# 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)
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DEAN

**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

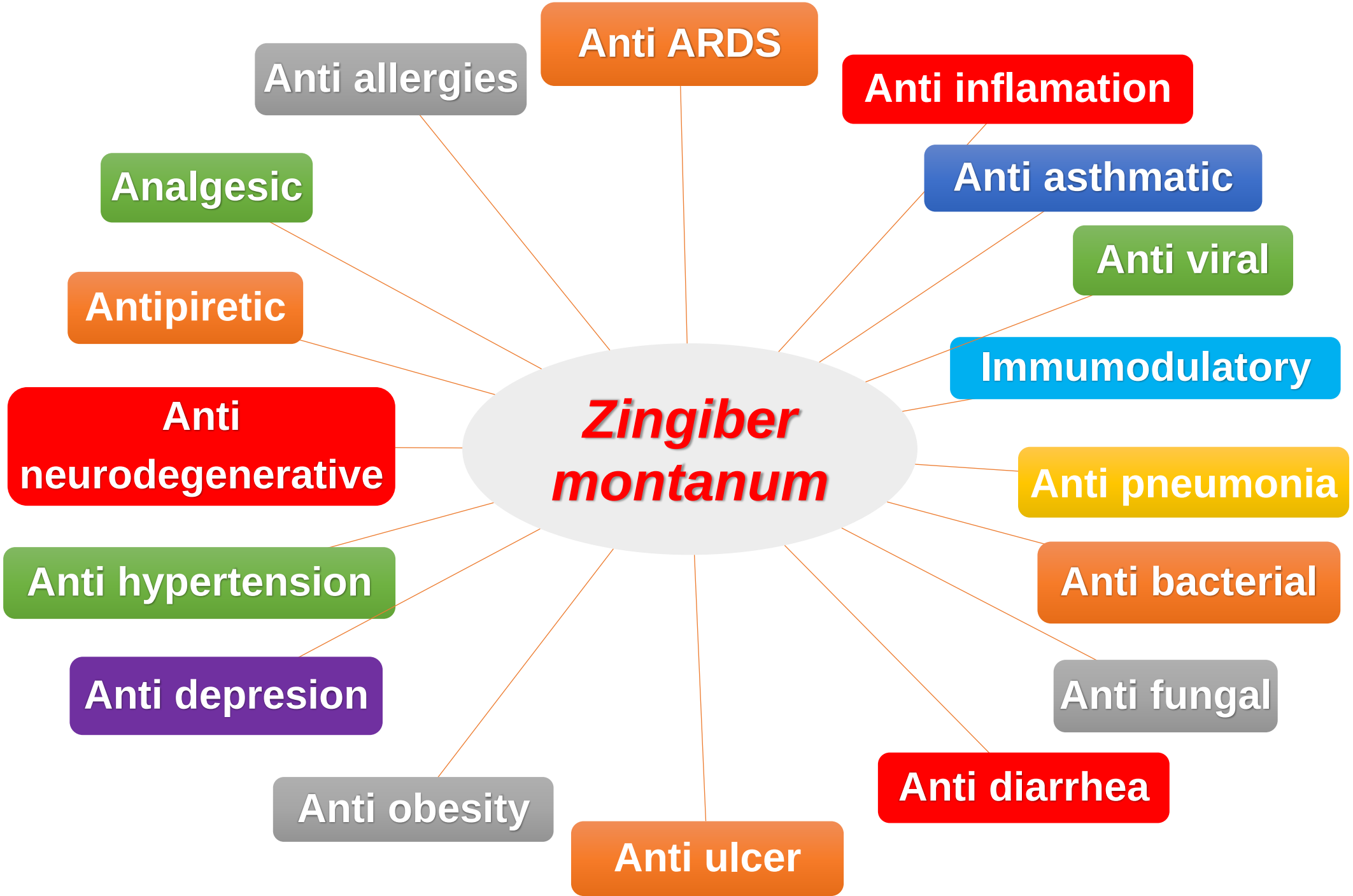
**M. Yanis Musdja, Ayu Haryati, Hendri Aldrat**

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


Zingiber montanum
Anti ARDS
Anti allergies
Anti inflamation
Analgesic
Anti asthmatic
Antipiretic
Anti viral
Anti neurodegenerative
Immumodulatory
Anti pneumonia
Anti hypertension
Anti bacterial
Anti depresion
Anti fungal
Anti diarrhea
Anti obesity
Anti ulcer

# 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

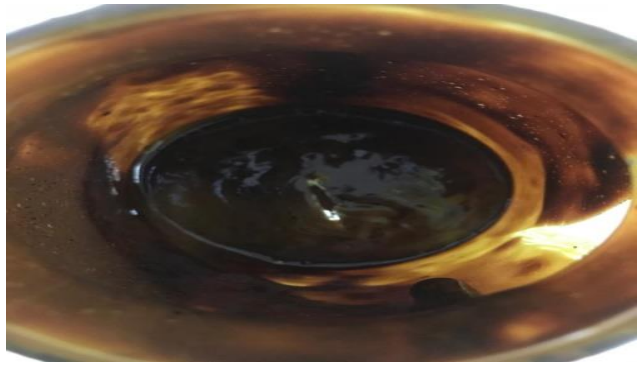
Di potong tipis-tipis lalu dikering anginkan


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

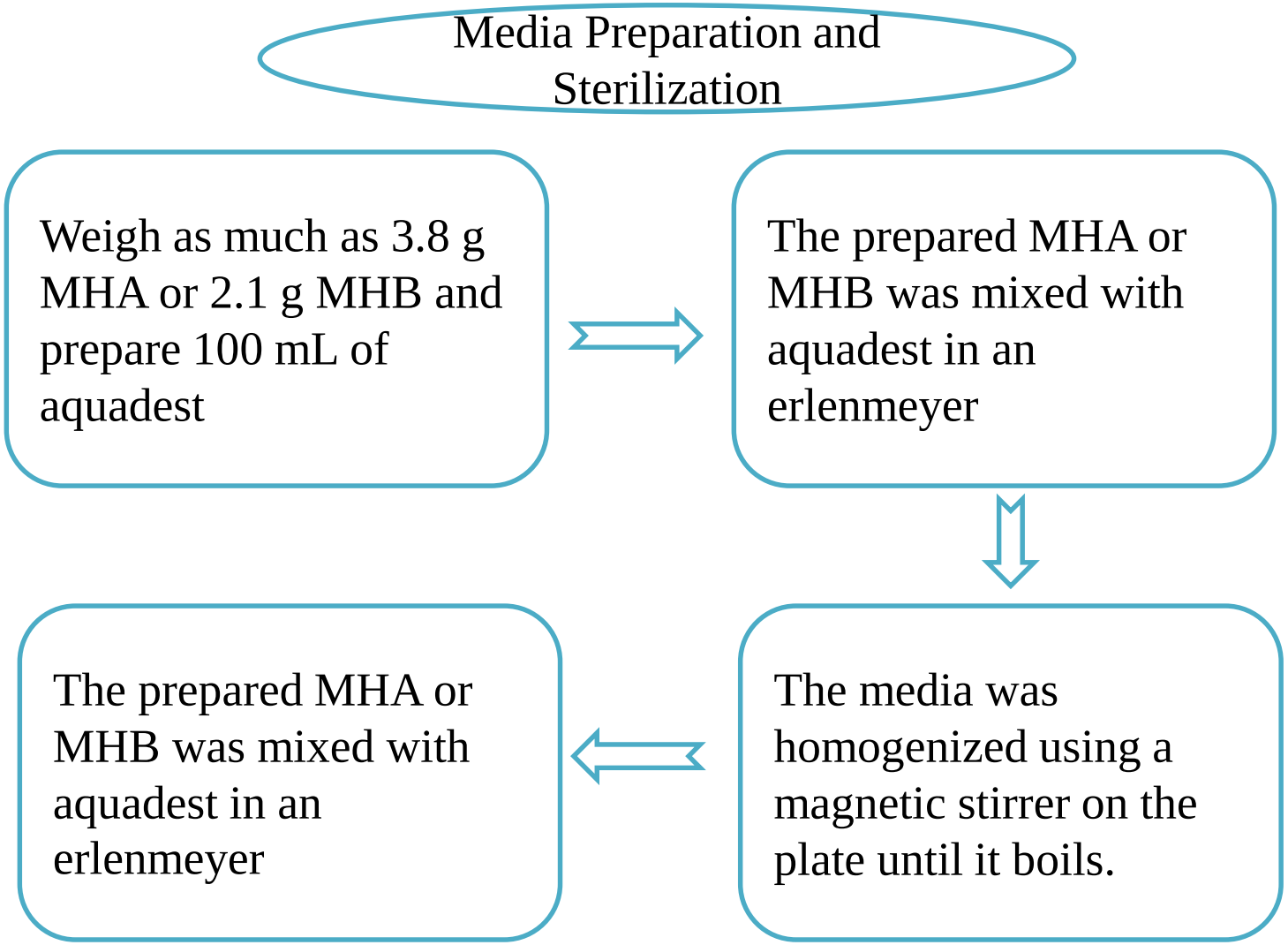
Filtered and obtained a liquid extract.



Viscous extract obtained as much as 56,62 g (Yield = ± 8%)

Evaporated with a vacuum evaporator at a temperature of ± 40 ºC

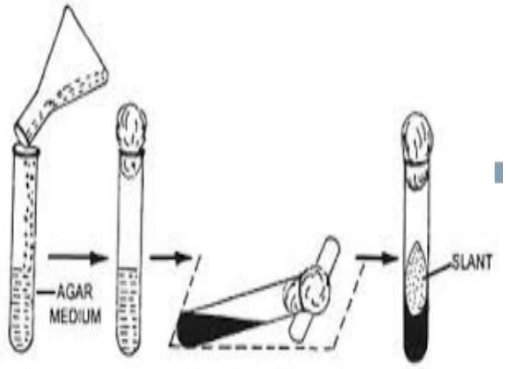
# Preparation of Antibacterial Activity Test with sterile materials and tools




Mpila, Fatimawali dan Wiyono, 2012

# Antibacterial Activity Test Preparation

## Bacterial Rejuvenation



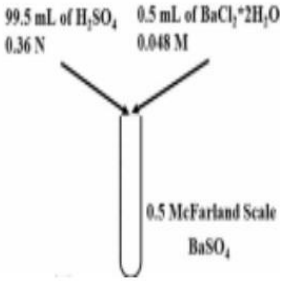

MHA which has been sterilized is poured into a test tube. Left at 45o C until hardened

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

Incubated at 37° C for 24 hours

Dwijoseputro, 2010; Mpila, Fatimawali dan Wiyono, 2012

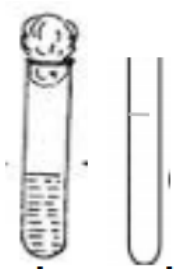
# Antibacterial Activity Test Preparation




## Bacteria Suspension Preparation

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


Vortex



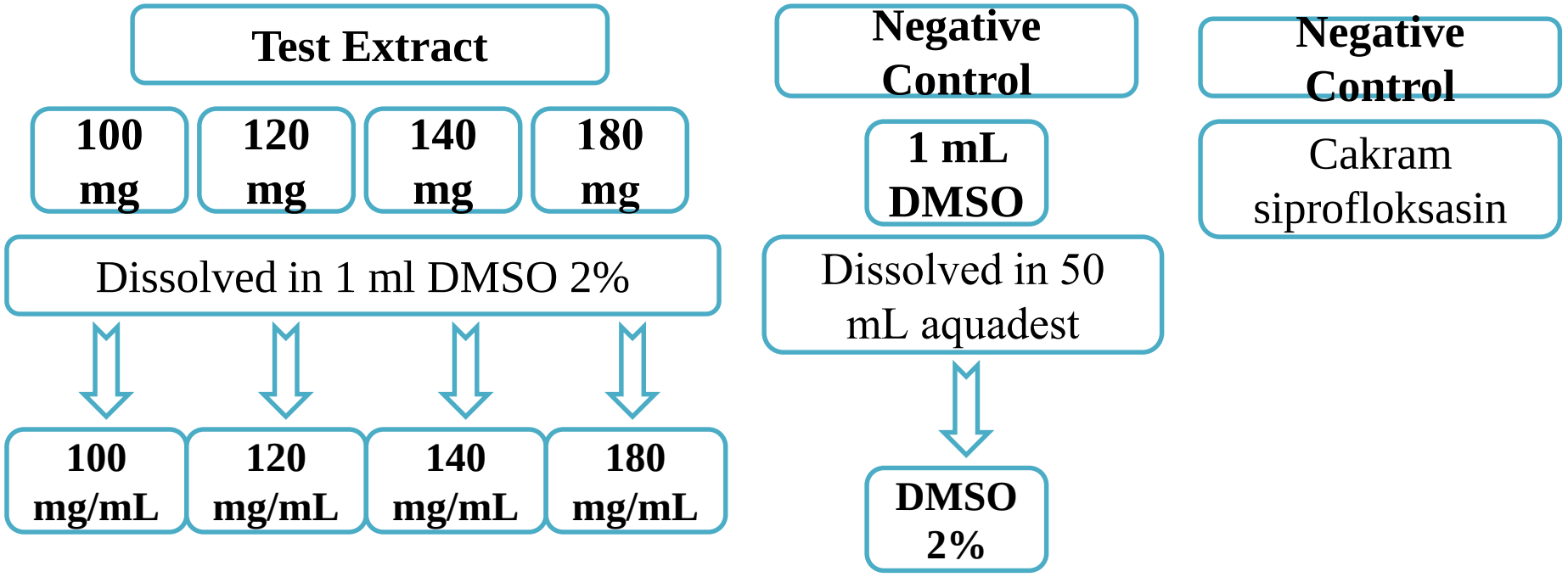
Made equivalent to 0.5 McFarland

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

Mpila, Fatimawali dan Wiyono, 2012

# Antibacterial Activity Test Preparation

Preparation of Test Solutions and Control Inhibitory Diameter Zone

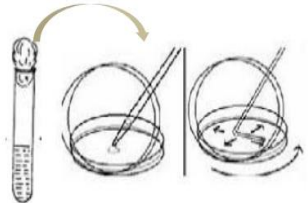



Balouiri, 2015

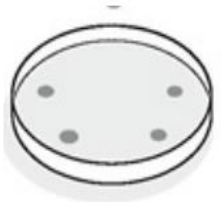
# Antibacterial Activity Test Preparation

## 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

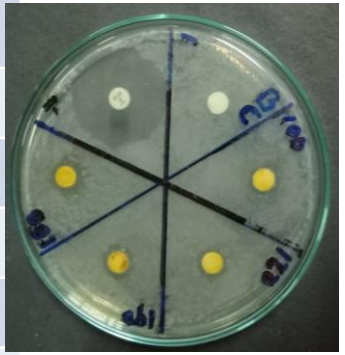


A disc containing 20 L of the test and control solution was placed. Incubated 24 hours at 37o C

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. | B. cereus | 28,96 | - | 9,26 | 10,66 | 10,73 | 11,73 |
| 2. | B. subtilis | 29,35 | - | 8,55 | 8,9 | 9,45 | 9,45 |
| 3. | P. acnes | 31,35 | - | 7,25 | 7,6 | 8,2 | 8,5 |
| 4. | S. aureus | 26,7 | - | 7,55 | 7,8 | 8,65 | 8,95 |
| 5. | S. epidermidis | 27,7 | - | 8,4 | 8,63 | 9,36 | 10,06 |



# Antibacterial Activity Test Preparation

Preparation of MIC Test and Control Solutions

**Test Extract**

**100 mg** | **200 mg** | **400 mg** | **800 mg**

Added 1 mL of MHB, then dilution

**100 mg/mL** | **120 mg/mL** | **140 mg/mL** | **180 mg/mL**

**Negative Control**

1 mL MHB + 1 mL Bacterial suspension $10^6$

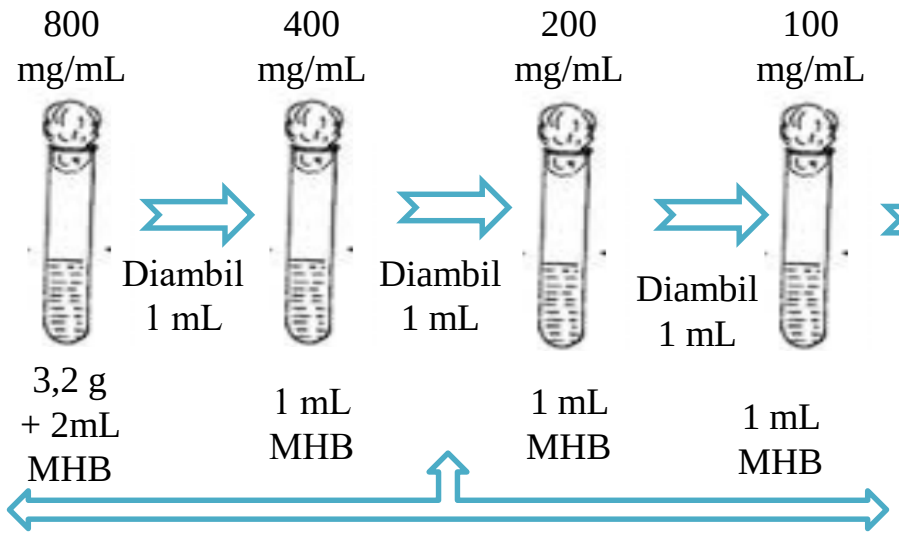
**Negative Control**

2 mL MHB

Pinta *et al.*, 2017

# 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

The MIC liquid is scratched on the MHA

Incubation for 19-24 hours at 35 °C

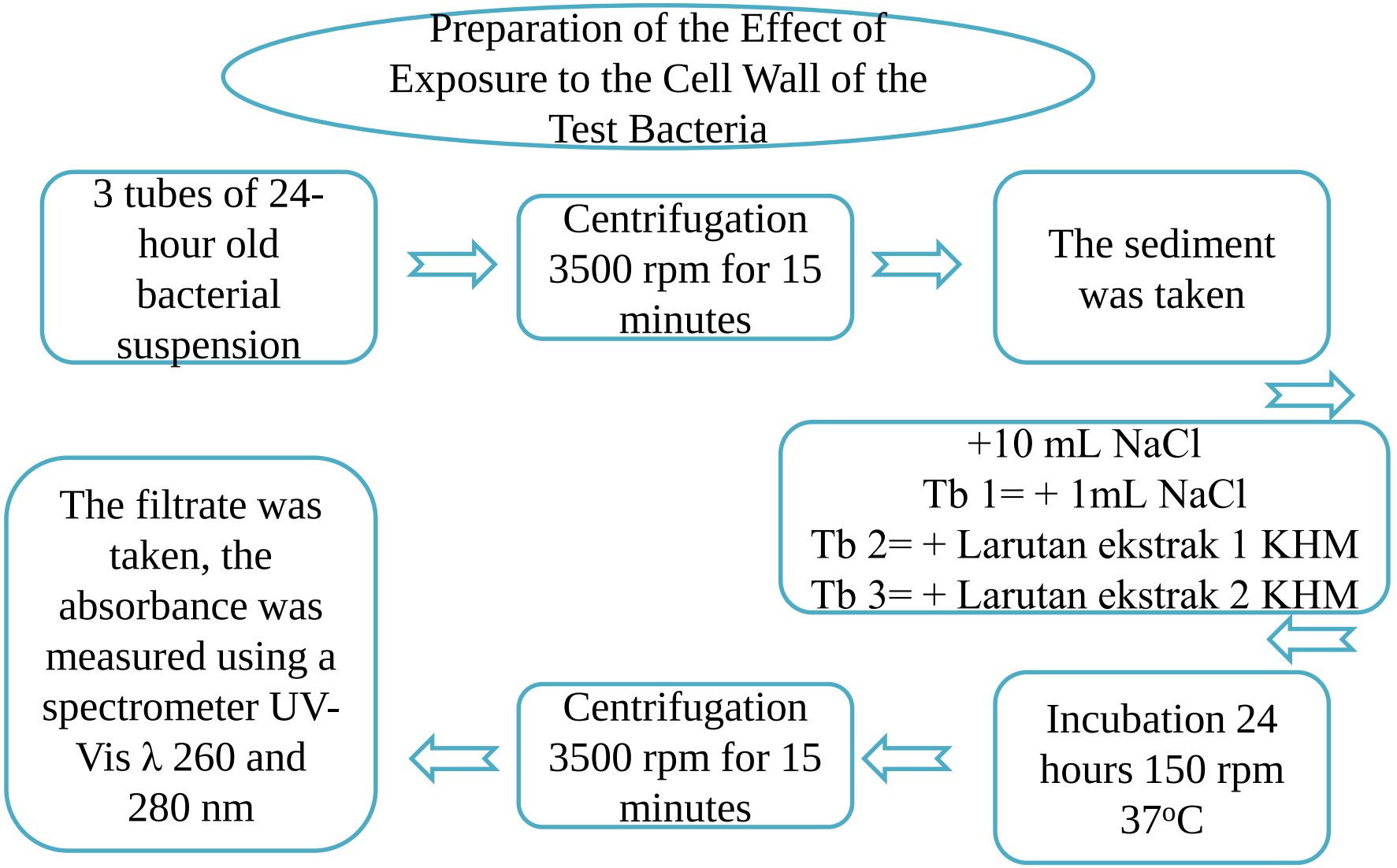
Observe:
MBC = absence of bacterial growth

# 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. | B. cereus | + | - | + | + | +* | -** |
| 2. | B. subtilis | + | - | + | + | +* | -** |
| 3. | P. acnes | + | - | + | + | +* | +** |
| 4. | S. aureus | + | - | + | + | +* | -** |
| 5. | S. epidermidis | + | - | + | + | +* | +** |

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

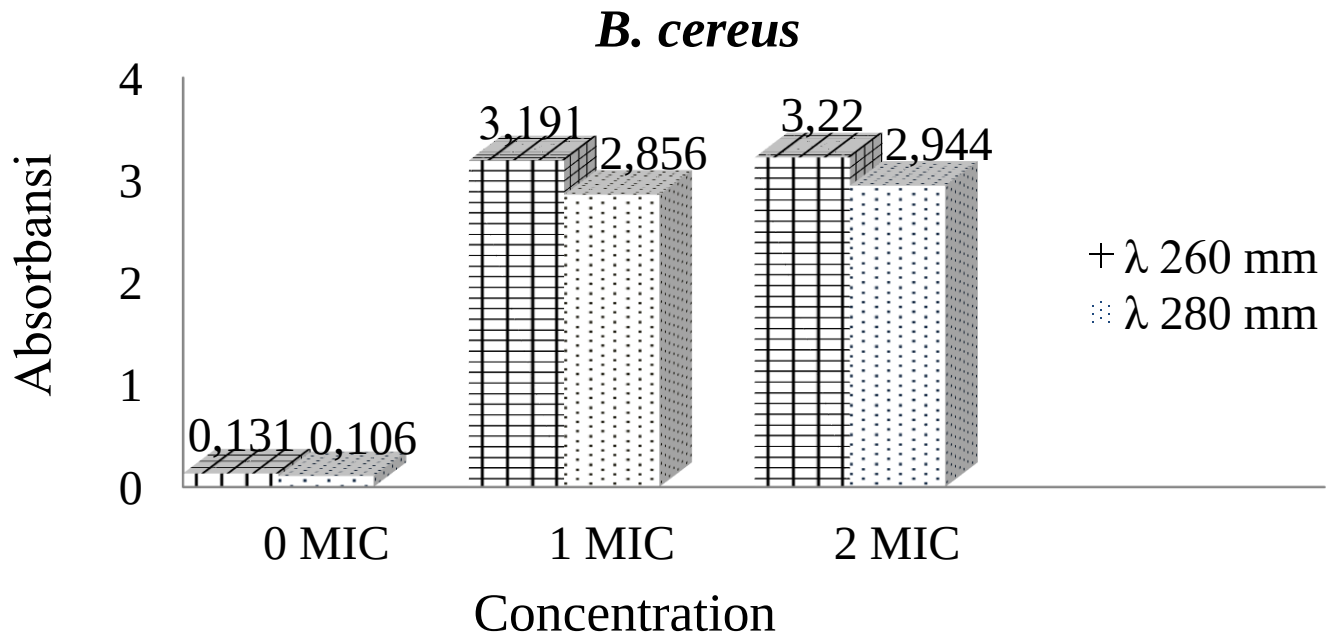
# Antibacterial Activity Test Preparation




Fariz, 2019

# Antibacterial Activity Test Preparation

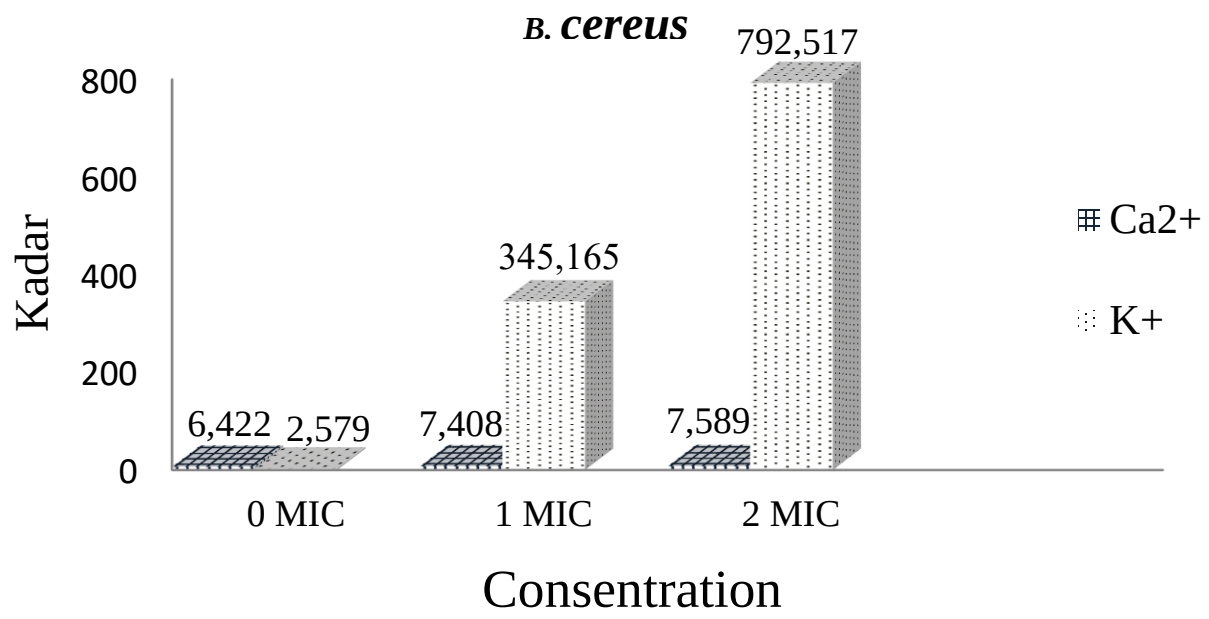
Analysis of the Effects of Exposure of Bangle Extract 70% on Bacterial Cell Walls (Spectro)




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 (Ca2+ and K+) from the cells.