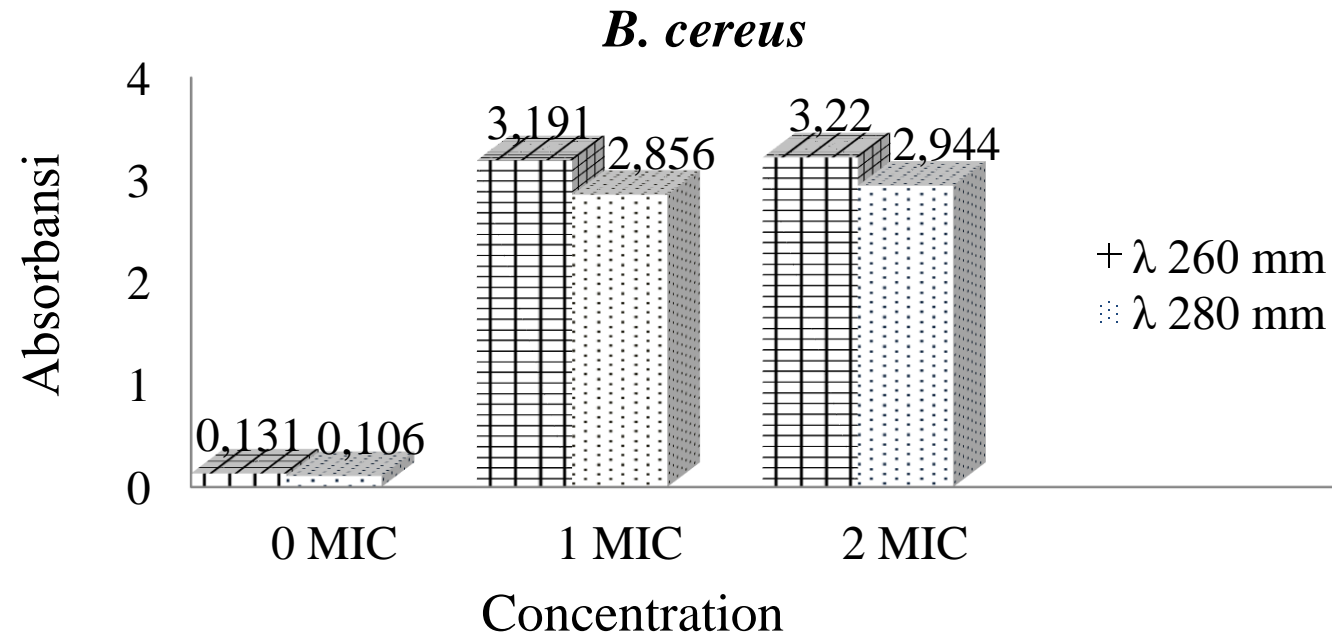
Antibacterial Activity Test Preparation

Preparation of the Effect of Exposure to the Cell Wall of the Test Bacteria



Antibacterial Activity Test Preparation

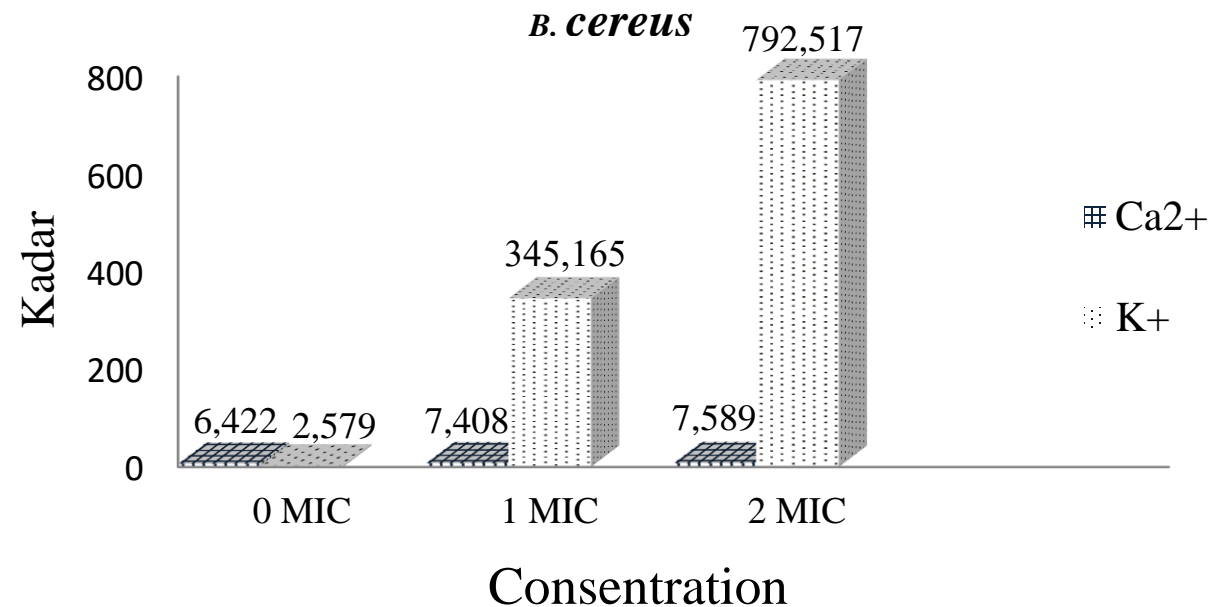
Analysis of the Effects of Exposure of Bangle Extract 70% on Bacterial Cell Walls (Spectro)



The absorbance value increases when the concentration of the given test extract increases → nucleic acid and protein leakage from bacterial cells also increases.

Antibacterial Activity Test Preparation

Analysis of the Effects of Exposure of Bangle Extract 70% on Bacterial Cell Walls (AAS)



The value increases when the concentration of the test extract given increases → Ca⁺ and K⁺ leaking from bacterial cells also increases.

CONCLUSION

1. The test results of antibacterial activity against 70% bangle ethanol extract showed that *B. cereus* was the most sensitive bacteria with a concentration of 140 mg/mL with a diameter of 11.73 mm. The MIC and MBC tests on *B. cereus*, *B. subtilis* and *S. aureus* test bacteria obtained values of 400 mg/mL and 800 mg/mL.
2. The administration of 70% bangle ethanol extract at a concentration of 2 MIC to the test bacteria *B. cereus* can damage cell walls and affect membrane permeability characterized by the release of nucleic acids, proteins and metal ions (Ca^{2+} and K^{+}) from the cells.