FYP FSB

ANTIMICROBIAL ACTIVITIES OF EXTRACT ISOLATED FROM *Alpinia javanica* (FAMILY ZINGIBERACEAE)

by

NURATIKAH IZZATI BINTI MD NOOR HISHAM

A report submitted in fulfillment of the requirements for the degree of Bachelor of Applied Science (Nature Resources Science) with Honours

FACULTY OF EARTH SCIENCE
UNIVERSITI MALAYSIA KELANTAN

2024

DECLARATION

I declare that this thesis entitled "Antimicrobial Activities of Extract Isolated From *Alpinia javanica* (Family Zingiberaceae)" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and has not concurrently submitted in candidature of any other degree.

Signature :

Name : Nuratikah Izzati Binti Md Noor Hisham

Date : 8 August 2024

FYP FSB

UNIVERSITI
MALAYSIA
KELANTAN

FYP FSB

ACKNOWLEDGEMENT

First and foremost, I would like to express my gratitude to Allah Almighty for giving me the strength and patience to accomplish this Final Year Project (FYP) Thesis throughout both semesters.

I'd like to express my greatest appreciation to my FYP supervisor, Assoc. Prof. Ts. Dr. Suganthi A/P Appalasamy, for allowing me to conduct this research and providing amazing advice and guidance throughout this journey. Her thoughtful guidance and inspiration, along with the time she gives me to correct my mistakes as well as enhance my knowledge and the quality of my research writing, are very much appreciated. I have learned a lot of new things that will benefit me in the future. I enjoyed my time under her supervision, and I hope that all of her sacrifices along with the process were worth the investment. I can come out with this research paper thanks to her major involvement in it.

Furthermore, I would love to express my thanks to both my parents, Siti Zauyah Binti Hamzah and Md Noor Hisham Bin Ahmad for their unconditional support and understanding during this journey. I would like to give my appreciation and endless love to my fellow members, especially Lavinyah Arimanan and Muzailinah Binti Moidin who helped me a lot throughout this journey and devoted their time to helping me accomplish this research. I would also like to thank Hassanal Fikri Abdul Gani for providing me to carry out this research using the extracts that I had obtained from him, as well as all of the lab assistants and anyone else who contributed directly or indirectly along this journey.

UNIVERSITI
MALAYSIA
KELANTAN

Antimicrobial Activities of Extract Isolated from *Alpinia javanica* (Family Zingiberaceae)

ABSTRACT

To find the efficiency of using herbs as part of alternative medicine against digestive bacteria involving *Escherichia coli* and *Staphylococcus aureus,* the extract of *Alpinia javanica* also known as "Lengkuas hutan" from different parts has been used. This monocotyledon plant belongs to the order Zingiberales where this plant consists of various compounds that potentially become antibiotic and have medicine properties. To test the efficiency and minimum Inhibition zone, the antimicrobial assay has been used throughout the Kirby-Bauer disk diffusion method by observing the inhibition zone of both bacteria that are present on the Petri dish. This assay involves three different parts of *A. javanica* involving leaf, pseudostem, and rhizome with different concentrations starting from 0 mg/ml, 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml. The minimum inhibitory concentration (MIC) has been determined after comparing the inhibition zone of three different parts of *A. javanica* at different concentrations. The finding shows that 2 mg/ml of extract from the leaf, pseudo stem, and rhizome was the minimum inhibitory concentration (MIC) as it shows the inhibition zone of both selected bacteria. In the concentration of 2 mg/ml, the leaf extract inhibits 7.06 ± 1.13 mm (*E. coli*) and 7.51 ± 0.48 mm (*S. aureus*) average diameter of the zone, pseudostem with 7.98 ± 0.78 mm (*E. coli*) and 8.01 ± 1.05 mm (*S. aureus*), and rhizome with 8.94 ± 0.54 mm (*E. coli*) and 8.71 ± 0.92 mm (*S. aureus*). The findings reveal that the plant extract of *A. javanica* for rhizome extract proved more efficient against *E. coli* and *S. aureus* through a larger inhibition zone of bacteria, while the pseudostem extract had the smallest inhibition zone compared to all three parts of the plant. The rhizome extract of 10 mg/mL (11.36 ± 0.29 mm) and 8 mg/mL (11.68 ± 0.56 mm) had the largest average diameter zone with nearly identical effects against *E. coli*, while 10 mg/mL had the highest diameter zone against *S. aureus* with a diameter of 11.47 ± 0.03 mm.

FYP FSB

UNIVERSITI MALAYSIA KELANTAN

Aktiviti Antimikrob Menggunakan Ekstrak Yang Dihasilkan daripada *Alpinia javanica* (Keluarga Zingiberaceae)

ABSTRAK

Bagi mencari keberkesanan penggunaan herba sebagai sebahagian daripada ubat alternatif terhadap bakteria penghadaman yang melibatkan *Escherichia coli* dan *Staphylococcus aureus*, ekstrak *Alpinia javanica* yang juga dikenali sebagai "Lengkuas hutan" dari bahagian yang berbeza telah digunakan. Tumbuhan monokotiledon ini tergolong dalam ordo Zingiberales di mana tumbuhan ini terdiri daripada pelbagai sebatian yang berpotensi menjadi antibiotik dan mempunyai khasiat ubat. Untuk menguji kecekapan dan Kepekatan Perencatan Minimum (MIC), ujian antimikrob telah digunakan melalui kaedah penyebaran cakera Kirby-Bauer dengan memerhati zon perencatan kedua-dua bakteria yang terdapat pada piring petri. Ujian ini melibatkan tiga bahagian berbeza *A. javanica* yang melibatkan daun, pseudostem, dan rizom dengan kepekatan berbeza bermula daripada 0 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, dan 10 mg/mL. Kajian menunjukkan bahawa 2 mg/mL ekstrak daripada daun, batang pseudo, dan rizom adalah kepekatan perencatan minimum (MIC) kerana ia menunjukkan zon perencatan kedua-dua bakteria terpilih. Dalam kepekatan 2 mg/mL, ekstrak daun menghalang 7.06 ± 1.13 mm (*E. coli*) dan 7.51 ± 0.48 mm (*S. aureus*) diameter purata zon, pseudostem dengan 7.98 ± 0.78 mm (E. coli) dan 8.01 ± 1.05 mm (*S. aureus*), dan rizom dengan 8.94 ± 0.54 mm (*E. coli*) dan 8.71 ± 0.92 mm (*S. aureus*). Penemuan menunjukan ekstrak tumbuhan *A. javanica* untuk ekstrak rizom terbukti lebih berkesan terhadap *E. coli* dan *S. aureus* melalui zon perencatan bakteria yang lebih besar, manakala ekstrak pseudostem mempunyai zon perencatan terkecil berbanding ketiga-tiga bahagian tumbuhan. Ekstrak rizom 10 mg/mL (11.38 ± 0.29 mm) dan 8 mg/mL (11.68 ± 0.56 mm) mempunyai zon diameter purata terbesar dengan kesan yang hampir sama terhadap *E. coli*, manakala 10 mg/mL mempunyai yang tertinggi. zon diameter terhadap *S. aureus* dengan diameter 11.47 ± 0.03 mm.

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

FYP FSB

TABLE OF CONTENT

	PAGES
DECLARATION	I
ACKNOWLEDGEMENT	II
ABSTRACT	III
ABSTRAK	IV
TABLE OF CONTENTS	V
LIST OF TABLES	VIII
LIST OF FIGURES	IX
LIST OF ABBREVIATIONS	XI
LIST OF SYMBOLS	XII
CHAPTER 1 INTRODUCTION	
1.1 Background of Study	1
1.2 Problem Statement	3
1.3 Objective	5
1.4 Scope of Study	5
1.5 Significant of Study	6
CHAPTER 2 LITERATURE REVIEW	
2.1 *Alpinia javanica* Morphology and Classification	7
2.2 Plant Extraction Method for Determination of Bioactive Compounds	10
2.3 Plant Biochemical Compound of *Alpinia javanica.*	11
2.4 Gram-Positive Bacteria	
2.4.1 *Staphylococcus aureus*	12
2.5 Gram-Negative Bacteria	
2.5.1 *Escherichia coli*	15
2.6 Minimum Inhibitory Concentration (MIC) of Plant Extracts and Its Potential in Phyto Medical	17
2.7 Antimicrobial Assay of Extract from *Alpinia javanica*.	19
CHAPTER 3 MATERIAL AND METHOD	

UNIVERSITI MALAYSIA KELANTAN

3.1 Preparation of Nutrient Agar 20
3.2 Preparation of Stock Culture of Bacteria 20
3.3 Preparation of Plant Extract 22
3.4 Determination of Bacteria Growth, Inhibition Zone, and Antimicrobial Activity of *Alpinia javanica* at Different Concentrations.
3.4.1 Antimicrobial Assay 23
3.4.2 Measurement of Inhibition Zone 25
3.5 Statistical Analysis of The Inhibition Zone of Bacteria 27
CHAPTER 4 RESULTS AND DISCUSSION
4.1 The Kirby-Bauer Disk Diffusion Method to Determined Minimum Inhibitory Concentration (MIC) 28
4.2 Antimicrobial Activities from Different Parts of *Alpinia javanica* 33
4.3 Determination of Minimum Inhibitory Concentration (MIC) of Extracts from Different Parts of *Alpinia javanica*. 38
4.4 The Comparison of Antimicrobial Activities from Different Parts of *Alpinia javanica*. 40
4.5 Factors That Affect the Outcomes of Antimicrobial Assays 45
CHAPTER 5 CONCLUSION AND RECOMMENDATION
5.1 Conclusion 54
5.2 Recommendations 55
REFERENCE 58
APPENDICES
APPENDIX A The Diameter of The Inhibition Zone of Tested Bacteria on Different Parts of *Alpinia javanica's* Extracts. 66
APPENDIX B The Concentration of 0 mg/mL Consists of 100% Methanol Works as a Negative Control 67
APPENDIX C The Dilution of Leaf Extract at Different Concentrations From 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL. 67

FYP FSB

APPENDIX D	The Dilution of Pseudostem Extract at Different Concentrations From 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL.	68
APPENDIX E	The Dilution of Rhizome Extract at Different Concentrations From 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL.	68

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

LIST OF TABLES

No.	TABLE	PAGE
2.1	Compound in *Alpinia javanica* in various parts of the plant	11
3.1	Test microorganism for antimicrobial activities assay	21
4.1	The inhibition zone of *Alpinia javanica* extracts from different parts which involve leaf, pseudostem, and rhizome against two different bacteria which are bacteria *E. coli* and S. *aureus*.	29

FYP FSB

UNIVERSITI
MALAYSIA
KELANTAN

LIST OF FIGURES

No.	TITLE	PAGE
2.1	*Alpinia javanica* leaf and stem.	8
2.2	The phylogenetic tree of *Alpinia*.	9
2.3	A closer look at *Alpinia javanica* flower with fruits.	9
2.4	*Staphylococcus aureus* under a higher magnification of 20,000x taken from a vancomycin intermediate resistant culture (VISA).	14
2.6	*Escherichia coli* under the microscope with rod-shaped in red color indicates gram-negative bacteria.	17
3.1	Continuous streaking method	22
3.2	The presence of the inhibition zone around the disk	25
3.3	Digital vernier caliper	26
3.4	Measurements for the normalized width of the inhibition zone.	26
4.1	The diameter of the inhibition zone of the bacteria that was measured.	30
4.2	The inhibition zones of *A. javanica* from different parts of plants 10µL extract which were leaf (A), pseudostem (B), and rhizome (C) against *E. coli*.	31
4.3	The inhibition zones of *A. javanica* from different parts of plants 10µL extract which were leaf (A), pseudostem (B), and rhizome (C) against *S. aureus*.	32
4.4	Discs containing 5µL extracts of *A. javanica* from leaf (A), pseudostem (B), and rhizome (C) against *E. coli* showed no inhibition zone around the discs containing diluted extracts.	50
4.5	Discs containing 5µL extracts of *A. javanica* from leaf (A), pseudostem (B), and rhizome (C) against *S. aureus* showed	51

FYP FSB

	no inhibition zone around the discs containing diluted extracts.	
4.6	Discs containing 20 µL extracts of *A. javanica* from leaf (A), pseudostem (B), and rhizome (C) against *E. coli* showed no inhibition zone around a few discs containing diluted extracts.	52
4.7	Discs containing 20 µL extracts of *A. javanica* from leaf (A), pseudostem (B), and rhizome (C) against *S. aureus* showed no inhibition zone around the discs and some of it cannot be measured.	53

FYP FSB

UNIVERSITI
MALAYSIA
KELANTAN

LIST OF ABBREVIATION

ANOVA	Analysis of Variance
MIC	Minimum Inhibitory Concentration
SD	Standard Deviation

FYP FSB

LIST OF SYMBOLS

No.	Symbol	Meaning
1.	mm	Millimetre
2.	µ	Micrometre
3.	Mg	Milligram
4.	mL	Millilitre
5.	l	Liter
6.	ºC	Degree Celsius
7.	cm	Centimetre
8.	m	Meter
9.	g	Gram
10.	%	Percent
11.	µg	Microgram

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

FYP FSB

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Antimicrobial was a product that could slow down or kill the spread of microorganisms involving bacteria, viruses, protozoa, and fungi. The antimicrobial component was well-known in various products that were widely manufactured. Antibiotics, one of the products of antimicrobials, have been used to overcome the spread of microorganisms. There are two main ways in which antibiotics target bacteria, which either prevent the reproduction of bacteria or kill the bacteria by stopping the mechanism responsible for building their cell walls (Dhakad et al., 2022). Still, the overuse of antibiotics could cause antimicrobial resistance, where microorganisms had no response to the agent of the antibiotic. Antibiotic-sensitive bacterial cells can become resistant via de novo gene mutation or through the acquisition of resistance genes from other bacterial cells resulting from massive overuse of antibiotics through selection pressure (Serwecińska, 2020). This was essential in the healthcare setting because it creates progress-targeted pressure for the development of resistance and creates an ideal setting for organism transmission and outbreaks because of the high antimicrobial usage and the significant number of immunocompromised patients who are nearby (Morrison & Zembower, 2020).

Bacteria can be gram-positive or gram-negative, and the identification was based on the staining method. Gram staining is a simple and quick method for classifying bacteria into gram-positive and gram-negative groups, providing valuable information on cell components and surface charges (Biswas et al., 1970). This method consists of a few reagents which were crystal violet dye, iodine solution, methylene blue dye, and safranin solution. Gram-positive organisms were those that, when observed under a microscope, appeared in blue or purple, while gram-negative organisms that were not absorbing the primary stain appeared red or pink. Gram-positive microorganisms that retain the primary dye (crystal violet and gram-negative microorganisms that take the color of the counterstain (usually Safranin O) result from their differences in the structure of the cell wall (Goldman & Green, 2015). Because of the different thicknesses of their peptidoglycan cell wall, gram-positive are thicker than gram-negative, which helps to hold the dye where it makes crystal violet more extensively entrapped in the peptidoglycan layer (Goldman & Green, 2015). Gram-positive organisms' cell walls retain this complex after exposure to alcohol and take on a purple color, whereas gram-negative organisms' cell walls turn pink (Coico, 2006). The method outlined here can be used to determine whether tissue culture samples consist of bacteria or to look at the Gram stain status and morphological characteristics of bacteria isolated from mixed or isolated bacterial cultures (Coico, 2006). To prevent bacterial infection, antibiotics inhibit the growth of bacteria.

A pantropical group of prominent broad-leaved plants with attractive flowers, Zingiberales was an order species of ginger, heliconias, prayer plant known as arrowroots and banana under the kingdom of Plantae consisting of 8

FYP FSB

UNIVERSITI MALAYSIA KELANTAN

families, 92 genera, and more than 2,100 species (Kress & Holttum, 2020). The order Zingiberales can be divided into the 'banana families': Musaceae (three genera including Musa, the bananas), Strelitziaceae (three genera including Strelitzia, the bird-of-paradise, and Ravenala, the traveller's palm), Lowiaceae (one genus), and Heliconiaceae (one genus, lobster claws); and the 'ginger families': Costaceae (four genera), Zingiberaceae (gingers, 51 genera), Cannaceae (one genus), and Marantaceae (prayer plants, 31 genera) (Prince & Kress, 2002,). They were different from other closely related monocots by having specialized isomorphic root hair cells, intracellular silica bodies, epigynous flowers, pollen grains with a reduced exine layer and an elaborated intine layer, nuclear endosperm development, and arillate seeds (Kress, 1990).

1.2 Problem Statement

Alpinia javanica is one of the herbs found to produce different types of biochemical compounds that are important for medical purposes. The biochemical compounds contained in *A. javanica* from the previous studies show anti-inflammatory, antibiotic, anti-cancer, and antioxidant properties. Ironically, the herb has been shown to have anti-inflammatory properties which can ease pain, reduce inflammation, and enhance overall well-being. All these medical properties that contain *A. javanica* can be one of the valuable ingredients to develop new drugs or become one of the alternative medicines to treat various illnesses. The use of *A.*

FYP FSB
UNIVERSITI MALAYSIA KELANTAN

javanica as alternative medicine can offer a more affordable and accessible option for healthcare. Traditional remedies based on herbs must be advertised and maintained because they promote personal health and well-being, diversity in culture, and preserve our cultural tradition.

Due to limited scientific research and evidence to support the efficacy, safety, quality control, and standardized regulations of herbal medicines, healthcare professionals and the general public will face a barrier to acceptance due to a lack of education and awareness about herbs' benefits and proper use in healthcare. A lack of knowledge about these herbs makes using them safely and effectively in medical care more difficult, which leads to variations in the potency and effectiveness of herbal medications becoming questionable, making it more difficult for alternative healthcare practices to embrace and include them. The urge to conduct research on *A. javanica* is important because it could help to become a valuable source of bioactive compounds in pharmaceuticals, promote preventive care and self-care, cultural diversity, and preserve traditional knowledge and practices.

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

1.3 Objective

The aim of this study: -

i. To determine the minimum inhibition concentration of extracts from various parts of *Alpinia javanica* for antimicrobial activity.

ii. To compare the inhibition activity of extracts from various parts of *Alpinia javanica* using selected microorganisms.

1.4 Scope of Study

The use of *Alpinia javanica* becomes the aim of this study to evaluate the antimicrobial activities of the extracts from various plant parts and identify these potential natural products that can serve as effective antimicrobial agents against drug-resistant pathogens. The study was conducted at the Microbiology and Biochemistry Laboratory, Faculty of Earth Science (FSB), Universiti Malaysia Kelantan (UMK), Jeli Campus. The extract samples of *A. javanica* from various parts and stock culture of selective bacteria were obtained from the Microbiology and Biochemistry Laboratory, Faculty of Earth Science (FSB), Universiti Malaysia Kelantan (UMK), Jeli Campus, where the sample of plant extracts was got from the previous study. The extracts were diluted with methanol and prepared at different concentrations, including 0 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL. The steaking method was used to transfer the stock culture of bacteria to

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

nutrient agar to grow the bacteria. The filter paper on a petri dish that contained selective bacteria was soaked with the plant extract at different concentrations to conduct an antimicrobial assay to determine the potential of the plant extract. The experiment was repeated three times for different parts of *A. javanica,* including the leaf, pseudo stem, and rhizome.

1.5 Significant of Study

The purpose of this research is to provide valuable knowledge and information that benefits the following entities, including researchers and the pharmaceutical sector. The findings allow other researchers to gather quality information for a clear understanding to analyze results precisely, provide a starting point for future studies, and offer validity for further research. Researchers can identify patterns and trends in the information they have collected and help determine those elements expected to contribute to successful outcomes for their research by analyzing the data. The findings of this study also benefit the pharmaceutical sector in developing new products or medicines to prevent outbreak diseases. It helps improve society's well-being with the advancement of healthcare as it offers various treatment options.

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

CHAPTER 2

LITERATURE REVIEW

2.1 *Alpinia javanica* Morphology and Classification

Alpinia javanica, also known as “Lengkuas hutan” has been spotted around Thailand and Peninsular Malaysia and recorded in Terengganu, Johore, Kedah, Kelantan, Pahang, and Perak. Recent studies have been conducted in Gunung Telapak Burok, Berembun Forest Reserve (FR), Negeri Sembilan, where *A. javanica* have been found (Appalasamy & Arumugam, 2020).

This monocotyledon plant belongs to the order Zingiberales, the genus of Alpinia, and under the Plantae kingdom, which was the family of angiosperm plants. This family of plants were pseudo stem (Figure 2.1), perennial, herbaceous, aromatic, and contain rhizomes (Larsen et al., 1998) and caulescent aromatic plant, vessel elements in roots (Mabberley, 1997) with creeping horizontal or tuberous rhizomes (Xu & Chang, 2017). A recent study has been carried out that shows the chloroplast genomes of Alpinia species reveal similarities in genetic contents, gene orders, and GC contents but differences in SSR and long repeat numbers, potentially aiding in species identification and phylogenetic analysis (Li et al., 2020).

Alpinia javanica (Figure 2.2) in Rafflesian Clade (Figure 2.2) could grow around 3.3m tall with long and lance-shaped leaves around 60–100 × (10–)16–21.5 cm, velvety hairy underneath and on the margins, but glabrescent or glabrous on the upper surface except on the midrib (Julius, 2011). According to Rayfiqa Maulidah et

FYP FSB

al. (2019), *A. javanica* had large flowers with a square ovary where the petiole (see Figure 2.3) is around 5 cm to 10cm shortly hairy, and the inflorescence was around 5cm to 31 cm long with red rachis, and the peduncle holds the droops slightly with its flowers facing down (Julius, 2011). The hairs on the lamina may occupy both surfaces equally and scattered over the whole surface, most frequently near the veins (Tomlinson, 1956). The sepal or calyx was tubular with pink-white and turned pink in fruiting specimens (Figure 2.3). Julius (2011) also states that the corolla had a broadly obovate labellum, which is concave and envelopes the stamen and crinkled on its margin. The red lateral staminodes with an irregular shape. The immature fruits were green and round shaped (Figure 2.3) and were covered with short hairs.

Figure 2.1 *Alpinia javanica* leaf and stem (Gan, 2013)

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

FYP FSB

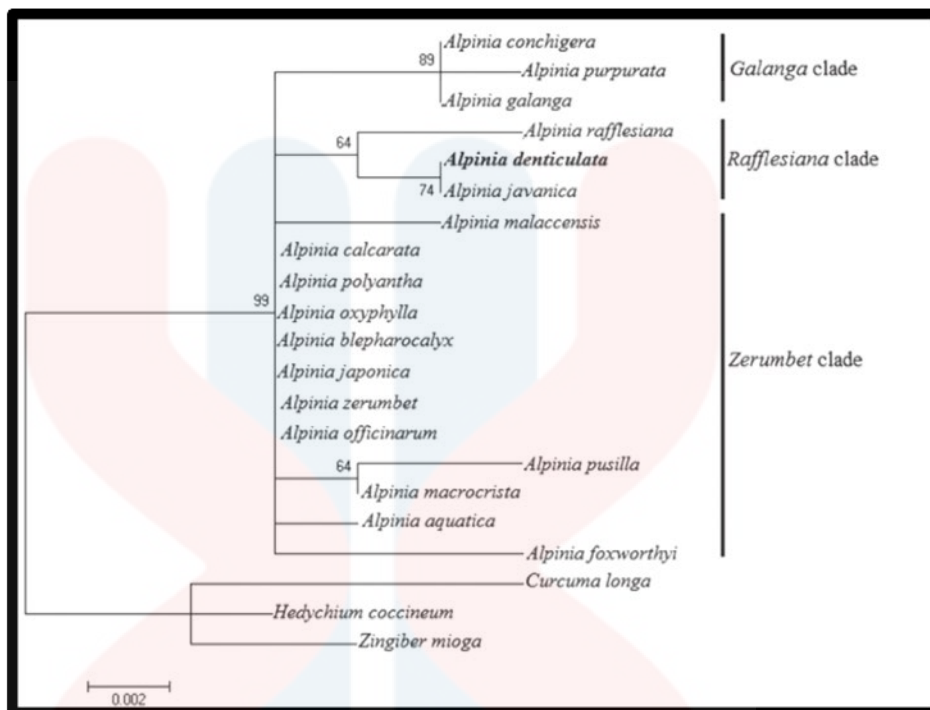


Figure 2.2 The phylogenetic tree of *Alpinia* (Kress et al., 2005)

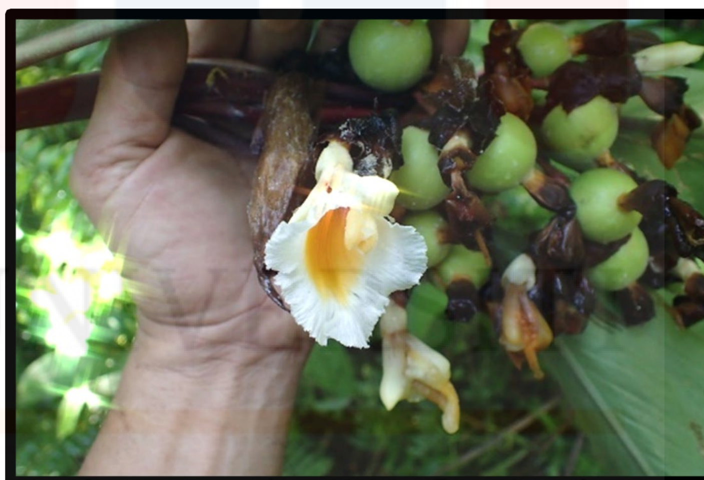


Figure 2.3 A closer look at *Alpinia javanica* flower with fruits. (Morad, 2014)

UNIVERSITI
MALAYSIA
KELANTAN

2.2 Plant Extraction Method for Determination of Bioactive Compounds

A study has been conducted that shows the most efficient extract method, which promotes easy extraction where the extract powder was suspended in distilled water, pH of the mixture was adjusted, incubated, and neutralized with diethyl ether and concentrated to dryness (Nostro et al., 2000). The research by Laveena and Chandra (2020) shows that methanol extract is the best solvent for detecting secondary metabolites in medicinal plant leaves, which has become helpful in evaluating their activity. This research also has been supported by another research that has been carried out by Al Farraj, Dunia A. et al. (2020), where *Dipcadi viride (L.)* Moench crude plant extracts have been used and resulting inhibitory effects against pathogenic bacteria and fungi, with ethanol and methanol showing better solvents than chloroform. This is because methanol was found to be the best to extract natural products to get the maximum health and medicinal benefits, resulting in increased extraction efficiency with the polarity of the solvents (Abdel-Hamid, Nermin S. et al., 2017). In the conventional implementation of Soxhlet extraction, the sample is placed in a thimble that is gradually filled with condensed fresh extractant (the term used to refer to the solvent used for extraction) from a distillation flask until the liquid reaches the overflow level, of a siphon aspirates it from the thimble and unloads it back into the distillation flask, thus carrying the extracted analytes into the bulk liquid and it would be repeated until the extraction process complete (López-Bascón & Luque de Castr, 2020).

FYP FSB

2.3 Plant Biochemical Compound of *Alpinia javanica*.

Alpinia javanica is a ginger species that has been used traditionally around Southeast Asia as it was known for its medical properties. There were a few studies have been conducted to identify the biochemical components contained in *A. javanica,* which was taken from any part of a plant involving leaf, stem, fruit, and rhizomes (Table 2.1). There a study conducted where Labda-8(17), 12-diene- l5,16-dial, and coronarin E had isolated the rhizome of *A. javanica* by using the same extraction process with chloroform (Hasnah M. Sirat et al. et al., 1994).

Table 2.1 Compound in *Alpinia javanica* in various parts of the plant (PubChem, 2023)

No.	Compound
1.	15Alpha-Hydroxy-Kaurenoic Acid
2.	Tagitinin A
3.	Tirotundin
4.	Deacetylovatifolin
5.	Grandifloric Acid
6.	Orizabin
7.	Niveusin C
8.	Coronarin E
9.	-4-hydroxy-6,10-dimethyl-3-methylidene-2-oxo-3a
10.	-4-hydroxy-10-methyl-3-methylidene-2-oxo-3a
11.	-1,12-dihydroxy-2-(hydroxymethyl)-11-methyl-7-methylidene-6-oxo-5
12.	-1,9,12-trihydroxy-2-(hydroxymethyl)-11-methyl-7-methylidene-5
13.	-1-hydroxy-2-(hydroxymethyl)-11-methyl-7-methylidene-6-oxo-5
14.	-6-hydroxy-6,10-dimethyl-3-methylidene-2
15.	-2-hydroxy-5,9-dimethyl-14-methylidene-4
16.	-8-hydroxy-4,9-dimethyl-14-methylidene-13-oxo-5,
17.	-1,12-dihydroxy-2,11-dimethyl-7-methylidene-6-oxo-5

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

Research conducted by Van et al. (2021) shows that Alpinia essential oils exhibit antimicrobial, cytotoxic, antioxidant, and anti-inflammatory properties, as well as slimming aromatherapy properties. There were a few studies carried out that show the effectiveness of a few compounds which potentially become one of medicine. According to the research conducted by Gonçalves et al. (2021) grandiflorenic acid (GFA) effectively reduces trypomastigote forms and eliminates intracellular parasites in Chagas disease patients without causing cellular cytotoxicity. Another research carried out by Ide et al. (2020) shows that orizabin from *Tithonia diversifolia* shows potential as a novel anti-atherosclerotic compound by suppressing Akt phosphorylation and promoting PTEN expression in cells, potentially benefiting vascular health maintenance.

2.4 Gram-Positive Bacteria

Gram-positive bacteria have a thick cell wall with 20 to 80 nm thick polymer (Sizar et al., 2023), which makes them able to hold the dye. These bacteria are classified by the colour they turn in the staining method, either blue or purple.

2.4.1 *Staphylococcus aureus*

Staphylococcus aureus is an adaptable pathogen that has become known as a worldwide threat to public health mainly because of its capability to build up

FYP FSB
UNIVERSITI MALAYSIA KELANTAN

antimicrobial resistance to several antibiotics. *S. aureus* is a mammalian commensal and flexible pathogen that grows in niches including the skin, nares, and mucosal membranes of approximately 20-30% of the human population, resulting in infection and diseases that are having an impact on the world's wellness (Haag et al., 2019). *S. aureus* leads to infection through the creation of toxins like δ-toxin and other cytolytic peptides, along with infectious factors that influence disease urgency (Cheung et al., 2021).

S. aureus can develop resistance against a few antibiotics, which causes a serious threat to human health, and makes it harder to cure infections that originate from this resilient bacterium. The study conducted by Zaghen et al. (2023) shows that *S. aureus* contains genes that carry out 35 different antibiotic resistance genes (ARGs), with each gene having the ability to resist different kinds of antibiotics. *S. aureus* appears to be immune to human cathelicidin LL-37 peptides through a complex molecular network, showing potential opportunities for developing more potent antibiotics against the community-associated pathogen (Golla et al., 2020). Since these bacteria had the potential to develop resistance to a few antibiotics, which can result in serious risks to human health, treating diseases triggered by these immune bacteria becomes more challenging. Further studies were necessary to identify effective treatment options and the right amount of dosage needed, prevent the spread of resistant infections, and eventually enhance patient outcomes. These assays were essential to discovering efficient options for treatment, analyzing bacterial resistance mechanisms, and leading the research and development of fresh antimicrobial drugs.

FYP FSB

A previous study conducted by Karunarathne et al. (2020) shows that the active biomolecule 1'-acetoxychavicolacetate contained in Galangal (*Alpinia galanga*) rhizome extract possesses antibiofilm activity that capable against *S. aureus*. Another study has found that *Alpinia monopleura* consists of properties such as α-caryophyllene, β-pinene, limonene, α-pinene, β-caryophyllene, and caryophyllene oxide where the leaf and fruits show antibacterial activities with minimal inhibition zone (MIC) of 31.3 μ g/mL against *S. aureus* (Agung Wibawa Mahatva Yodha et al., 2023). Using *S. aureus* strains in antimicrobial assays makes it possible to study the effectiveness of different drugs in combating drug-resistant strains, providing valuable insights into the mechanisms of drug resistance and identifying potential new targets for antimicrobial treatment.

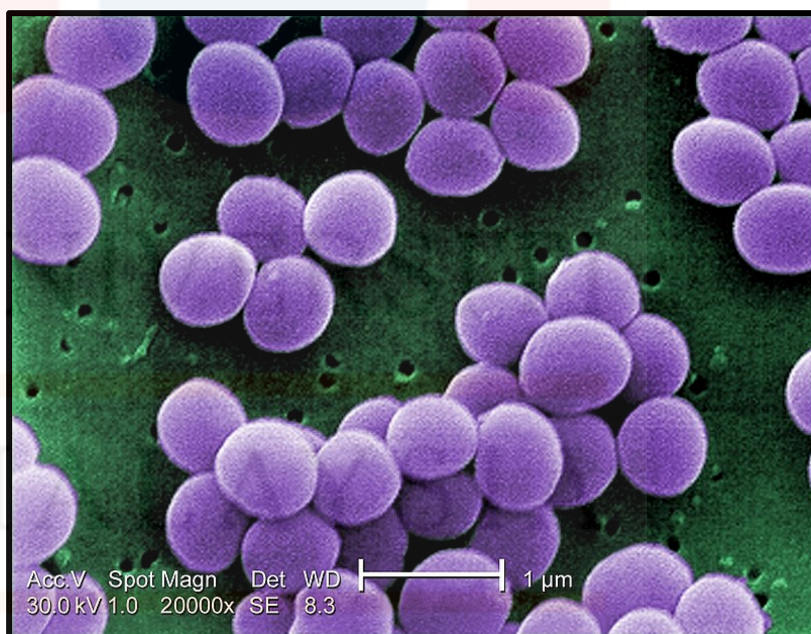


Figure 2.4 *Staphylococcus aureus* under a higher magnification of 20,000x taken from a vancomycin intermediate resistant culture (VISA). (Carr, 2001)

FYP FSB

2.5 Gram-Negative Bacteria

Gram-negative bacteria are well known for their resistance to a few antibiotics. It contains bacteria that do not retain the crystal violet dye meanwhile, they were able to retain it in safranin dye, which makes them appear in pink or red that is used in the gram staining method.

2.5.1 Escherichia coli

Antimicrobial assays implement several kinds of approaches, from traditional agar diffusion methods to novel high-throughput screening approaches, all intended for determining the inhibitory effects of antimicrobial compounds on *Escherichia coli* growth and reproduction. *E. coli* has a wide range of pathogenic potential, resulting in hybrid strains such as Enteroaggregative *E. coli* (EAEC)/ Shiga Toxin-producing *E. coli* (STEC), extraintestinal pathogenic *E. coli* (EPEC)/ Enterotoxigenic *E. coli* (ETE), and Shiga Toxin-producing *E. coli* (STEC)/ Extraintestinal Pathogenic *E. coli* (EPEC) hybrid strains which make clear pathotype identification difficult to do (Geurtsen et al., 2022). Previous research by Sellera et al. (2018) shows that the presence of extended-spectrum β-lactamase (ESBL) leading to *E. coli* in wild fish from polluted coastal waters in South America causes a public health risk. This could impact the safety of seafood supply and recreational use of these waters.

FYP FSB

E. coli is essential in antimicrobial assays due to its widespread distribution along with its significance in human health, affecting everyone from mild gastrointestinal issues to severe illnesses, making it an important pathogen to investigate in antimicrobial assays. Certain strains of *E. coli* have virulence factors, which have particular characteristics or genes that increase their capability to cause disease. Ramos et al. (2020) stated that the rapid spread of extended-spectrum β-lactamase (ESBL)s among commensal and pathogenic *E. coli* strains in humans and food-producing animals causes an alarming risk to public health.

A study conducted by Busayo et al. (2023) shows that the effect of extract from *Alpinia oxyphylla* leaves contains organic properties including nootkatone, chrysin, tectochrysin and alpha-tocotrienol extracted from the leaves is effective against *E. coli.* Another research conducted by Anisa Lutfia et al. (2021) on the rhizome of *Zingiber griffithii* where the finding shows antibacterial activity against *E. coli* as it contains unknown properties proposed as a novel compound in the group of alkaloid-terpenoid. Understanding how *E. coli* responds to different antimicrobial agents would assist with the development of new drugs and treatment procedures.

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

FYP FSB

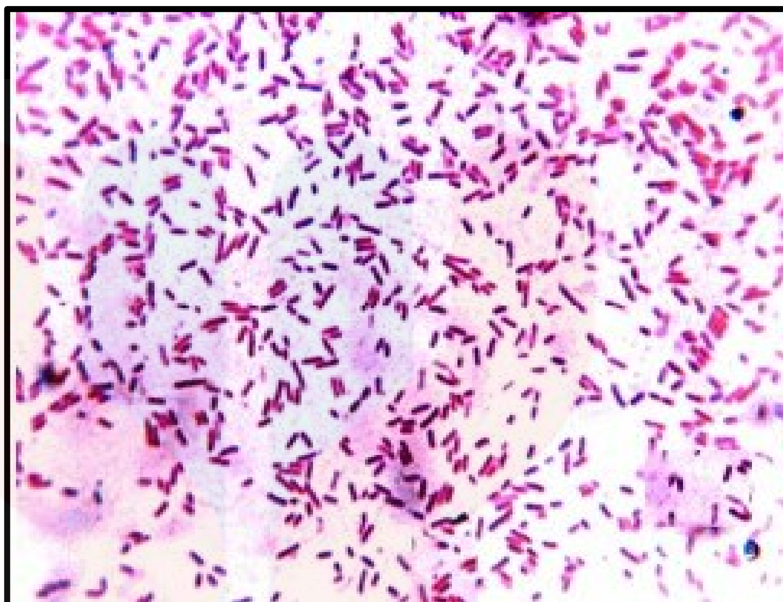


Figure 2.5 *Escherichia coli* under the microscope with rod-shaped in red color indicates gram-negative bacteria. (Yonis, 2017)

2.6 Minimum Inhibitory Concentration (MIC) of Plant Extracts and Its Potential in Phyto medical

Minimum Inhibitory Concentration is an important parameter when determining the antimicrobial activity of plant extracts. A few Studies have been conducted using extract of Alpinia species to identify the Minimum Inhibitory Concentration (MIC) value to determine appropriate dosages in Phyto-medicine. A study conducted by Yasin et al. (2019) shows that the extracts of two different plants, including *Piper betle* and *Alpinia galanga* were effective in inhibiting *Fusobacterium nucleatum* and *Streptococcus mutans* where *F. nucleatum* can be inhibited with the MIC value around 1.56 mg/mL to 6.25 mg/mL while the MIC value for *S. mutant* at range of 1.56 mg/mL to 25.00 mg/mL. Another study conducted by Siti Nur Izaty Che

UNIVERSITI MALAYSIA KELANTAN

Humaidi et al. (2020) shows that *Alpinia conchigera Griff.* which contains phenylpropanoid 1'S-1'-acetoxychavicol acetate (ACA) had the highest antimicrobial activity against *Mycobacterium smegmatis* with a MIC value of 62.5 µg/mL. Research conducted by Chakrabartty et al. (2019) shows (E)-labda-8(17), 12-diene-15,16-dial isolated from the seeds of *Alpinia nigra* resulting in ≤ 0.44 mg/mL as the maximum dosage of labdane for gram-negative bacteria (*Salmonella paratyphi* and *Escherichia coli*) and fungus (*Candida albicans*) inhibition. Another study conducted by Tian et al. (2022) indicates that the essential oil of *Alpinia galanga* showed strong-to-moderate antibacterial activities with various diameters of inhibition zone around 8.79 mm to 14.32 mm and minimal inhibitory concentration (MIC) around 3.13 mg/mL 6.25 mg/mL against *Escherichia coli*.

The findings from the study mentioned in the sources highlight the effectiveness of *Alpinia* plant extracts against bacteria and explore new compounds that were possible to overcome bacterial infection. The minimum inhibitory concentration of plant extracts was an important parameter in Phyto-medicine as it helps to provide important information to determine the antimicrobial potency of these extracts and dosage formulation and contributes to the development of targeted treatments.

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

2.7 Antimicrobial Assay of Extract from *Alpinia javanica*.

Antimicrobial assays were important in developing new antibiotics to test the effectiveness of potential antibiotic candidates against various strains of bacteria. Datar et al. (2021) stated that recent advances in antimicrobial susceptibility testing methods have improved accuracy and rapidity but require further improvements for routine clinical microbiological detection of antimicrobial resistance. Studies conducted by Leonard et al. (2018) show that recent advances in antimicrobial susceptibility testing (AST) contribute to guiding physicians to the correct antibiotic for an infection, potentially reducing antibiotic resistance and the evolution of superbugs. The previous study conducted by Taylor et al. (2018) provides a cost-effective strategy for analyzing antibiotic-induced changes in respiratory tract microbiology, providing valuable insights for monitoring antibiotic resistance and assessing the impact of antimicrobial therapies in clinical trials. There was research conducted by Usharani S. (2023) on the *Alpinia* genus where *Alpinia officinarum* was used in antimicrobial assays and it shows that this species has compounds that were capable against gram-negative bacteria with half-maximal inhibitory concentration (IC50) concentration of 253.83µg/mL. By studying the specific pathways resistance of bacteria development, new approaches would develop to disrupt these mechanisms and restore the effectiveness of antibiotics.

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

FYP FSB

CHAPTER 3

MATERIAL AND METHOD

3.1 Preparation of Nutrient Agar

Media bacteria was prepared using 28g of Nutrient agar (Oxoid, England) added with one litter of distilled water. The nutrient agar was dissolved and sterilized using an autoclave (Tomy, Japan) at 121°C for 15 to 20 minutes and let cool at temperatures around 40°C to 45°C in laminar flow. The sterilized agar solution (20 mL) was transferred into individual sterilized petri dishes and left to cool down inside the laminar flow until it solidified around 27°C. Then, the agar plates were stored in the chiller in the Microbiology and Biochemistry Laboratory, Faculty of Earth Science (FSB), Universiti Malaysia Kelantan (UMK), Jeli Campus, at 2°C to 8°C.

UNIVERSITI

3.2 Preparation of Stock Culture of Bacteria

MALAYSIA

Four different types of microorganisms, which are *Staphylococcus aureus* and *Escherichia coli,* were chosen as test anti-microorganisms and were obtained from (Table 3.1). These microorganisms were used to observe antimicrobial activities in the Microbiology and Biochemistry Laboratory, Faculty of Earth Science (FSB), Universiti Malaysia Kelantan (UMK), Jeli Campus. From the main culture, 100µL

KELANTAN

of bacteria was transferred to nutrient agar using the continuous streaking method (Figure 3.1) (Testbook Edu Solutions, 2023). To perform the streaking method, the inoculation loop was flamed with a Bunsen burner for sterilization and let it cool down. The selected bacteria (Table 3.1) were transferred by inserting the inoculation loop into the tube containing bacteria culture and streak on nutrient agar started from the edge of the plate (A) in back-and-forth movement in a continuous movement to the centre of the plate with putting light pressure on the plate. The plate was rotated at 180 degrees and continued streaking on another side, starting from the edge of the plate (B) to the centre of the plate with the same loop as shown in Figure 3.1. The inoculation loop was flamed after finishing the streaking to prevent contamination. Two different bacteria (Table 3.1) went through the same streaking technique three times.

Table 3.1 Test microorganism for antimicrobial activities assay

Microorganism	Classification of Microorganism	Scientific Name	Culture Media
Bacteria	Gram Positive	*Staphylococcus aureus*	Nutrient Agar
	Gram Negative	*Escherichia coli*	

FYP FSB
UNIVERSITI MALAYSIA KELANTAN

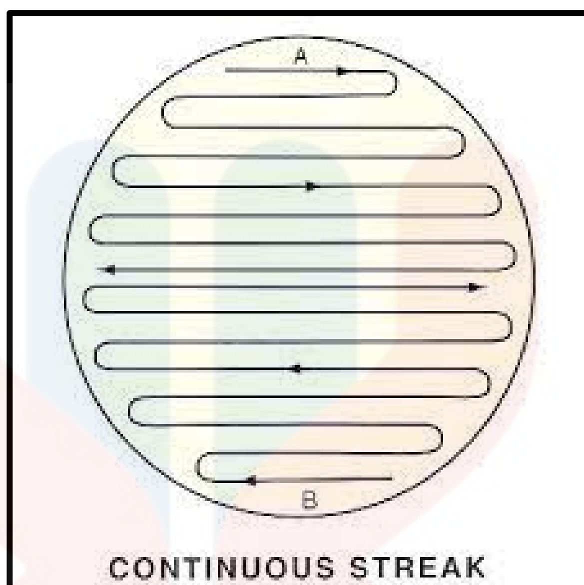


Figure 3.1 Continuous streaking method (Hussam M. Al-Imam, 2019)

3.3 Preparation of Plant Extraction

This study involves the use of *Alpinia javanica* extracts to determine the ways microorganisms respond to the compound of this herb plant at different parts of the plant, including the leaf, pseudo stem, and rhizome. The dried extracts were obtained from the previous study completed by Hassanal FikriAbdul Gani (2023) from the Microbiology and Biochemistry Laboratory, Faculty of Earth Science (FSB), Universiti Malaysia Kelantan (UMK), Jeli Campus. Plant extracts were prepared at different concentrations starting at 0 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL, where the extract was diluted with analysis methanol (HmbG, Malaysia) to determine the susceptibility of bacteria to various concentration antibiotics. According to Paulsen et al. (2015) state that the frozen material was extracted with methanol/diluted acetic acid (pH 3.5; 75:25, v/v).

FYP FSB
UNIVERSITI MALAYSIA KELANTAN

The extracts of leaf, pseudostem, and rhizome were prepared using 0.05g of each extract added with 1000 µL of methanol inside a 10 mL falcon tube to get 5 mL solutions. Mixed the solution by using the single-tube vortex mixer to make sure the extracts and methanol were well diluted. To prepare a one mL dilution of 2 mg/mL extracts, take 200 µL of extract from 5 mL of extracts and add 800 µL of methanol. Continued with 4 mg/mL with 400 µL of extract added with 600 µL of methanol, 6 mg/mL with 600 µL of extract, with 400 µL of methanol, 8 mg/mL with 800 µL of extract added with 200 µL of methanol,10 mg/mL with 1000 µL of plant extracts, 0mg/mL with 1000 µL of methanol. After the preparation of the plant extract, the concentration was stored in a refrigerator from the same laboratory with a temperature of 4 °C (Arullappan et al., 2009).

3.4 Determination of Bacteria Growth, Inhibition Zone, and Antimicrobial Activity of *Alpinia javanica* at Different Concentrations.

3.4.1 Antimicrobial Assay

To determine antimicrobial assay, the bacteria from gram-negative and gram-positive were used nutrient agar (Oxoid, England), as stated in Table 3.1, where both bacteria culture and nutrient agar (Oxoid, England) were obtained from the Microbiology and Biochemistry Laboratory, Faculty of Earth Science (FSB),

FYP FSB
UNIVERSITI MALAYSIA KELANTAN

Universiti Malaysia Kelantan (UMK), Jeli Campus. Streptomycin (Oxoid, England) was used as a known antibiotic, and different extract concentrations of *Alpinia javanica* from different parts, including the rhizome, pseudo stem, and leaf extract, work as the positive control (Ni Nyoman Rupiasih et al., 2019) for testing antimicrobial susceptibility which was also obtained from the same laboratory.

The disk was prepared by using Whatman® filter paper no. 1 (Sigma Aldrich, Damstadt, Germany). The filter papers were cut out using a hole puncher to get disks at an approximate diameter of 6mm. The disk filter papers were placed into a petri dish, wrapped using aluminum foil, autoclaved, and let it dry in the laboratory oven (Binder, United States of America) from the same laboratory.

The sterilized forceps were used to hold the disk and placed on nutrient agar containing selective bacteria (Table 3.1). Disk filter papers were soaked individually with plant extract at different concentrations starting 0 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, 10 mg/mL, and 10 mg/mL Streptomycin (Oxoid, England), and analytical methanol (HmbG, Malaysia) as negative control placed on top of the solid nutrient agar medium by using the micropipette. The bacteria were inoculated onto agar plates with a further 18 to 24-hour incubation at 37°C (Chikezie, 2017). After the incubation period, the zone of inhibitions was present around the filter paper containing the antimicrobial agents (Figure 3.2). Each nutrient agar was divided into eight sections, with each section containing a single disk of filter paper holding the negative and positive control agents. The diffusion test was performed in

FYP FSB

triplicates for each concentration and was repeated three times for three extracts from different parts of *A. javanica.*

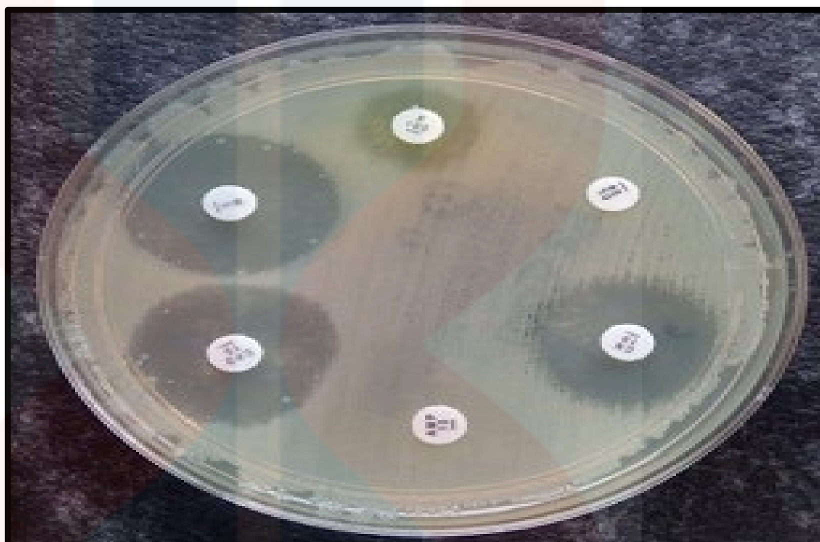


Figure 3.2 The presence of the inhibition zone around the disk (Open University, n.d.)

3.4.2 Measurement of Inhibition Zone

To measure the inhibition zone, the diameter of the inhibition zone (d_{iz}) and the disk diameter (d) (Figure 3.3) were measured in millimeters (mm) by using a Digital Vernier Caliper (Standard Gage, America) (Figure 3.4) obtained from Microbiology and Biochemistry Laboratory, Faculty of Earth Science (FSB), Universiti Malaysia Kelantan (UMK), Jeli Campus, from edge to edge or measure the diameter of the inhibition zone (d_{iz}) which also includes the disk diameter (d) (Figure 3.3) and divided by two to calculate the radius and use formula πr^2 where r is the radius of the inhibition zone.

FYP FSB

FYP FSB

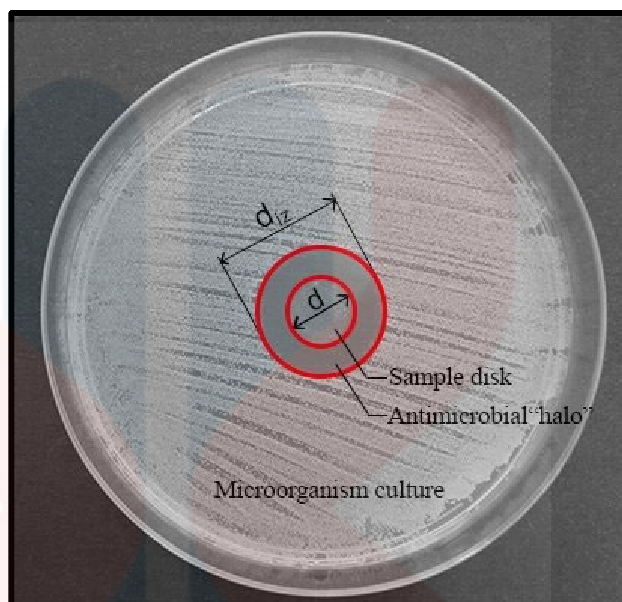


Figure 3.3 Measurements for the normalized width of the inhibition zone. (Serrano-Aroca, 2018)

Figure 3.4 Digital vernier calliper

UNIVERSITI
MALAYSIA
KELANTAN

3.5 Statistical Analysis of The Inhibition Zone of Bacteria

The statistical analysis was conducted to compare extract inhibition activities at different parts of *Alpinia javanica* for different selective bacteria. The test was done for triplicates of each concentration, and the mean value was calculated to evaluate the results where huge variability is covered, the probability due to chance alone was drastically reduced, and the data became more trustworthy (Deo, 2017). Comparison analysis was determined using the mean and standard deviation. Two-way analysis of variance (ANOVA) was used to compare the mean of the data, whereas two-way analysis of variance (ANOVA) was often used to determine the differences and possible interaction when the variable was present from the perspective of two or more categories (MacFarland, 2012). Two-way analysis of variance (ANOVA) was conducted using IBM® Software (SPSS®) Statistics version 29.0 to compare differences in the inhibition zone of three different plant parts extract involving leaf, pseudo stem, and rhizome. The data was considered significantly different when the P-value was smaller than 0.05, which is $P < 0.05$. The significant result of the inhibition zone between various parts of *A. javanica* extracts was assessed using a two-way analysis of variance (ANOVA) with a confidence level of 95% to show the comparison. The potential of antimicrobial compounds on selective bacteria was determined using an ANOVA.

FYP FSB

UNIVERSITI MALAYSIA KELANTAN

CHAPTER 4

RESULT AND DISCUSSION

4.1 The Kirby-Bauer Disk Diffusion Method to Determined Minimum Inhibitory Concentration (MIC).

In this study, the 10 microliters of *Alpinia javanica*'s extracts started from 0 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL were tested with *Escherichia coli* and *Staphylococcus aureus* using the Kirby-Bauer disk diffusion method for 10µL of extract. After the incubation period, there was the presence of an inhibition zone around the disks for petri dishes. The diameter of the inhibition zone of both bacteria (Figure 4.1) was measured to determine the effectiveness of the plant extract. The inhibition zones were different for every part of the plant and type of bacteria (Table 4.1). The presence of the inhibition zone of bacteria around the disk containing diluted extract from different parts of *A. javanica* can be seen in Figure 4.2 and Figure 4.3 where different types of bacteria react differently at different concentrations.

FYP FSB

Table 4.1 The inhibition zone of *Alpinia javanica* extracts from different parts which involve leaf, pseudostem, and rhizome against two different bacteria which were bacteria *E. coli* and S. *aureus*.

The Inhibition zone of Bacteria (mm)			
Plant Parts	Concentration (mg/mL)	*E. coli*	*S. aureus*
		Mean ± SD	Mean ± SD
Leaf	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2	7.06 ± 1.13^{bc}	7.51 ± 0.48^{cd}
	4	6.67 ± 0.64^{b}	8.64 ± 1.81^{ef}
	6	8.14 ± 0.38^{de}	9.02 ± 0.07^{fg}
	8	9.60 ± 0.37^{fg}	9.71 ± 0.37^{fg}
	10	8.79 ± 0.28^{fg}	8.98 ± 0.33^{fg}
	10 (Streptomycin)	16.27 ± 1.15^{i}	14.71 ± 0.24^{i}
Pseudostem	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2	7.98 ± 0.78^{de}	8.01 ± 1.05^{de}
	4	7.84 ± 0.42^{de}	7.74 ± 0.50^{de}
	6	6.79 ± 0.06^{bc}	8.59 ± 0.10^{de}
	8	7.52 ± 0.30^{cd}	7.48 ± 0.08^{cd}
	10	6.97 ± 0.26^{bc}	7.60 ± 0.43^{cd}
	10 (Streptomycin)	15.40 ± 1.22^{i}	13.60 ± 2.09^{hi}
Rhizome	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2	8.94 ± 0.54^{fg}	8.71 ± 0.92^{ef}
	4	9.72 ± 0.27^{fg}	10.64 ± 0.37^{gh}
	6	9.64 ± 0.27^{fg}	9.17 ± 0.01^{fg}
	8	11.68 ± 0.56^{gh}	10.26 ± 0.45^{fg}
	10	11.38 ± 0.29^{gh}	11.47 ± 0.03^{gh}
	10 (Streptomycin)	15.69 ± 0.22^{i}	16.51 ± 1.94^{i}

The same lowercase alphabets indicate that there were no statistical differences ($p > 0.05$) while the different lowercase alphabets indicate statistical differences ($p < 0.05$).

FYP FSB

UNIVERSITI MALAYSIA KELANTAN

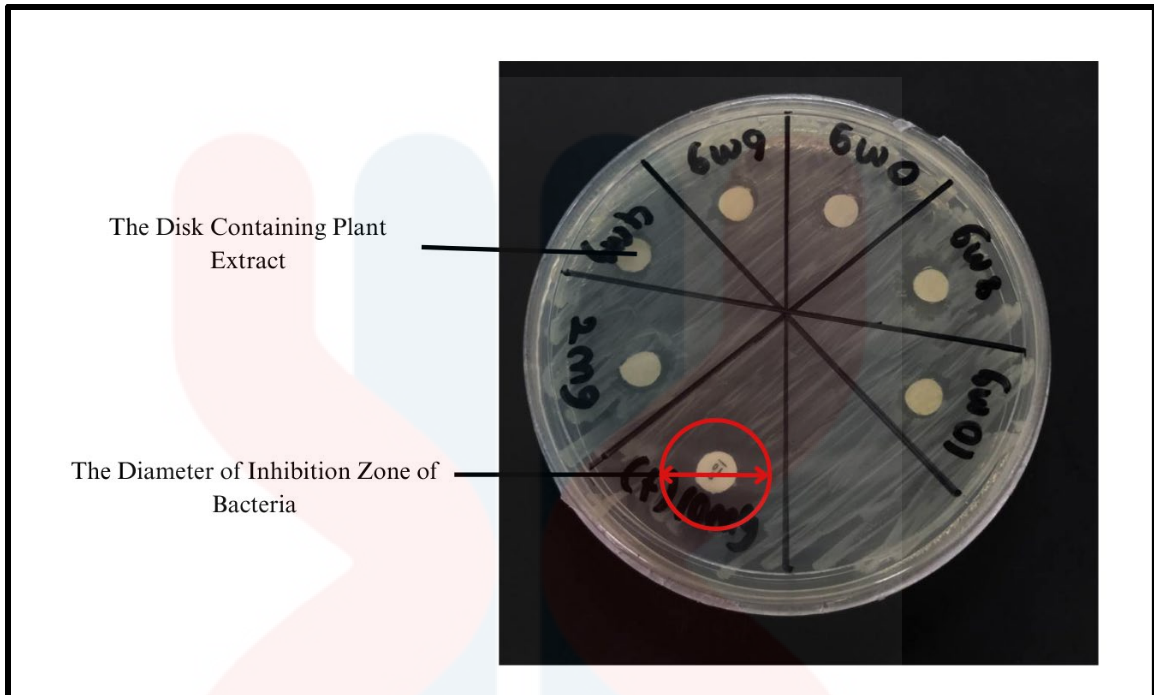


Figure 4.1 The diameter of the inhibition zone of bacteria that was measured.

FYP FSB

UNIVERSITI
MALAYSIA
KELANTAN

FYP FSB

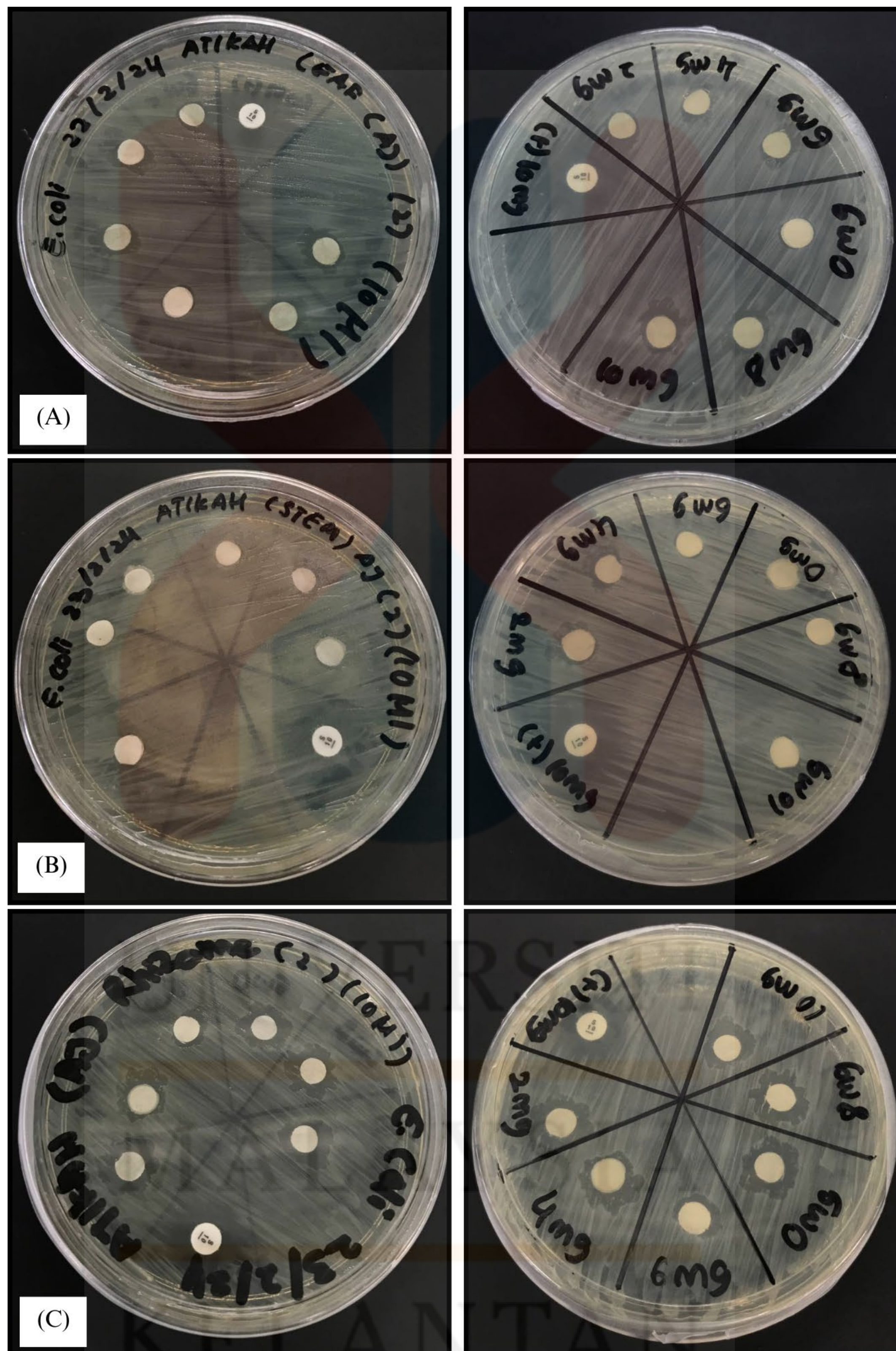


Figure 4.2 The inhibition zones of *A. javanica* from different parts of plants 10µL extract which were leaf (A), pseudostem (B), and rhizome (C) against *E. coli.*

FYP FSB

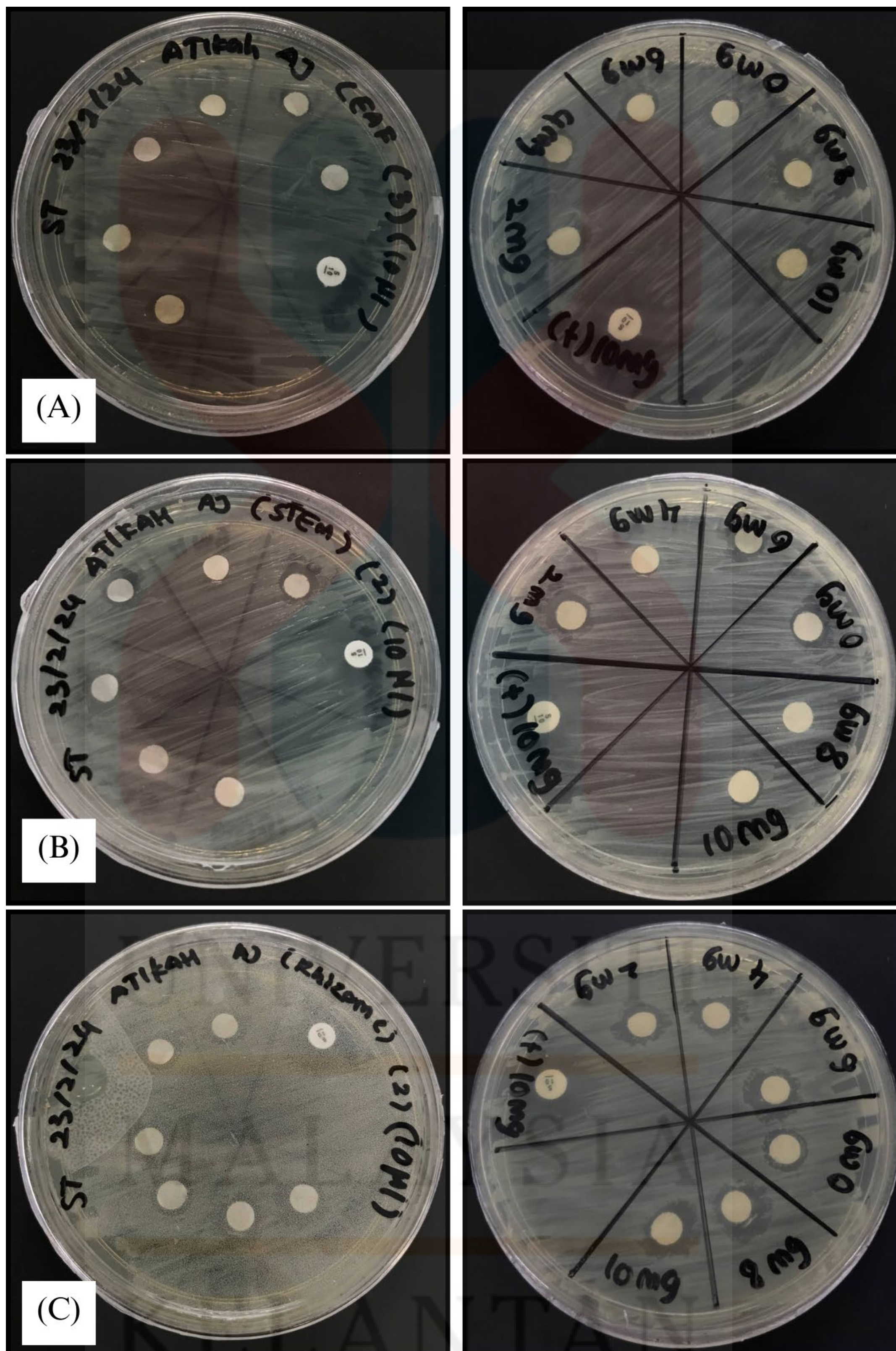


Figure 4.3 The inhibition zones of *A. javanica* from different parts of plants 10µL extract which were leaf (A), pseudostem (B), and rhizome (C) against *S. aureus.*

4.2 Antimicrobial Activities from Different Parts of *Alpinia javanica.*

The bacteria that have been used in this antimicrobial assay have been shown to the inhibition zone after 24 hours of incubation which indicates that both bacteria *Escherichia coli* and *Staphylococcus aureus* showed the reaction to the plant extract that had been soaked by the disk. The negative control which consists of only methanol (0 mg/mL) shows no inhibition zone around the disk and the positive control shows a significant amount of inhibition zone (10 mg/mL Streptomycin) as it should show the efficiency of an antimicrobial assay where the result (Table 4.1) shows all the petri dish that consists of both negative and positive controls shows the statistically same result.

Antimicrobial assays using leaf part show statistically no difference inhibition zone for *E. coli* (8 mg/mL and 10 mg/mL) and *S. aureus* (6 mg/mL, 8 mg/mL, and 10 mg/mL) while another concentration for *E. coli* (2 mg/mL, 4 mg/mL, 6 mg/mL) and *S. aureus* (2 mg/mL and 4 mg/mL) in table 4.1 shows the differences of inhibition zone. In pseudostem, the inhibition zone shows no difference in concentration of 2 mg/mL and 4 mg/mL (*E. coli*). The concentration of 4 mg/mL showed no significant difference inhibition zone of *S. aureus* where (2 mg/mL and 4 mg/mL) (8 mg/mL and 10 mg/mL) (Table 4.1) showed the same range of inhibition zone. However, another concentration including 6 mg/mL, 8 mg/mL, and 10 mg/mL for *E. coli* and 6 mg/mL and 10 mg/mL for *S. aureus* shows differences in the inhibition zone. In Rhizome, all concentrations for *E. coli* and *S. aureus* showed no differences in the inhibition zone.

FYP FSB

UNIVERSITI MALAYSIA KELANTAN

The result of this study (Table 4.1) shows that the inhibition zone in the leaf part indicates that the concentration of 2 mg/mL shows the inhibition zone with an average inhibition of 7.06 ± 1.13 mm (*E. coli*) and 7.51 ± 0.48 mm (*S. aureus*) which it was slightly smaller compared to 4 mg/mL against *E. coli* with an average 6.67 ± 0.64 mm but bigger against *S. aureus* with average 8.64 ± 1.81 mm of inhibition zone. The concentration of 6 mg/mL shows *S. aureus* had a higher average compared to *E. coli* where *E. coli* had an average of 8.14 ± 0.38 mm while *S. aureus* had an average of 9.02 ± 0.07 mm. The concentration of 8 mg/mL and 10 mg/mL shows there were slight differences in the inhibition zone where 8 mg/mL and 10 mg/mL for *E. coli* had an average of 9.60 ± 0.37 mm and 8.79 ± 0.28 mm while *S. aureus* had each 9.71 ± 0.37 mm and 8.98 ± 0.33 mm (Table 4.1). The leaf extracts showed that the concentration of 8 mg/mL had the highest average diameter inhibition zone against *E. coli* and *S. aureus* compared to another concentration with the same extract. This result was slightly different statistically with a concentration of 10 mg/mL of the extract. It shows that the concentration of 8 mg/ml shows the same effectiveness as the concentration of 10 mg/ml against both tested bacteria. However, The Karuk leaf (*Piper Sarmentosum* Roxb) had a strong inhibition zone of bacteria at a concentration of 100% of the extract which same as the concentration of 10 mg/mL extract with a diameter of inhibition zone at 34.2 mm against *Microsporum Gypseum* and 16.3 mm against *Candida Albicans* (Khusnul et al., 2018) which supposedly shows the efficiency of 10 mg/mL of leaf extract to have largest inhibition zone against tested bacteria. The research conducted by Mahdavi et al. (2017) on *Etlingera sayapensis*, shows that the leaf methanolic

FYP FSB
UNIVERSITI MALAYSIA KELANTAN

extract was the most active extract with a value of 1.17 mg/mL against *Bacillus subtilis* which shows the effectiveness of leaf extract from the plant with the same family to inhibit the bacteria. Even though leaf extract at the concentration of 8 mg/mL had the highest mean inhibition zone for both bacteria compared to other concentrations, it was still a weak concentration compared to the 10 mg/mL of Streptomycin (positive control) with a mean diameter inhibition zone of 16.27 ± 1.15 mm (*E. coli*) and 14.71 ± 0.24 mm (*S. aureus*) (Table 4.1).

In the pseudostem of *Alpinia javanica*, the concentration of 2 mg/mL and 4 mg/mL showed slight differences for each concentration and each bacterium where 2 mg/mL had an average of 7.98 ± 0.78 mm (*E. coli*) and 8.01 ± 1.05 mm (*S. aureus*) along with 4 mg/mL with an average of 7.84 ± 0.54 mm (*E. coli*) and 7.74 ± 0.50 mm (*S. aureus*). The concentration of 6 mg/mL consists of an average of 6.79 ± 0.06 mm (*E. coli*) which was smaller than *S. aureus* with an average of 8.59 ± 0.92 mm. The concentration of 8 mg/mL had slightly the same average size of inhibition zone for both bacteria where 7.52 ± 0.30 mm (*E. coli*) and 7.48 ± 0.08 mm (*S. aureus*). In a concentration of 10 mg/mL, both bacteria showed differences in the size of the inhibition zone where *E. coli* had an average of 6.97 ± 0.26 mm while *S. aureus* had an average of 7.60 ± 0.43 mm where *S. aureus* had a larger size of inhibition zone compared to *E. coli* in this concentration. The least diameter size of the inhibition zone of bacteria using *A. javanica* extract could be related to the study involving *Zingiber zerumbet* (under the same order) where it showed that pseudostem was ineffective against *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Le T. Huong et al., 2020). Although pseudostem extract

FYP FSB

showed the least size of inhibition zone compared to leaf and rhizome extract, pseudostem extract was capable against both tested bacteria. The concentration of 2 mg/mL shows the higher average of inhibition with 7.98 ± 0.78 mm for *E. coli* (Table 4.1) while the concentration of 6 mg/ml shows the highest average of inhibition zone with 8.59 ± 0.10 mm (Table 4.1) against *S. aureus*. Another research conducted on one of the herbal plants, *Ochrosia oppositifolia* showed that the stem-bark alkaloid extract had low antibacterial activity while the leaf alkaloid extract showed no antibacterial activity against *S. aureus* and *E. coli* (Puteri I.A.M. Mahmud et al., 2017). The extract of pseudostem at the different concentrations on both tested bacteria shows significant differences compared to 10 mg/ml Streptomycin that was placed on the same agar where the diameter of positive control was 15.40 ± 1.22 mm (*E. coli*) and 13.60 ± 2.09 mm (*S. aureus*) (Table 4.1) which indicates that the extract was not efficient as Streptomycin against these gram-positive and gram-negative bacteria.

The result of the rhizome extract of *A. javanica* shows a concentration of 2 mg/mL showing only slight differences in both bacteria with an average of 8.94 ± 0.54 mm (*E. coli*) and 8.71 ± 0.92 mm (*S. aureus*) (Table 4.1). In the concentration of 4 mg/mL, there was a difference in the result where *E. coli* with average diameter of 9.72 ± 0.27 mm while *S. aureus* had an average of 10.64 ± 0.38 mm average which was larger than *E. coli*. The concentration of 6 mg/mL shows a slight difference in size in the inhibition zone of bacteria with *E. coli* having an average diameter of 9. 64 ± 0.27 mm and *S. aureus* with an average of 9.17 ± 0.01 mm. The concentration of 8 mg/mL consists of an average of 11.68 ± 0.56 mm (*E. coli*) and 10.26 ± 0.45 mm (*S. aureus*) where

FYP FSB

the inhibition zone of E. *coli* was slightly larger than *S. aureus*. The concentration of 10 mg/mL had a similar average inhibition zone where *E. coli* has an average diameter of 11.38 ± 0.29 mm against *E. coli* and 11.47 ± 0.03 mm against *S. aureus.* The finding shows that the extract of the rhizome at a concentration of 8 mg/ml had the highest diameter of the inhibition zone with an average diameter of 11.68 ± 0.56 mm and the average was slightly different with the concentration of 10 mg/ml with an average diameter of 11.38 ± 0.29 mm against *E. coli* while the concentration of 10 mg/mL had the highest average of diameter zone with 11.47 ± 0.03 mm. Although the concentration of 10 mg/mL had the highest average against *S. aureus*, the result statistically shows that the average of 10 mg/mL against *S. aureus* was slightly different from the concentration of 4 mg/mL against the same bacteria (Table 4.1). Another research using the rhizome of Galangal (*Alpinia Galanga*) shows the best result in the presence of an inhibition zone with a concentration of 100% with a diameter of 12.3 mm against *Candida albicans* and 32.06 mm against *Microsporum gypseum* (Khusnul et al., 2018) which supposedly have similar result that obtained from 10 mg/mL of rhizome against *E. coli* which using 100% of the diluted extract. This finding indicated that the concentration of 8 mg/ml to 10 mg/ml of rhizome extract was more potent among other concentrations against both tested bacteria. However, the largest diameter of the inhibition zone for both bacteria at a concentration of 8 mg/ml (*E. coli*) and 10 mg/ml (*S. aureus*) was still weak compared to 10 mg/ml Streptomycin with a diameter of 15.69 ± 0.22 mm (*E. coli*) and 16.51 ± 1.94 mm (*S. aureus*) (Table 4.1).

FYP FSB

FYP FSB

4.3 Determination of Minimum Inhibitory Concentration (MIC) of Extracts from Different Parts of *Alpinia javanica.*

Based on the findings show that 2 mg/mL extract of leaf, pseudostem, and rhizome inhibit the *Escherichia coli* and *Staphylococcus aureus* where the diameter of the zone was present in Figure 4.2 and Figure 4.3 and the measurement have been stated in Table 4.1. This indicates that the Minimum inhibitory concentration (MIC) of *Alpinia javanica* extracts from different parts of the plant which were the leaf, pseudostem, and rhizome was 2 mg/mL as at this concentration there was a presence of inhibition zone of bacteria around the disk.

The minimum inhibitory concentration (MIC) value of an ethanolic leaf extract from *Curcuma longa* on *Pseudomonas aeruginosa* shows 3.129% at the concentration of 6.25 mg/mL (Singh et al., 2017) where the concentration was higher than leaf extract of *A. javanica* with the minimum inhibitory concentration (MIC) of 2 mg/mL with average diameter of 7.06 ± 1.13 mm against *E. coli* and 7.51 ± 0.48 mm against *S. aureus*. The leaves essential oil nanoemulsion of *Zingiber officinale* shows a MIC value of 62.5 µL/mL against the *Streptococcus mutans* (Mostafa, 2018). It shows that the extract of *A. javanica* was more effective and potent compared to *C. longa* against targeted bacteria with a higher MIC value. However, the leaves essential oil nanoemulsion of *Z. officinale* was more potent compared to *A. javanica* due to the lowest MIC value against the bacteria.

Huong et al. (2020) found that the minimum inhibitory concentration (MIC) value of pseudo stem essential oil of *Zingiber castaneum* was 12.5 µg/mL against *Pseudomonas aeruginosa* and 50 µg/mL against *Fusarium oxysporum* and *Aspergillus niger*. Tongwanichniyom et al. (2024) stated that the biologically green synthesis of silver nanoparticles (AgNPs) from pseudostem extract of *Alpinia nigra* shows the MIC value of 7.9 $\mu g \cdot mL^{-1}$ against *E. coli* and *S. aureus* which was lower compared to *Z. castaneum*. The pseudostem of *Alpinia rafflesiana* shows the MIC value of 31.25 $\mu g \cdot mL^{-1}$ against *Bacillus subtilis*, 62.5 $\mu g \cdot mL^{-1}$against *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Candida albicans*, and 125 $\mu g \cdot mL^{-1}$ against *Aspergillus niger* (Van et al., 2021). Another concentration containing a component of pseudostem from ginger plant shows there were lowest minimum inhibitory concentration (MIC) value compared to the MIC value of pseudostem extract from *A. javanica* which at a concentration of 2 mg/mL with an average diameter of zone 7.98 ± 0.78 mm against *E. coli* and 8.01 ± 1.05 mm against *S. aureus*.

The minimum inhibitory concentration (MIC) value of an ethanolic rhizome extract from *Curcuma longa* on *Pseudomonas aeruginosa* shows 3. 991% at concentration of 6. 25 mg/ml (Singh et al., 2017) while Lara et al. (2021) found that the MIC of rhizome from *Zingiber officinale* Roscoe had MIC around 7.81 mg/mL to 15.62 mg/mL which were high compared to MIC value of rhizome extract from *A. javanica*. Momoh and Olaleye (2022) found the MIC value of rhizome extract from *Zingiber officinale* Roscoe was 125 mg/mL against *E. coli* and 250 mg/mL against *S. aureu*s. However, the research conducted on rhizome extract on *Zingiber zerumbet* Linn where the MIC value

FYP FSB

shows the highest antimicrobial activity against two Gram-positive and four Gram-negative bacteria including *E. coli* within the value of 128 µg/mL to 256 µg/mL (Golam Kader et al., 2011). It shows that 2 mg/mL of rhizome extract isolated from *A. javanica* was more potent against *E. coli* and *S. aureus* with an average diameter each of 8.94 ± 0.54 mm and 8.71 ± 0.92 mm compared to *Zingiber officinale* Roscoe and *Curcuma longa* as it was more effective against bacteria at the lowest concentration while less potent to compare to *Zingiber zerumbet* Linn.

4.4 The Comparison of Antimicrobial Activities from Different Parts of *Alpinia javanica.*

The extract of *Alpinia javanica* from different parts which includes the leaf, stem, and rhizome shows significant differences in the inhibition zone of both bacteria *E. coli* and *S. aureus*. The result shown in Table 4.1 indicated that the extract of the leaf at the concentration of 8 mg/mL for both bacteria shows a higher average of inhibition zone 9.60 ± 0.37 mm (*E. coli*) and 9.71 ± 0.37 mm (*S. aureus*) among other concentrations within the same extract but less potent compared to rhizome extract at a concentration of 6 mg/mL where the average size of inhibition zone was slightly different with an average diameter of 9.64 ± 0.27 mm (*E. coli*) and 9.17 ± 0.01 mm (*S. aureus*) which shows that the efficiency of 6 mg/mL of rhizome extract was

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

same as 8 mg/mL of leaf's extract which shows that the extract of the rhizome was stronger compared to leaf. The concentration of 4 mg/mL of rhizome extract shows a similar average diameter zone as 8 mg/mL of leaf extract, indicating that rhizome was more efficient than leaf extract because 4 mg/mL of rhizome extract, which was a lower concentration than leaf extract, came up with the same effect, suggesting that it was stronger than the leaf extract against *E. coli*. Rasha Saad Suliman et al. (2012) proved that another plant under the same family (*Zingiberceae*), *Zingiber officinale*, had higher antimicrobial activity in rhizome extract compared to the leaf extract against the four different pathogenic bacteria which were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp., and *Salmonella* spp. There was also a study conducted using the plant from the same genus by Kochuthressia et al. (2010) which showed *Alpinia purpurata* rhizomes had a wide range of antimicrobial activity with the highest inhibition zone discovered against *Enterobacter aerogens.* Singh et al. (2017) indicate that *Curcuma longa* (family *Zingiberaceae*) shows the rhizome was more potent antimicrobial potency compared with the leaf extract against gram-negative and positive bacteria although the minimal inhibition zone (MIC) of leaf and rhizome extract shows very less variation which was 3.129 % and 3.991% whereas the percent inhibition at highest concentration 200mg/ ml was found to be 93.125% for rhizome extract and 91.058% for leaf extract.

However, the leaf extracts show that the concentration of 8 mg/mL was capable of inhibiting both tested bacteria as the result (Table 1) shows the highest average inhibition zone size with 9.60 ± 0.37 mm (*E. coli*) and

FYP FSB

UNIVERSITI MALAYSIA KELANTAN

9.71 ± 0.37 mm (*S. aureus*) (Table 4.1). This result was slightly different statistically with a concentration of 10 mg/mL with an average inhibition zone of 8.79 ± 0.28 mm (*E. coli*) and 8.98 ± 0.33 mm (*S. aureus*) (Table 4.1). It showed that the concentration of 8 mg/mL shows the same effectiveness as the concentration of 10 mg/ml against both tested bacteria. The Karuk leaf (*Piper Sarmentosum* Roxb) also had a strong inhibition zone of bacteria at a concentration of 100% of the extract with a diameter of inhibition zone at 34.2 mm against *Microsporum Gypseum* and 16.3 mm against *Candida Albicans* (Khusnul et al., 2018) which showed that leaf parts capable to works as antibacterial against targeted bacteria.

The concentration of 10 mg/mL from leaf extract had a similar average of inhibition zone against both tested bacteria with an average of 8.79 ± 0.28 mm (*E. coli*) and 8.98 ± 0.33 mm (*S. aureus*) (Table 4.1) which was similar average to the concentration of 2 mg/ml of rhizome extract with an average of 8.94 ± 0.54 mm (*E. coli*) and 8.71 ± 0.92 mm *(S. aureus*) (Table 4.1). It shows that 2 mg/mL of rhizome extracts could give the same effect as 10 mg/mL of leaf extract which indicates that 2 mg/mL of rhizome were more efficient against both tested bacteria. *Curcuma zedoaria* and *Curcuma aeruginosa* Roxb. leaves showed a high antibacterial activity against *Streptococcus mutans* with the minimum inhibition zone of bacteria (MIC) at concentration as low as 15.6 µg/mL (Irmanida Batubara et al., 2019).

The concentration of 2 mg/ml of pseudostem against *E. coli* shows slight differences with the concentration of 6 mg/ml of leaf extract where the

FYP FSB

UNIVERSITI MALAYSIA KELANTAN

pseudostem shows an average of 7.84 ± 0.42 mm while the leaf extract shows 8.14 ± 0.36 mm. This finding shows that the 2 mg/ml of pseudostem was more efficient against *E. coli* compared to leaf extract. The pseudostem extract's concentrations of 8 mg/ml and 10 mg/ml were slightly different from the leaf extract with a concentration of 2 mg/ml against *S. aureus*. The leaf extract of 2 mg/mL with an average of 7.51 ± 0.48 mm shows slight differences with pseudostem extract with an average of 7.48 ± 0.08 mm (8 mg/ml) and 7.60 ± 0.43 mm (10 mg/ml) against the same bacteria. These results indicate that the concentration of 2 mg/ml of leaf extract was more effective against *S. aureus* since it gave the same effect as the concentration of 8 mg/ml and 10 mg/ml.

To compare their part of *A. javanica*, pseudostem shows the smaller size of inhibition zone for both bacteria compared to leaf and rhizome. In this part of the plant extract, the concentration of 2 mg/ml for *E. coli* shows the highest inhibition zone compared to another concentration using the same bacteria with an average of 7.98 ± 0.78 mm and 6 mg/ml for *S. aureus* with an average of 8.59 ± 0.10 mm with the highest among another concentration using the same bacteria. Comparison from various concentrations of leaf and rhizome against *E. coli* and *S. aureus*, it was found that the pseudostem extract was weaker and less potent than the leaf and rhizome. In summary, the pseudostem extract had the lowest average diameter zone where most of the concentrations, including 2 mg/mL, 4 mg/mL, 8 mg/mL, and 10 mg/mL, showed significant differences. Although the pseudostem extract was the weakest of the three, it was still able to

FYP FSB

UNIVERSITI MALAYSIA KELANTAN

inhibit *E. coli* and *S. aureus* at a concentration of 2 mg/mL, with the pseudostem extract having the same MIC value as the leaf and rhizome extracts. The leaf extracts from a different herbal plant, *Carissa bispinosa* also showed better inhibitory activities than the stem extracts where the minimum inhibitory concentration (MIC) of methanol leaf extract showed the lowest value of 0.31 mg/mL while the stem ethanol extract had the least minimum inhibitory concentration (MIC) value of 0.31 mg/mL against fungus and bacteria (Shekwa et al., 2023).

The findings indicate that the extract of rhizome from *A. javanica* proved to be more potent and effective against the bacteria considering this part of the plant MIC value with the better cumulative diameter of the zone at the majority of the concentration between leaf and pseudostem. Overall, the result shows that *E. coli* had the lowest inhibition zone compared to *S. aureus* which was proven where there was research stated that in both the leaf and rhizome ginger extract (Zingiber officinale) against *E. coli* was the lowest compared to *S. aureus* (Rasha Saad Suliman et al., 2012).

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

4.5 Experimental Errors and The Factor that Influences the False Outcome in Antimicrobial Assays

Antimicrobial assays are essential to determine the potency of antimicrobial compounds against microorganisms. However, errors and mistakes might have happened throughout the experiment's process, resulting in unreliable outcomes. In this study, 20 microliters and 5 microliters of diluted plant extracts from different plant parts at different concentrations which were 0 mg/ml, 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml were piped on the disks. The findings from this experiment involving different types of parts from *Alpinia javanica* which include leaf (A), pseudostem (B), and rhizome (C) for *Escherichia coli* and *Staphylococcus aureus* showed slight minimum inhibition zones (5 microliter extracts) (Figure 4.4) (Figure 4.5) that cannot be measured meanwhile there were a few extracts that show inhibition zone for samples that used 20 microliters extracts (Figure 4.6) (Figure 4.7) while some of it did not show any presence of inhibition zone. The result indicates a few factors that lead to unreliable outcomes.

Through figures 4.4, 4.5, 4.6, and 4.7, only 10 mg/ml Streptomycin, being used as a positive control in this study, shows a noticeable inhibition zone, while the plant extract from leaf (A), pseudostem (B), and rhizome (C) shows a slight inhibition zone, and some of it did not have an inhibition zone around the disks which indicates to negative outcomes. When a negative

FYP FSB

result occurs in an antimicrobial assay, it may mislead the overall understanding of antimicrobial efficacy with inaccurate and unreliable data, which leads to the inaccurate allocation of resources towards ineffective antimicrobial agents, eventually slowing down the finding of stronger antibiotics and targeting the amount to be taken.

The absence of an inhibition zone around the discs in antimicrobial assays was possible due to several factors. The antimicrobial agent's strength was not always closely related to the area of the inhibition zone (Sipert et al., 2005). The absence of an inhibition zone around the discs in this study could be due to cross-contamination throughout the handling and incubation procedures. Cross-contamination in antimicrobial assays may happen in the absence of a zone of inhibition around the discs, influencing the accuracy of results due to the unintentional transferred of microorganisms or antimicrobial compounds between samples (Filippitzi et al., 2016). Cross-contamination in antimicrobial assays may happen in the absence of an inhibition zone around the discs, influencing the accuracy of results due to the unintentional transferred of microorganisms or antimicrobial compounds between samples (Filippitzi et al., 2016). Contamination during the procedures throughout the experiments could cause misleading results or negative readings, affecting the results and possibly invalidating the whole study. According to Gaugain et al. (2020), Cross-contamination in antimicrobial susceptibility testing can happen through different procedures, which include the transfer of antimicrobial compounds from one sample to another, which might affect the growth of microbes and the formation of

FYP FSB

inhibition zones. Cross-contamination in antimicrobial assays may affect the reliability of results through the introduction of unintended microorganisms or antimicrobial compounds into the samples, possibly resulting in no presence of inhibition zones around the disks and changing the entire interpretation of the antimicrobial activity of the extract from *Alpinia javanica*.

Additionally, the way it was placed of the disk on the agar medium, and the type, size, and thickness of the disks may considerably affect the spread of the antimicrobial agent and the spread of bacteria, thus affecting the outcome's zone of inhibition. The correct placement prevents uneven diffusion or obstacles of the antimicrobial substance, which could lead to unreliable outcomes and having no visible inhibition zones (Balouiri et al., 2016). According to Scorzoni et al. (2007), differences in disk composition or properties could impact the absorption rate and diffusion pattern of the antimicrobial substance, potentially leading to inconsistent or unreliable outcomes. The overall thickness of the disc could also have an impact on the amount of absorption where the thick discs may absorb less plant extract, resulting in no inhibition zone. Standardization of disk properties, which include type, size, and thickness, became essential to ensure the reproducibility and accuracy of antimicrobial assays. (Bauer et al., 1966). Disk selection and handling require extreme care and attention to detail to achieve reliable and accurate results from antimicrobial assays.

Furthermore, Unreliable data in this study was caused by errors during conducting the procedure in the laboratory such as improper agar plate

FYP FSB

inoculation or uneven spread of the bacteria, which could all result in false-negative results in antimicrobial tests. If the agar plate was improperly inoculated, the microorganisms could be spread unevenly across the surface, resulting in variations in microbial growth and potentially influencing the formation of inhibition zones. when the microbial lawn spreads unevenly, the microorganisms' growth patterns would be different. This might have been caused by factors such as uneven streaking of the bacteria culture on the surface of the nutrient agar or using an overheated inoculation loop, which eliminates some of the microbes. When the microbial lawn was not evenly distributed, some areas of the plate could have dense growth while others may have bare growth. This could have a direct effect on how results were interpreted, especially if testing for antibiotic susceptibility and the size of the zone of inhibition is extremely important.

Next, the outcome of this study involving Figure 4.3, Figure 4.4, Figure 4.5, and Figure 4.6 was due to the inconsistencies in disk placement, especially the disk being unable to stay still. If the disk moves or shifts during placement or incubation, it can also interfere with the diffusion of the antimicrobial compound into the agar, resulting in inaccurate results. The movement of the disk could cause uneven zones of inhibition around the disk, making it difficult to determine the bacteria's susceptibility to the antimicrobial compounds in extracts of *A. javanica*. As soon as discs containing antimicrobial compounds move around, the spreading pattern changes, causing the antimicrobial substance to be distributed irregularly in the agar. This irregular distribution could result in inconsistent inhibition

FYP FSB
UNIVERSITI MALAYSIA KELANTAN

zones around the discs, making it difficult to accurately determine zone diameters and interpret bacteria susceptibility to antimicrobial agents (Hoo & Drew, 1974). Disc movement could disturb the growth of clear and well-defined inhibition zones, complicating the interpretation of results and possibly leading to incorrect conclusions about bacterial susceptibility to antimicrobial compounds. If the areas were not well-defined due to the disk movement, it could be difficult to accurately assess the bacteria's susceptibility to the antimicrobial agents.

As a result of the experimental error that occurred throughout the experiment, the value of the standard deviation based on the results (Table 4.1) indicates that some of the values exceeded one, showing that inconsistency and instability during the experiment affected the result. The standard deviation provides information about the consistency or variability of data values within a set of data. If the standard deviation was near one, it indicates low variability due to the data points being close together around the mean while a higher standard deviation shows that data values were further dispersed from the mean. In this experiment, a high standard deviation may be viewed as biased since this experiment needs a low variance for the accuracy of the result especially involved with analyses that were precision-focused. Overall, the result from this study indicates that a standard deviation value over one could be interpreted as biased and inconsistent because of the experimental error that occurred throughout the activities in the laboratory.

FYP FSB
UNIVERSITI
MALAYSIA
KELANTAN

FYP FSB

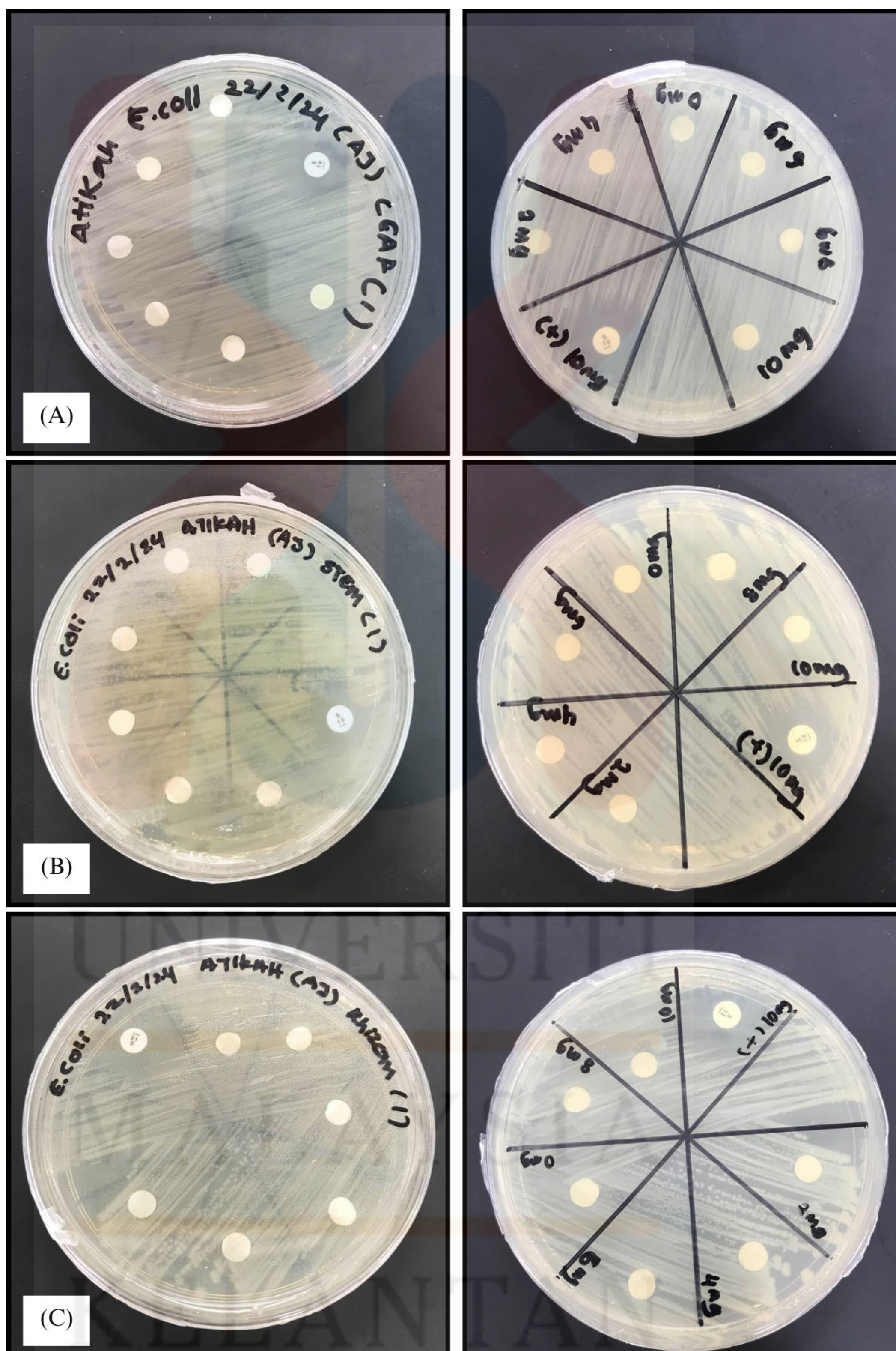


Figure 4.4 Discs containing 5µL extracts of *A. javanica* from leaf (A), pseudostem (B), and rhizome (C) against *E. coli* showed no inhibition zone around the discs containing diluted extracts.

FYP FSB

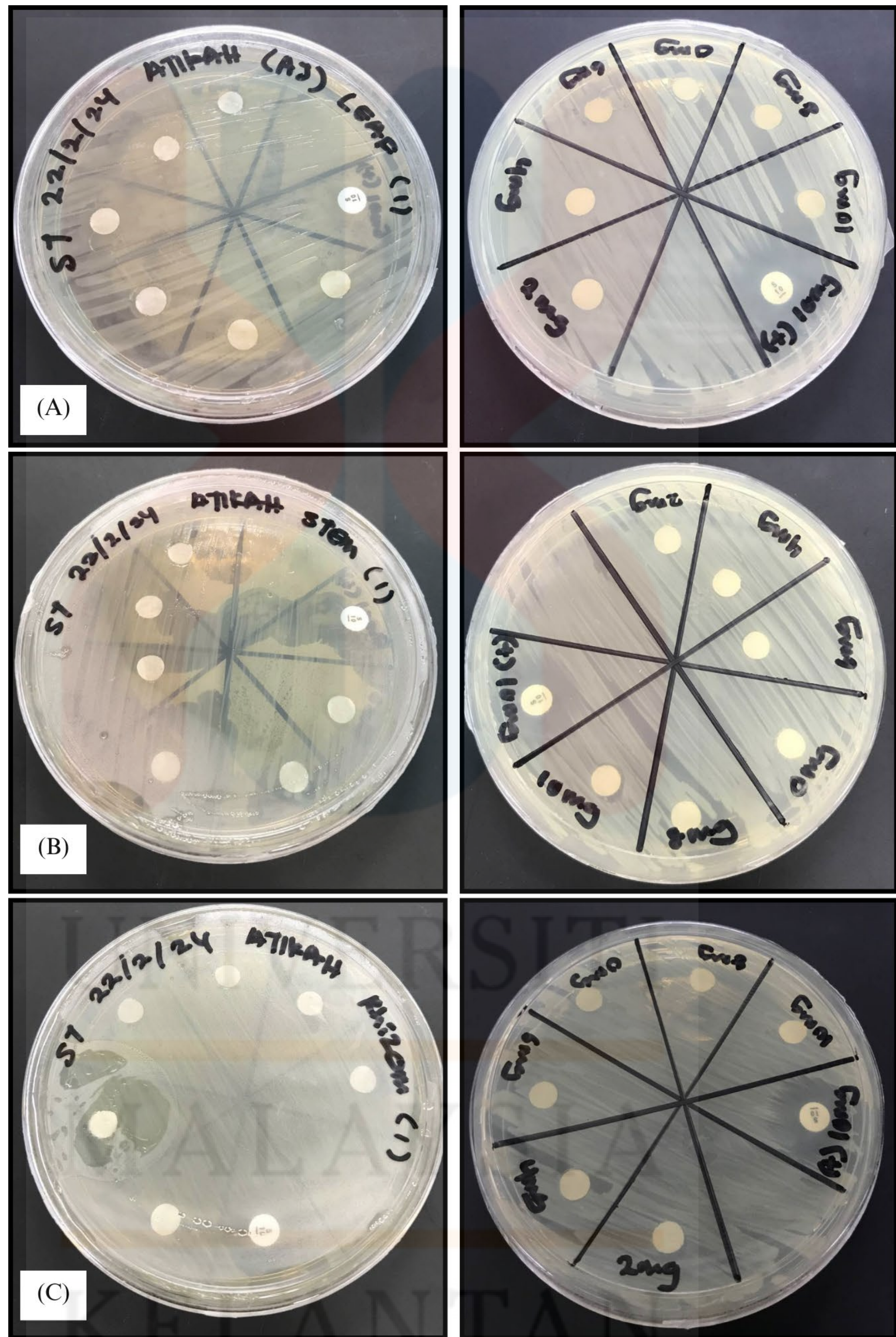


Figure 4.5 Discs containing 5µL extracts of *A. javanica* from leaf (A), pseudostem (B), and rhizome (C) against *S. aureus* showed no inhibition zone around the discs containing diluted extracts.

UNIVERSITI MALAYSIA KELANTAN

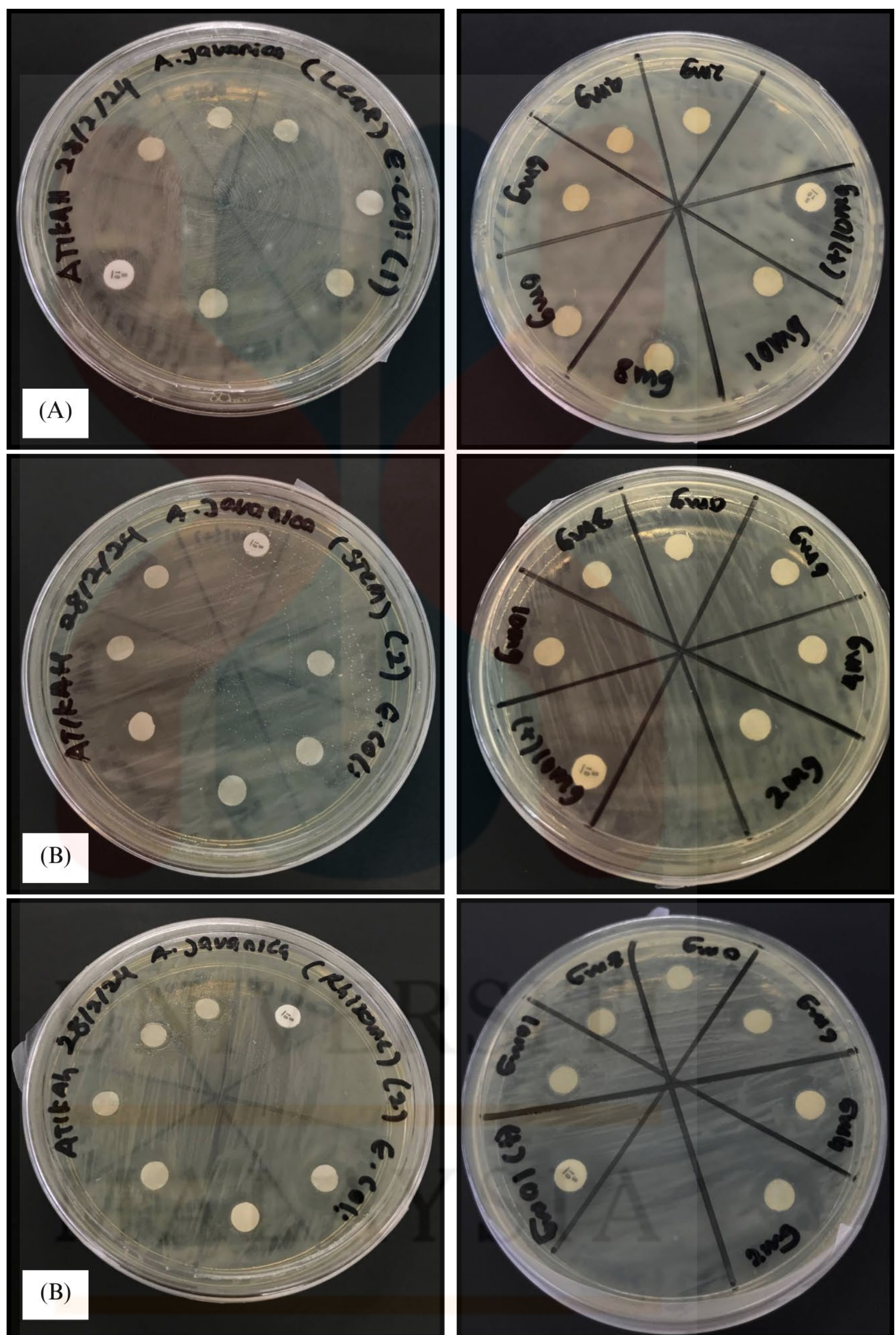


Figure 4.6 Discs containing 20 µL extracts of *A. javanica* from leaf (A), pseudostem (B), and rhizome (C) against *E. coli* showed no inhibition zone around a few discs containing diluted extracts.

FYP FSB

FYP FSB

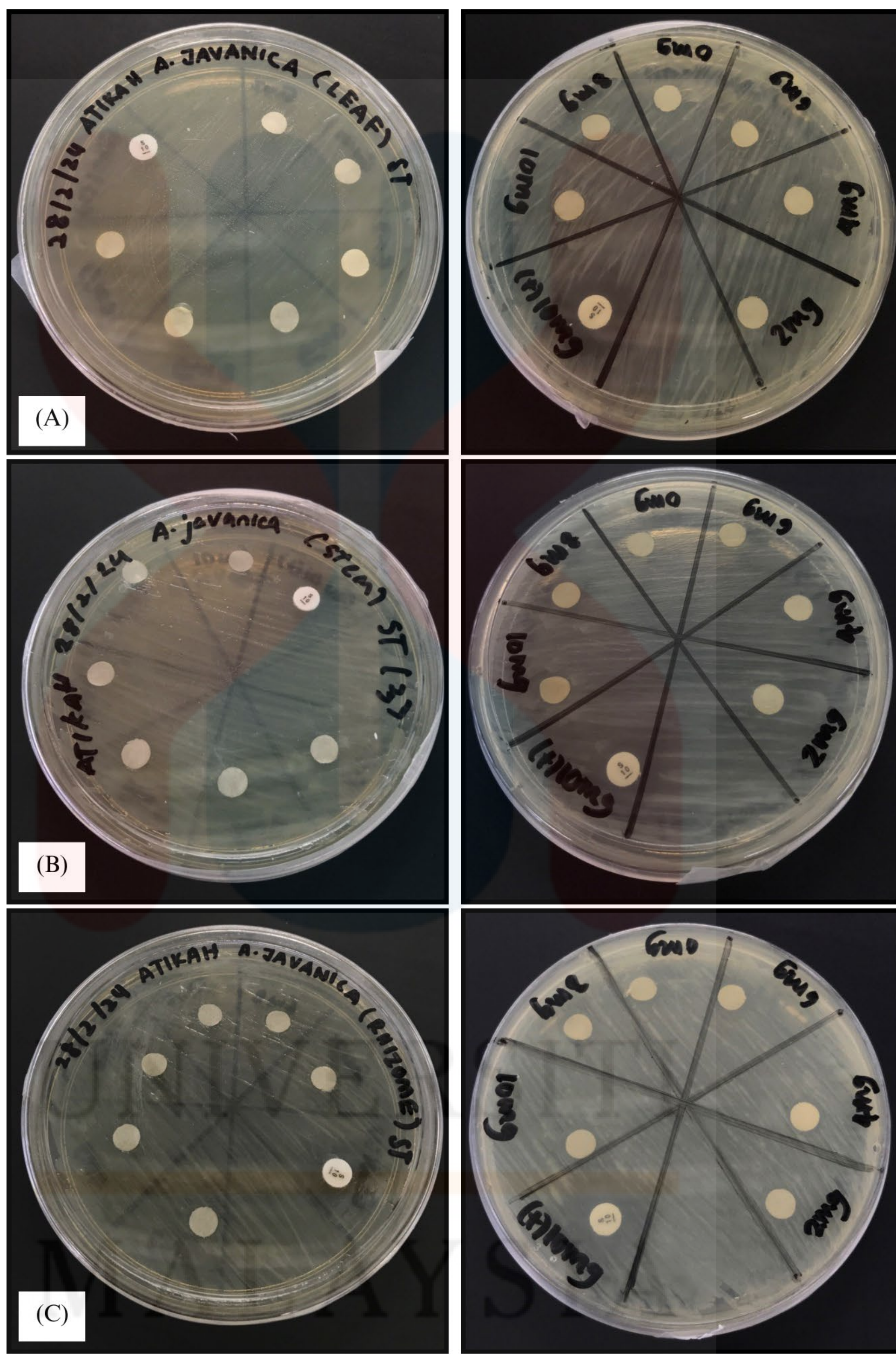


Figure 4.7 Discs containing 20 µL extracts of *A. javanica* from leaf (A), pseudostem (B), and rhizome (C) against *S. aureus* showed no inhibition zone around the discs and some of it cannot be measured.

UNIVERSITI MALAYSIA KELANTAN

FYP FSB

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Based on the findings show that the rhizome extract was more efficient against both bacteria, *Escherichia coli* and *Staphylococcus aureus*. Another part of *Alpinia javanica* also showed the inhibition zone but was not as significant as the rhizome extract. The Minimum Inhibitory Concentration (MIC) against both tested bacteria showed that the concentration of 10 mg/ml of rhizome shows the highest inhibition zone among the two extracts. Among these different parts of *A. javanica*'s extracts, pseudostem appeared minimal inhibition zone compared to the extract of leaf and rhizome. Although there were a few experimental errors throughout the assays, reliable data was obtained from the disk containing 10 microliters of extract of each part. The outcomes reveal that the minimum inhibitory concentration (MIC) of the leaf, pseudostem, and rhizome extracts against *E. coli* and *S. aureus* were at a concentration of 2 mg/mL where, the leaf extract inhibits 7.06 ± 1.13 mm (*E. coli*) and 7.51 ± 0.48 mm (*S. aureus*) average diameter of the zone, pseudostem with 7.98 ± 0.78 mm (*E. coli*) and 8.01 ± 1.05 mm (*S. aureus*), and rhizome with 8.94 ± 0.54 mm (*E. coli*) and 8.71 ± 0.92 mm (*S. aureus*). The findings reveal that the plant extract of *A. javanica* for rhizome extract proved more efficient against *E. coli* and *S. aureus* through a larger inhibition zone of bacteria, while the pseudostem extract had the smallest

inhibition zone compared to leaf and pseudostem. The rhizome extract of 10 mg/mL with an average of diameter zone 11.38 ± 0.29 mm and 8 mg/mL average diameter zone of 11.68 ± 0.56 mm had the largest average diameter zone nearly identical against *E. coli*, while 10 mg/mL had the highest diameter zone against *S. aureus* with a diameter of 11.47 ± 0.03 mm. Although rhizome extract had the largest average diameter of inhibition zone when inhibiting *E. coli* and *S. aureus* compared to leaf and pseudostem extracts, it was not as efficient and strong as Streptomycin to inhibit both tested bacteria and had the same impact on replacing commonly used antibiotics.

5.2 Recommendation

It is recommended that future studies use a variety of microorganism strains in the assays to discover new potential and indicate the study's validity and relevance. It's suggested to use bacteria that are related to the gastrointestinal tract since ginger plants are very well known for their potential to overcome digest problems and are widely used as an alternative to traditional medicine. Deciding on a variety of clinically important pathogens and multidrug-resistant strains allows for an accurate assessment of the plant extract's antimicrobial potential and applicability in combating infectious diseases. This approach allows an in-depth evaluation of the extract's efficacy in inhibiting the growth of various pathogens, leading to a more comprehensive understanding of its antimicrobial properties. The findings of such studies may contribute to the

FYP FSB

UNIVERSITI MALAYSIA KELANTAN

development of novel antimicrobial medications or alternative treatments for infectious diseases, enhancing both medicine and public health.

A further suggestion for future antimicrobial assay studies is to better understand and consider the composition and properties of plant extracts, which are associated with many different kinds of biological activities. These compounds can consist of flavonoids, alkaloids, terpenoids, and phenolic compounds, among others. Different phytochemicals possess various levels of antimicrobial activity, with a few being more effective than others. Understanding how individual phytochemicals in a plant extract react against microorganism targets can provide insights into plant-part mechanisms of action. Finding and analyzing these specific phytochemicals will assist researchers in determining which extract components are most effective.

It recommended exploring different approaches to carrying out antimicrobial assays, such as biofilm inhibition assays and synergy testing, in order to obtain a better understanding of plant extracts' medicinal properties. These methods allow researchers to determine the effectiveness of plant extracts in more accurate infection situations, where biofilm formation and antibiotic resistance are important factors. These methods enable researchers to evaluate the efficacy of plant extracts in more accurate infection scenarios, where biofilm formation and antibiotic resistance are important factors.

FYP FSB
UNIVERSITI MALAYSIA KELANTAN

It is also recommended that all laboratory equipment used in the antimicrobial assay appear maintained properly and the environment sanitized throughout the assay to avoid contamination. Proper equipment maintenance and a clean environment help to ensure that the antimicrobial assay results are valid. Contamination can result in false results, limiting the study's accuracy and reliability.

FYP FSB

UNIVERSITI
MALAYSIA
KELANTAN

REFERENCES

Abdel-Hamid, Nermin S., Abdel-Khalek, Hannan H., Mattar, Z. A., Abou-Taleb, Khadiga A., & Ramadan, El. M. (2017). Screening of Some Egyptian Plant Extracts for Biological Activity Against Some Pathogenic Bacteria. *Arab Universities Journal of Agricultural Sciences*, 25(2), 377–386.

Agung Wibawa Mahatva Yodha, Esti Badia, Musdalipah Musdalipah, Muhammad Azdar Setiawan, Nur Saadah Daud, Angriani Fusvita, Adryan Fristiohady, & Sahidin Sahidin. (2023). Essential Oils of Alpinia monopleura and Their Antibacterial and Antioxidant Activity. *Molekul*, *18*(1), 80–80.

Al Farraj, Dunia. A., Gawwad, Mohamed Ragab Abdel, Adee Mehmood, Ali Alsalme, Noura M. Darwish,Nabil Al-Zaqri, & Ismail Warad. (2020). In-vitro Antimicrobial Activities of Organic Solvent Extracts Obtained from Dipcadi viride (L.) Moench. *Journal of King Saud University - Science*, 32(3), 1965–1968.

Amira M. El Farakh, Ragaa A. Hamouda, Abdel Fatah, Mohamed O., & Saad I. Amar. (2017). Detecting The Anti-Streptococcuc Pyogens Activity of Cyanobacterium (Phanocapsa Species) By Using Plackett-Burman Experimental Design. *Research Journal of Applied Biotechnology*, 3(1), 36–46.

Anisa Lutfia, Erman Munir, Yurnaliza Yurnaliza, & Mohammad Basyuni. (2021). Chemical Analysis and Anticancer Activity of Sesterterpenoid from An Endophytic Fungus Hypomontagnella monticulosa Zg15SU and Its Host Zingiber griffithii Baker. *Heliyon*, 7(2), e06292.

Appalasamy., S., & Arumugam, N. (2020). Four New Records of Zingiberaceae in Gunung Telapak Burok, Berembun Forest Reserve (Fr), Negeri Sembilan. *Internasional Conference on Science and Technology*.

Arullappan, S., Zubaidah Zakaria, & Dayang Fredalina Basri. (2009). Preliminary Screening of Antibacterial Activity Using Crude Extracts of Hibiscus rosa sinensis. *Tropical Life Sciences Research*, 20(2), 109-118.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in Vitro Evaluating Antimicrobial activity: a Review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology*, *45*(4_ts), 493–496.

FYP FSB

FYP FSB

Berwal, A., Chawla, K., Shetty, S., & Gupta, A. (2019). Trend of Antibiotic Susceptibility of Streptococcus pyogenes Isolated from Respiratory Tract Infections In Tertiary Care Hospital In South Karnataka. *Iranian Journal of Microbiology*, 11(1).

Biswas, B. B., Basu, P. S., & Pal, M. K. (1970). *Gram Staining and Its Molecular Mechanism*. International Review of Cytology, 29, 1–27.

Chakrabartty, I., Vijayasekhar, A., & Rangan, L. (2019). Therapeutic Potential of Labdane Diterpene Isolated from Alpinia nigra: Detailed Hemato-Compatibility and Antimicrobial Studies. *Natural Product Research*, 36(6).

Cheung, G. Y. C., Bae, J. S., & Otto, M. (2021). Pathogenicity and Virulence of Staphylococcus aureus. *Virulence*, *12*(1), 547–569.

Chikezie, I. O. (2017). Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Using A Novel Dilution Tube Method. *African Journal of Microbiology Research*, 11(23), 977–980.

Coico, R. (2006). *Gram staining*. Current protocols in microbiology, (1), A-3C

Datar, R., Orenga, S., Pogorelcnik, R., Rochas, O., Simner, P. J., & Belkum, A. van. (2021). Recent Advances in Rapid Antimicrobial Susceptibility Testing. *Clinical Chemistry*, 68(1), 91–98.

Deo, S. (2017). *World of Triplicates, Statistics and Research*. Research Gate. Retrieved March 24, 2024, from https://conceptpharma.wordpress.com/2017/06/21/world

Dhakad, G. G., Patil, R. V., Girase, D. S., Amrutkar, S. P., & Jain, R. S. (2022). Review on Antibiotics. *International Research Journal of Pharmacy*, 15(1), 91–96.

Filippitzi, M. E., Sarrazin, S., Imberechts, H., Smet, A., & Dewulf, J. (2016). Risk of Cross-Contamination Due to The Use of Antimicrobial Medicated Feed Throughout The Trail of Feed From The Feed Mill To The Farm. *Food Additives & Contaminants: Part A*, *33*(4), 1–12.

Gaugain, M., Fourmond, M.-P., Fuselier, R., Verdon, E., Roudaut, B., & Pessel, D. (2020). Control of Antimicrobials in Feed Using Liquid Chromatography-Tandem Mass Spectrometry: Assessment of Cross-Contamination Rates at the Farm Level. *Journal of Agricultural and Food Chemistry*, 9033–9042.

Geurtsen, J., Been, M. D., Weerdenburg, E., Zomer, A., McNally, A., & Poolman, J. (2022). Genomics and Pathotypes of the Many Faces of Escherichia coli. *FEMS Microbiology Reviews*, *46*(6).

Goldman, E., & Green, L. H. (2015). *Practical Handbook of Microbiology*. In Google Books (Third Edition). Taylor & Francis Group.

Gonçalves, M. D., Bortoleti, B. T. da S., Tomiotto-Pellissier, F., Concato, V. M., Matos, R. L. N. de, Silva, T. F., Rodrigues, A. C. J., Carlos, A. C. M., Costa, I. N., Lazarin-Bidóia, D., Miranda-Sapla, M. M., Pavanelli, W. R., Arakawa, N. S., & Conchon-Costa, I. (2021). Grandiflorenic Acid Isolated from Sphagneticola trilobata Against Trypanosoma cruzi: Toxicity, Mechanisms of Action and Immunomodulation. *Toxicology in Vitro*, 78, 105267–105267.

Golam Kader, Farjana Nikkon, Mohammad Abdur Rashid, & Tanzima Yeasmin. (2011). Antimicrobial Activities of The Rhizome Extract of Zingiber zerumbet Linn. *Asian Pacific Journal of Tropical Biomedicine*, *1*(5), 409–412. https://doi.org/10.1016/s2221-1691(11)60090-7

Golla, R. M., Mishra, B., Dang, X., Lakshmaiah Narayana, J., Li, A., Xu, L., & Wang, G. (2020). Resistome of Staphylococcus aureus in Response to Human Cathelicidin LL-37 and Its Engineered Antimicrobial Peptides. *ACS Infectious Diseases*, 1866–1881.

Haag, A. F., Fitzgerald, J. R., & Penadés, J. R. (2019). Staphylococcus aureus in Animals. *Microbiology Spectrum*, *7*(3).

Hasnah M. Sirat, Dian Masri, & Ahmad A. Rahman. (1994). The Distribution of Labdane Diterpenes in The Zingiberaceae of Malaysia. *Phytochemistry*, *36*(3), 699–701.

Hassanal Fikri Abdul Gani. (2023). *Screening of Bioactive Compounds of Alpinia Javanica (Family Zingiberaceae) Using Liquid Chromatography*. Final Year Project Unpublish. Universiti Malaysia Kelantan.

Hoo, R., & Drew, W. L. (1974). Potential Unreliability of Nitrofurantoin Disks in Susceptibility Testing. *Antimicrobial Agents and Chemotherapy*, *5*(6), 607–610.

Huong, L. T., Huong, T. T., Bich, N. T., Viet, N. T., Abdulatif Olufemi Giwa-Ajeniya, & Ogunwande, I. (2020). Antimicrobial Efficacy and Chemical Constituents of Pseudo-stem Essential Oils from Zingiber castaneum. *Trends in Phytochemical Research*, 4(2), 93.

Ide, M., Yoshida, I., Kumagai, M., Mishima, T., Takahashi, Y., Fujita, K., Igarashi, T., & Matsuura, E. (2020). Tithonia Diversifolia -Derived Orizabin Suppresses Cell Adhesion, Differentiation, and Oxidized LDL Accumulation by Akt Signaling Suppression Via PTEN Promotion in THP-1 Cells. *Journal of Food Biochemistry*, 44(7).

Irmanida Batubara, Dian Yunita, & Irma Herawati Suparto. (2019). Antibacterial and Biofilm Degradation Activity of Extract from Steam Distillation Residue of Zingiberaceae Leaves Against Streptococcus mutans. *Journal of the Indonesian Chemical Society*, *2*(1).

FYP FSB

FYP FSB

Iqbal Nisa, Muhammad Qasim, Nusrat Yasin, Rafi Ullah, & Anwar Ali. (2020). Shigella flexneri: An Emerging Pathogen. *Folia Microbiol*, 65, pages275-291.

Jespersen, M. G., Lacey, J. A., Tong, S. Y. C., & Davies, M. R. (2020). Global Genomic Epidemiology of Streptococcus pyogenes. *Infection, Genetics and Evolution,* 86, 104609–104609.

Julius, A. (2011). *Alpinia javanica*. Flora of Peninsular Malaysia Online, 76(8). Retrieved February 2, 2024, from https://www.researchgate.net/publication/262181751_Alpinia_javanica

Karunarathne, Thammitiyagodage, & Weerakkody. (2020). Antibiofilm Activity of Galangal (Alpinia galanga) Against Staphylococcus aureus. *Food Processing & Nutritional Science*, 1(2), 123–131.

Khusnul, Suhartati, R., Virgianti, D., Fathurohman, M., & Pratita, A. (2018). Effect of Karuk Leaves (Piper Sarmentosum Roxb) and White Galangal Rhizome (Alpinia Galanga L) Ethanol Extract on the Growth of Microsporum gypseum and Candida Albicans in Vitro. *Journal of Physics: Conference Series*, 1179, 012168.

Kochuthressia, K., Britto, S., Jaseentha, M., Raj, L., & Senthilkumar, S. (2010). Antimicrobial Efficacy of Extracts from Alpinia purpurata (Vieill.) K.Schum. Against Human Pathogenic Bacteria and Fungi. *Agriculture and Biology Journal of North America*, *1*(6), 1249–1252.

Kress, W. J., & Holttum, R. E. (2020). *Zingiberales*. Encyclopedia Britannica. Retrieved April 20, 2024, from https://www.britannica.com/plant/Zingiberales

Kress, W. J. (1990). *The Phylogeny and Classification of Zingiberanae*. Ann. Mo. Bot.Gard. 77:698721.

Li, D., Zhu, G., Xu, Y.-C., Ye, Y.-J., & Liu, J. (2020). Complete Chloroplast Genomes of Three Medicinal Alpinia Species: Genome Organization, Comparative Analyses and Phylogenetic Relationships in Family Zingiberaceae. *Plants*, 9(2), 286–286.

Laveena, K. B., & Chandra, M. (2020). International Journal of Advanced Research. *International Journal of Advanced Research.*

Lara, A., Santos, I. C. dos, Soares, A. A., Otutumi, L. K., Jacomassi, E., Lovato, E. C. W., Gazim, Z. C., Rahal, I. L., Oliva, L. R., Gonçalves, J. E., Barbosa, L. N., & Gonçalves, D. D. (2021). Composition and Antimicrobial Activity of Ginger (Zingiber officinale Roscoe). *Australian Journal of Crop Science*, *15*(06), 882–889.

FYP FSB

Larsen, K., Lock, J. M., Maas, H., & Maas, P. J. M. (1998). Zingiberaceae. In K. Kubitzki (Ed.), *Flowering Plants · Monocotyledons. The Families and Genera of Vascular Plants* (Vol. 4, pp. 474–495). Springer, Berlin, Heidelberg.

Leonard, H., Colodner, R., Halachmi, S., & Segal, E. (2018). Recent Advances in the Race to Design a Rapid Diagnostic Test for Antimicrobial Resistance. *ACS Sensors*, 3(11), 2202–2217.

Le T. Huong, Hoang V. Chinh, Do. N. Dai, Olanrewaju I. Eresanya, & Isiaka A. Ogunwande. (2020). *Antimicrobial Activity and Constituents of Leaf and Pseudo-Stem Essential Oils of Zingiber zerumbet*.

Liu, H., Zhu, B., Qiu, S., Xia, Y., Liang, B., Yang, C., Dong, N., Li, Y., Xiang, Y., Wang, S., Xie, J., Mahe, M., Sun, Y., & Song, H. (2018). Dominant Serotype Distribution and Antimicrobial Resistance Profile of Shigella spp. in Xinjiang, China. *Plos One*, 13(4)

López-Bascón, M. A., & Luque de Castro, M. D. (2020). Soxhlet Extraction. In C. F. Poole (Ed.), *Liquid-Phase Extraction* (pp. 327–354). United Kingdom: Susan Dennis.

MacFarland, T. W. (2012). *Two-Way Analysis of Variance: Statistical Tests and Graphics Using R. In Google Books.* Springer Science & Business Media.

Mabberley, D. J. (1997). *The plant-book*. Cambridge University Press.

Mahdavi, B., W.A., Y., & Laily B. Din. (2017). Antioxidant and Antimicrobial Activity of the Extracts from Different Parts of Etlingera sayapensis (Zingiberaceae). *Sains Malaysiana*, *46*(9), 1565–1571.

Morrison, L., & Zembower, T. R. (2020). *Antimicrobial resistance*. Gastrointestinal Endoscopy Clinics, 30(4), 619-635.

Momoh, J. O., & Olaleye, O. N. (2022). Evaluation of Secondary Metabolites Profiling of Ginger (Zingiber officinale Roscoe) Rhizome using GC-MS and Its Antibacterial Potential on Staphylococcus aureus and Escherichia coli. *Microbiology Research Journal International*, 32(7), 7–31.

Mostafa, N. M. (2018). Antibacterial Activity of Ginger (Zingiber officinale) Leaves Essential Oil Nanoemulsion against the Cariogenic Streptococcus mutans. *Journal of Applied Pharmaceutical Science*, *8*(9), 34–41.

Nandu, T. G., Ganapathy, A. S., Sugathan, S., Viszwapriya, D., Iyer, P. M., Balamurugan, K., Rameshkumar, K. B., & Pandian, S. K. (2018). Fukugiside, a biflavonoid from Garcinia travancorica Inhibits Biofilm Formation of Streptococcus pyogenes and

Its Associated Virulence Factors. *Journal of Medical Microbiology*, 67(9), 1391–1401.

Nostro, A., Germano, M. P., D'Angelo, V., Marino, A., & Cannatelli, M. A. (2000). Extraction Methods and Bioautography for Evaluation of Medicinal Plant Antimicrobial Activity. *Letters in Applied Microbiology*, *30*(5), 379–384.

Ni Nyoman Rupiasih, Gusti Ayu Putu Cyntia Dewi, I Wayan Supardi, & I Ketut Putra. (2019). The Potency of Biologically Synthesized Silver Nanoparticles Using Andrographis Paniculata Extract (S-AgNp) as an Antibacterial Agent. *IOP Conference Series: Materials Science and Engineering*.

Paulsen, J., Yahyazadeh, M., Hänsel, S., Kleinwächter, M., Ibrom, K., & Selmar, D. (2015). 13,14-Dihydrocoptisine: The Genuine Alkaloid from Chelidonium majus. *Phytochemistry*, 111(111), 149–153.

Prince, L. M., & Kress, W. J. (2002). *Zingiberales (gingers and bananas)*. Encyclopedia of Life Sciences. Macmillan Publisher.

Puteri I.A.M. Mahmud, W.A.Yaacob, & Nazlina Ibrahim. (2017). Antibacterial Activity of Alkaloid Extracts from Ochrosia oppositifolia. *Sains Malaysiana*, *46*(8), 1279–1284.

Ramos, S., Silva, V., Dapkevicius, M. de L. E., Caniça, M., Tejedor-Junco, M. T., Igrejas, G., & Poeta, P. (2020). Escherichia coli as Commensal and Pathogenic Bacteria Among Food-Producing Animals: Health Implications of Extended Spectrum β-lactamase (ESBL) Production. *Animals: An Open Access Journal from MDPI*, *10*(12).

Rasha Saad Suliman, Eddy Yusuf, Norfazrin Mohd Hanif, & Fadli Asmani. (2012). Comparative Studies of Zingiber Officinale Leaves and Rhizomes on The Antibacterial Effect. *Internasional Journal of Pharmacy and Analytical Research*, 3(3).

Rayfiqa Maulidah, Suci Erta Fitri, Nurainas, Syamsuardi, & Dayar Arbain. (2019). Two New Records of Alpinia Roxb. (Zingiberaceae) In Sumatra, Indonesia And Phylogenetic Relationship To Their Allied Species. *The Journal of Biodiversity Data,* 15(1), 109–117.

Scorzoni, L., Benaducci, T., Almeida, A. M. F., Silva, D. H. S., Bolzani, V. d. S., & Gianinni, M. J. S. M. (2007). The Use of Standard Methodology for Determination of Antifungal Activity of Natural Products Against Medical Yeasts Candida Sp. and Cryptococcus Sp. *Brazilian Journal of Microbiology*, 38(3), 391-397.

FYP FSB

Sellera, F. P., Fernandes, M. R., Moura, Q., Carvalho, M. P. N., & Lincopan, N. (2018). Extended-spectrum-β-lactamase (CTX-M)-Producing Escherichia coli in Wild Fishes from A Polluted Area in The Atlantic Coast of South America. *Marine Pollution Bulletin*, *135*, 183–186.

Shekwa, W., Maliehe, T. S., & Masoko, P. (2023). Antimicrobial, Antioxidant and Cytotoxic Activities of The Leaf and Stem Extracts of Carissa bispinosa Used for Dental Health Care. *BMC Complementary Medicine and Therapies*, *23*(462).

Sipert, C. R., Hussne, R. P., Nishiyama, C. K., & Torres, S. A. (2005). In vitro antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez. *International Endodontic Journal*, *38*(8), 539–543.

Siti Nur Izaty Che Humaidi1, Nur Shazieyatul Nadiah Shalan, Mohamad Nurul Azmi Mohamad Taib, Al-Shammary, Aemen Ali Kudayr, Nursyazwani Anuar, Khalijah Awang, & Khayriyyah Mohd Hanafiah. (2020). Antimicrobial and Drug-Synergistic Potential of Alpinia conchigera Griff. -Derived Phenylpropanoids Against Mycobacterium smegmatis. *Malaysian Journal of Microbiology*, 16(6).

Singh, N., Gupta, S., & Rathore, V. (2017). Comparative Antimicrobial Study of Ethanolic Extract of Leaf and Rhizome of Curcuma longa Linn. *Pharmacognosy Journal*, *9*(2), 208–212.

Serwecińska, L. (2020). Antimicrobials and Antibiotic-Resistant Bacteria: A Risk to the Environment and to Public Health. *Water*, *12*(12), 3313.

Shannon, B. A., McCormick, J. K., & Schlievert, P. M. (2019). Toxins and Superantigens of Group A Streptococci. *Microbiology Spectrum*, *7*(1).

Shen, Y., Qian, H., Gong, J., Deng, F., Dong, C., Lu, Z., … & Guo, H. (2013). High Prevalence of Antibiotic Resistance and Molecular Characterization of Integrons Among Shigella Isolates in Eastern China. *Antimicrobial Agents and Chemotherapy*, 57(3), 1549-1551.

Sizar, O., Unakal, C. G., & Leslie, S. W. (2023, February 14). *Gram Positive Bacteria*. Nationa Library of Medicine; StatPearls Publishing LLC.

Taylor, S. L., Leong, L. E. X., Mobegi, F. M., Choo, J. M., Burr, L. D., Wesselingh, S., & Rogers, G. B. (2018). Understanding The Impact of Antibiotic Therapies On The Respiratory Tract Resistome: A Novel Pooled-Template Metagenomic Sequencing Strategy. *Multidisciplinary Respiratory Medicine*, *13*(Supplement 1).

Testbook Edu Solutions. (2023). *Streak Plate Technique: Principle, Types, Procedure and Advantages*. Testbook.

FYP FSB

FYP FSB

Tian, Y., Jia, X., Wang, Q., Lu, T., Deng, G., Tian, M., & Zhou, Y. (2022). Antioxidant, Antibacterial, Enzyme Inhibitory, and Anticancer Activities and Chemical Composition of Alpinia galanga Flower Essential Oil. *Pharmaceuticals*, 15(9), 1069.

Tongwanichniyom, S., Phewrat, N., Rangsarikorn, N., Leasen, S., Luangkamin, S., & Chumnanvej, N. (2024). Green Synthesis of Silver Nanoparticles Using Mature-Pseudostem Extracts of Alpinia nigra and Their Bioactivities. *Green Processing and Synthesis*, *13*(1).

Tomlinson, P. B. (1956). Studies in the Systematic Anatomy of Zingiberaceae. *Botanical Journal of the Linnean Society*, 55(361), 547-592.

Usharani S. (2023). Isolation, Identification, and Bioactivity Evaluation of Novel Flavonoids from Alpinia Officinarum. *International Journal for Multidisciplinary Research*, *5*(3).

Van, H. T., Thang, T. D., Luu, T. N., & Doan, V. D. (2021). An Overview of The Chemical Composition and Biological Activities of Essential Oils from Alpinia Genus (Zingiberaceae). *Royal Society of Chemistry Advances*, 11, 37767–37783. https://doi.org/10.1039/d1ra07370b

Xu, Z., & Chang, L. (2017). Zingiberaceae. *Identification and Control of Common Weeds: Volume 3*, *3*, 909–911.

Yasin, P., Wanachantararuk, P., Jidaphatinoi, J., & Dumri, K. (2019). Antimicrobial Activity of Some Thai Aromatic Plants Against Oral Pathogens Inducing Halitosis. *Asian Journal of Pharmaceutical and Clinical Research*, *12*(1).

Zaghen, F., Sora, V. M., Meroni, G., Laterza, G., Martino, P. A., Soggiu, A., Bonizzi, L., & Zecconi, A. (2023). Epidemiology of Antimicrobial Resistance Genes in Staphyloccocus aureus Isolates from a Public Database in a One Health Perspective—Sample Characteristics and Isolates' Sources. *Antibiotics*, *12*(7), 1225–1225.

Zhu, Z., Zhou, X., Li, B., Wang, S., Cheng, F., & Zhang, J. (2018). Genomic Analysis and Resistance Mechanisms in Shigella flexneri2a Strain 301. *Microbial Drug Resistance*, *24*(3), 323–336.

APPENDICES

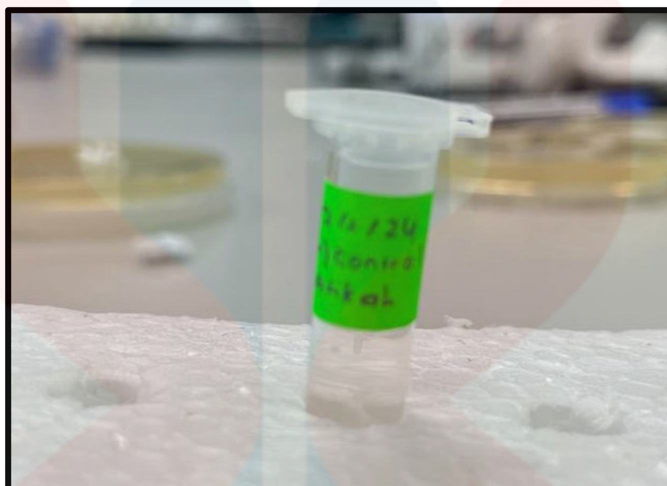
Appendix A The Diameter of The Inhibition Zone of Tested Bacteria on Different Parts of *Alpinia javanica's* Extracts.

		The Inhibition Zone of Bacteria (mm)			
Plant Parts	Concentration (mg/mL)	*E. coli*		*S. aureus*	
		First Replicate	Second Replicate	First Replicate	Second Replicate
Leaf	0	0.00	0.00	0.00	0.00
	2	7.86	6.26	7.17	7.85
	4	6.21	7.12	7.36	9.92
	6	7.87	8.40	8.97	9.07
	8	9.86	9.34	9.45	9.97
	10	8.98	8.59	9.21	8.74
	10 (Streptomycin)	17.08	15.46	14.88	14.54
Pseudostem	0	0.00	0.00	0.00	0.00
	2	7.43	8.53	7.27	8.75
	4	7.54	8.13	7.38	8.09
	6	6.75	6.83	8.66	8.52
	8	7.31	7.73	7.53	7.42
	10	6.78	7.15	7.90	7.29
	10 (Streptomycin)	16.26	14.53	15.07	12.12
Rhizome	0	0.00	0.00	0.00	0.00
	2	8.56	9.32	9.36	8.06
	4	9.91	9.53	10.37	9.18
	6	9.45	9.83	9.16	9.18
	8	11.28	12.07	9.94	10.58
	10	11.58	11.17	11.45	11.49
	10 (Streptomycin)	15.84	15.53	17.88	15.14

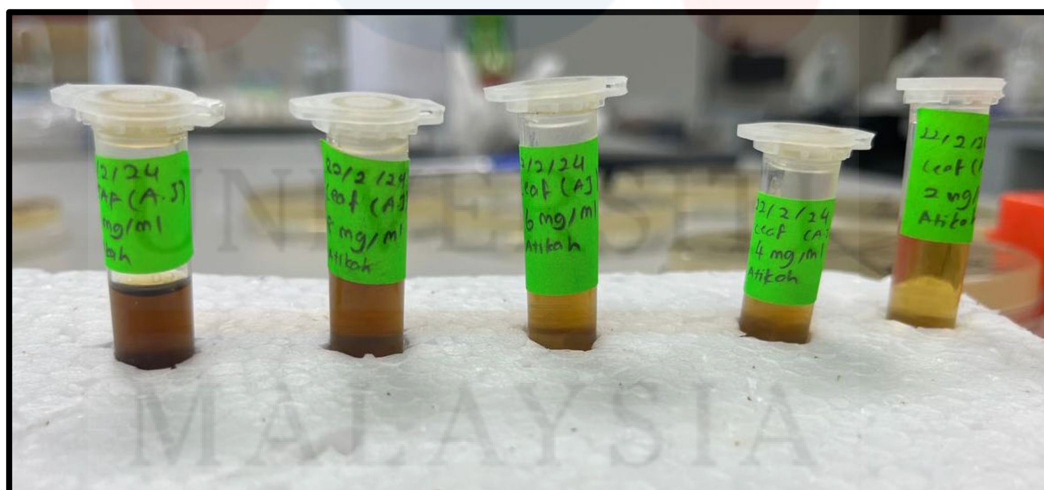
FYP FSB
UNIVERSITI MALAYSIA KELANTAN

FYP FSB

Appendix B The Concentration of 0 mg/mL Consists of 100% Methanol Works as a Negative Control



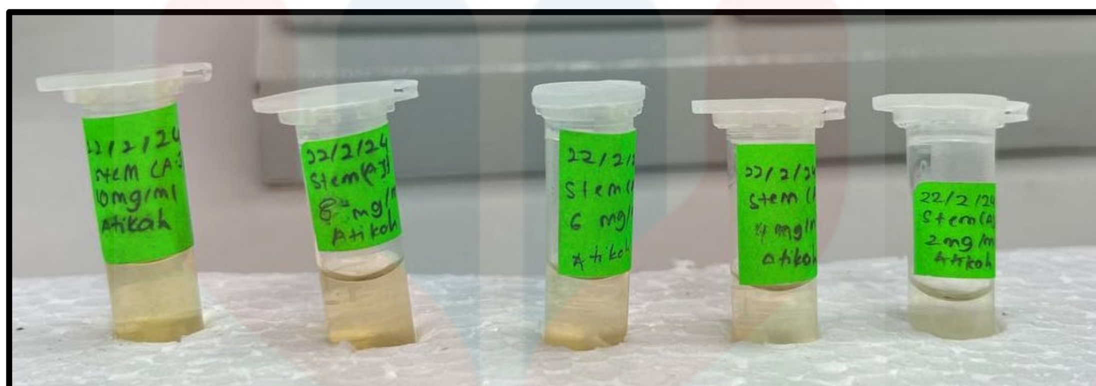
Appendix C The dilution of leaf extract at different concentrations started from 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL.



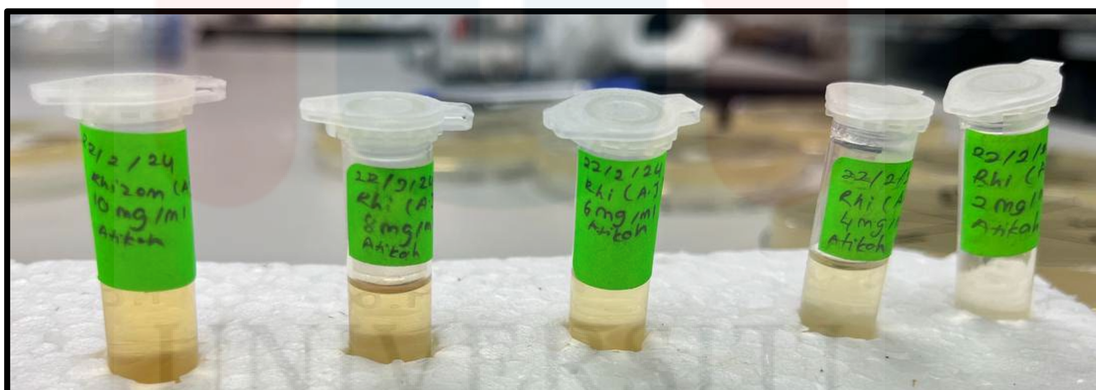
UNIVERSITI MALAYSIA KELANTAN

FYP FSB

Appendix D The Dilution of Pseudostem Extract at Different Concentrations From 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL.



Appendix E The Dilution of Rhizome Extract at Different Concentrations From 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL.



UNIVERSITI
MALAYSIA
KELANTAN