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**ANTIMICROBIAL ACTIVITIES OF EXTRACT
AND COMPOUNDS ISOLATED FROM
Kaempferia parviflora AND *Curcuma caesia***

By

IRMI FARHANA BINTI NAZARUDIN

A research submitted in fulfilment of the requirements for the Degree of
Bachelor of
Applied Science (Natural Resources Science) with Honours

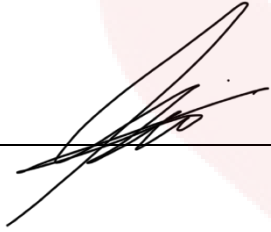
**FACULTY OF EARTH SCIENCE
UNIVERSITI MALAYSIA KELANTAN**

2024

Declaration.

I declare that this thesis entitled "Antimicrobial Activity of Extracts and Compounds Isolated from *Kaempferia parviflora* and *Curcuma caesia*" is the record of work carried out by me during the course of my research. This thesis has been neither submitted for a degree nor is being submitted for a degree or another qualification in any University or other institution of learning.

Signature: _____



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Date: 7.8.2024

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**ANTIMICROBIAL ACTIVITIES OF EXTRACT AND
COMPOUNDS ISOLATED FROM *Kaempferia parviflora* AND
*Curcuma caesia***

ABSTRACT

This study aimed to evaluate the efficacy of herbal remedies against bacterial pathogens such as *Escherichia coli* and *Staphylococcus aureus*. *Kaempferia parviflora* and *Curcuma caesia*, commonly known as 'Black Ginger' and 'Black Turmeric,' respectively, belong to the Zingiberaceae family and have been reported to possess antibacterial and therapeutic properties. The Minimum Inhibitory Concentration (MIC) of *K. parviflora* rhizome extract was determined to be 8 mg/ml, resulting in growth inhibition of both *E. coli* and *S. aureus*, with inhibition zones of 10.64 ± 0.453 mm and 11.46 ± 0.200 mm, respectively. Similarly, for *C. caesia* rhizome extract, the MIC was found to be 2 mg/ml, with inhibition zones of 11.37 ± 0.100 mm for *E. coli* and 11.38 ± 0.134 mm for *S. aureus*. The results indicated that lower concentrations of the extracts were effective and statistically significant compared to higher concentrations. This study underscores the importance of *K. parviflora* and *C. caesia* as antibacterial agents in both traditional and modern therapeutic applications

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**AKTIVITI ANTIMIKROB EKSTRAK DAN SEBATIAN YANG
DIASINGKAN DARIPADA *Kaempferia parviflora* DAN *Curcuma
caesia***

ABSTRAK

Kajian ini bertujuan untuk menilai keberkesanan ubat herba terhadap patogen bakteria seperti *Escherichia coli* dan *Staphylococcus aureus*. *Kaempferia parviflora* dan *Curcuma caesia*, yang biasanya dikenali sebagai 'Halia Hitam' dan 'Kunyit Hitam', masing-masing tergolong dalam keluarga Zingiberaceae dan telah dilaporkan mempunyai sifat antibakteria dan terapeutik. Kepekatan Rencana Minimum (MIC) ekstrak rizom *K. parviflora* ditentukan sebagai 8 mg/ml, menyebabkan perencatan pertumbuhan kedua-dua *E. coli* dan *S. aureus*, dengan zon perencatan masing-masing 10.64 ± 0.453 mm dan 11.46 ± 0.200 mm. Begitu juga, untuk ekstrak rizom *C. caesia*, MIC didapati 2 mg/ml, dengan zon perencatan 11.37 ± 0.100 mm untuk *E. coli* dan 11.38 ± 0.134 mm untuk *S. aureus*. Keputusan menunjukkan bahawa kepekatan ekstrak yang lebih rendah adalah berkesan dan signifikan secara statistik berbanding dengan kepekatan yang lebih tinggi. Kajian ini menggariskan kepentingan *K. parviflora* dan *C. caesia* sebagai agen antibakteria dalam kedua-dua aplikasi terapeutik tradisional dan moden.

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TABLE OF CONTENT

	PAGE
ACKNOWLEDGEMENTS	i
ABSTRACT	ii
ABSTRAK	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF SYMBOLS	viii
CHAPTER 1 INTRODUCTION	
1.1 Background of Study	1-3
1.2 Problem Statement	3-4
1.3 Expected Outcome	4-5
1.4 Objective	5
1.5 Scope of Study	5
1.6 Significant of Study	6
CHAPTER 2 LITERATURE REVIEW	
2.1 <i>K. parviflora</i> Description	7
2.1.1 Taxonomy of <i>K. parviflora</i>	7-8
2.2 <i>C. caesia</i> Description	8
2.2.1 Taxonomy of <i>C. caesia</i>	8-11
2.3 Morphology	12-16
2.4 Distribution	16-17
CHAPTER 3 MATERIAL AND METHOD	
3.1 Preparation of Stock solution <i>K. parviflora</i> and <i>C. caesia</i>	18-21
3.2 Preparation of Nutrient Agar	22
3.3 Preparation of Stock Culture of Bacteria	22-23
3.4 Preparation of Plant Extraction	24
3.4.1 Disc diffusion assay to evaluate the efficiency of antimicrobial agents	24
3.5 Determination of Bacteria Growth, Inhibition Zone, and Antimicrobial Activity of <i>K. parviflora</i> and <i>C. caesia</i> at Different Concentrations	24

3.5.1 Antimicrobial Assay	24-26
3.5.2 Measurement of Inhibition Zone	27-28
3.6 Statistical Analysis	28
CHAPTER 4 RESULT AND DISCUSSION	
4.1 Microbial Growth of <i>Escherichia coli</i> and <i>Staphylococcus aureus</i>	29-31
4.2 Preparation the Extract of <i>K. parviflora</i> and <i>C. caesia</i>	31-34
4.3 Antimicrobial Activity of <i>K. parviflora</i> and <i>C. caesia</i>	34-40
4.4 Determination of Minimum Inhibition (MIC) of <i>K. parviflora</i> and <i>C. caesia</i>	40
CHAPTER 5 CONCLUSION AND RECOMMENDATION	
5.1 Conclusion	41
5.2 Recommendation	42
6.0 REFERENCES	43-52

LIST OF TABLES

Table		Pages
2.1	Morphological characteristics of <i>K. parviflora</i> sample collected in southern Thailand	13
2.2	Morphological characteristics of <i>C. caesia</i> sample that was collected in southern India	15
4.1	Characteristics of the extract of <i>K. parviflora</i> and <i>C. caesia</i>	24
4.2	The difference in the width of the inhibition zone of the liquid <i>K. parviflora</i> extract	34
4.3	The difference in the width of the inhibition zone of the liquid <i>C. caesia</i> extract	35

LIST OF FIGURES

Figure		Page
2.1	Phylogenetic tree of family Zingiberaceae	10
2.2	Distribution <i>K. parviflora</i> at Thailand and Vietnam as shown in red box	11
2.3	Distribution <i>C. caesia</i> at India and Bangladesh as shown in red box	11
2.4	Comparison of <i>K. parviflora</i> with other species	14
2.5	<i>K. parviflora</i> rhizomes	14
2.6	Comparison of <i>C. Caesia</i> with other species	15
3.1	The continuous streak method for microorganisms culture	19
3.2	The presence of the inhibition zone around the disk	21
3.3	Digital Vernier Caliper	22
3.4	Measurements for the normalized width of the inhibition zone	22
4.1	Depicts the microorganisms contained in the universal bottle	28
4.2	Illustrates the growth of bacteria on an agar plate	28
4.3	The extract <i>K. parviflora</i> that has been mixed into a Microcentrifuge tube	30
4.4	The extract <i>C. caesia</i> that has been mixed into a Microcentrifuge tube	31
4.5	1mL methanol into a microcentrifuge tube.	31
4.6	Disk diffusion plate of <i>K. parviflora</i> and <i>C. caesia</i> deference Fluid extract against	33
4.7	Disk diffusion plate of <i>K. parviflora</i>	36
4.8	Disk diffusion plate of <i>C. caesia</i>	36

LIST OF SYMBOLS

No.	Symbol	Meaning
1.	mm	Milimetre
2.	μ	Micrometre
3.	ml	Millilitre
4.	l	Liter
5.	$^{\circ}\text{C}$	Degree Celsius
6.	cm	Centimetre
7.	m	Meter
8.	g	Gram



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1.0 INTRODUCTION

1.1 Background of Study

Kaempferia parviflora Wall. Ex Baker and *Curcuma caesia* Roxb. (William Roxburgh,) are two plants in the Zingiberaceae family that are known as "black ginger" (Toda k, 2017), "halia hitam" (Jalil m.a, 2018) and "black turmeric" (Bohra et al., 2021), "kunyit hitam" (Aznm, 2023). Zingiberales is a family with roughly 56 genera and about 1,300 species (Simpson, 2019). This is a kind of herbal plant endemic to Southeast Asia, particularly Thailand and Malaysia. For the ginger species, which includes *K. rubromarginata* (S.Q.Tong), R.J.Searle and *Stahlianthus rubromarginatus*. *C. ceratotheca*, *C. larsenii*, *C. parvula*, *C. yunnanensis*, *C. euchroma*, *C. neilgherrensis*, and *C. aeruginosa* are the name species of turmeric (Burapan et al., 2020).

This medicinal herb is essential for human existence. Many of the medications available today are originated from plants. *K. parviflora* is a medicinal plant with a long history of use (Techaprasan et al., 2010). The common names for *K. parviflora* include, "kra chai dam", "black ginger", and "kalahood" (Saokaew et al., 2016). The therapeutic usefulness of *K. parviflora* is rising, however this leads to major difficulties such as the plant's conservation in the wild due to overharvesting, as well as the quality and consistency of *K. parviflora* products. Superior genetic traits and high and constant secondary metabolite content affect the quality of medicinal plants (Labrooy et al., 2016). Black ginger was traditionally used to treat indigestion, ulcers, blood vessel relaxation, gout, and osteoarthritis.

There is one country, however, that has a long history of employing plants for therapeutic purposes (Ibrahim et al., 2023). *C. caesia* has evolved into an important source of natural goods for always maintaining human health (Tamang, 2022). *C. caesia* is a genus of roughly 70 rhizomatous herbs found mostly in Southeast Asia as wild and cultivated plants. *C. caesia* is a plant with thick rhizome that grows as a monocot (Tamang, 2022). The root stock is a big, sessile tuber with grey interior. *C. caesia* is a blue black rhizomatous plant herbaceous to Northeast Central India (Tamang, 2022). *C. caesia* is used in the treatment of leucoderma, asthma, tumours, haemorrhoids, bronchitis, and bruising in Manipur (Ibrahim et al., 2023).

In addition to the description given above, there are certain shortages of *K. parviflora* in this nation, making it difficult to get (Saokaew et al., 2016). The major reason for this is because it is not as popular as other herbs such as ‘Tongkat Ali’ and ‘Kacip Fatimah’ (Teh et al., 2021). However, the tree should not be planted in clay soil and should be put in a shady location to avoid overexposure to sunshine (Baeulieu, 2021). *K. parviflora* matures in 10 to 12 months and requires regular management, particularly in terms of fertiliser and air (Thukham-mee et al., 2021). Similarly, *C. caesia* is in low supply in this nation, making it extremely difficult to procure since a certain variety of *C. caesia* is rare (Munda et al., 2023). This *C. caesia* tree is a unique seasonal plant found in our nation. It requires healthy soil that is high in organic matter and has a strong drainage system (Kime, 2012). When turmeric rhizomes are buried in water for an extended period, they decay. *C. caesia* only grows to a height of 30 cm and reproduces via rhizomes (Lal et al., 2022).

Rizon is a plant stem that develops horizontally beneath the earth. *C. caesia* prefers a planting spacing of 30-40 cm and one tree per planting container (Lal et al., 2022).

1.2 Problem statement

K. parviflora, has been traditionally used in Southeast Asian Cultural Freedom for its perceived health benefits ranging from performance enhancement to potential anti-radar properties, there is a strong body of evidence from systematic science researchers to support this goal. *C. caesia*, has long been recognized in different traditional medical systems for health. Not only that, but this plant is also an antimicrobial agent that has been found in many plants. Plants can protect themselves from microbes such as bacteria, fungi and viruses by using natural antimicrobial chemicals (Chassagne et al., 2020). Each plant component, such as leaves, pseudostems, inflorescences, and rhizomes, contains a different amount of bioactive compounds (Zou et al., 2022). Parts of herbaceous plants can be used in a variety of traditional medical treatments.

A group of researchers who were all team members, but not all clearly performed a specific antimicrobial activity against *K. parviflora* and *C. caesia*, which is important information that needs to be filled concerning its ability to serve as a source of new antimicrobial agents. This has gained significance because of the belief in the development of active and inactive antimicrobials because of the increasing role of antibiotics. The purpose of this study is to see if extensive research on the antimicrobial activity of extract *K. parviflora* and *C. caesia* against several pathogenic microorganisms can help. Again, they could be candidates for positions as managers of innovative antimicrobial agents derived from natural

sources. Nevertheless, antimicrobial Sebastian's correct profile of which was used to study some pharmaceuticals in the future does not exist scientifically anymore. Also, whether the percentage of important antimicrobial compounds in different parts of the plant is known and still unknown for this species. In addition, scientific data is as important as traditional knowledge recorded by T. Sivaram where it comes to potential medicinal applications of *K. parviflora* and *C. caesia* that this report aims at exploring.

1. 3 Expected outcomes

The findings of this study will indicate that antibacterial active components exist in methanol extracts of *K. parviflora* and *C. caesia*. Methanol extracts and their constituents are expected to have strong antimicrobial activities against various microorganisms such as *Staphylococcus aureus* and *Escherichia coli*. The minimum inhibitory concentration (MIC) for the extract and its isolates can be determined by determining the lowest concentration that inhibits the growth of the tested microorganism by the compound. As a result, their compounds may be developed into new medicinal products to treat microbial diseases. This study will also shed light on the scientific aspects of *K. parviflora* and *C. caesia* in terms of antibacterial activity.

1.4 Objectives

- To determine the minimum inhibition concentration of *K. parviflora* and *C. caesia* extracts for antimicrobial activity.
- To compare inhibition activity of *K. parviflora* and *C. caesia* extracts using selected microorganisms.

1.5 Scope of study

The study was carried out at the Microbiology and Biochemistry Laboratory, Faculty of Earth Sciences (FSB) Universiti Malaysia Kelantan (UMK) Jeli campuses. *K. parviflora* and *C. caesia* samples, on the other hand, were gathered from Universiti Malaysia Kelantan (UMK), Jeli Campus, Jeli District, Kelantan. The rhizomes of *K. parviflora* and *C. caesia* were harvested. Each of these components will weigh 500g in total. The samples were separated one at a time. The Microbiology and Biochemistry Laboratory, Faculty of Earth Sciences (FSB), Universiti Malaysia Kelantan (UMK), Jeli Campus, were prepare the samples. The Minimum Inhibitory Concentration (MIC) method, on the other hand, were carried out at the Universiti Malaysia Kelantan (UMK) Jelly Campus. The antibacterial activity of extracts and components isolated from *K. parviflora* and *C. casiea* were determined using the Minimum Inhibitory Concentration (MIC).

1.6 Significance of study

This research can assist to improve the economy's quality. Because both *K. parviflora* and *C. caesia* have been utilises in traditional medicinal systems, the data obtains can offer validation data for traditional use. Their antibacterial qualities have been scientifically validated, providing evidence-bases support for their traditional usage. Furthermore, if this plant is shown to have considerable antibacterial capabilities, it may drive its production, resulting in economic benefits for farmers and the herbal sector (Bittner Fialova et al., 2021). Products containing recognises constituents will be produces using extracts and components from *K. parviflora* and *C. caesia*.

2.0 LITERATURE REVIEW

2.1 *Kaempferia parviflora*

2.1.1 Taxonomy

Kaempferia parviflora belongs to the Zingiberales order (Singh et al., 2023). Zingiberales is a class of plant in the Liliopsida family (Liliopsida, 2019). Zingiberales members are typically restricted to the tropics. According to most taxonomists, the phylogeny of Zingiberales is the natural or monophyletic lineage of plants (Sass et al., 2016). The rhizome, the underground part of the plant that is the main source of bioactive components for the *K. parviflora* plant, is one of the most important structures in this plant (Hashiguchi et al., 2022). The leaves of *K. parviflora* are large and grow horizontally or close to the soil surface (Thawtar et al., 2023). Ginger extract powder, on the other hand, is derived from the species *Zingiber officinale* (Mao et al., 2019). It has a natural ginger aroma and a yellow powder that is water soluble. It contains numerous important substances such as vitamin C, flavonoids, and phenol compounds.

The order Zingiberales has multiple families, including Musaceae (Thipe et al., 2023), Heliconiaceae (Arias-Bernal, 2019), Strelitziaceae (Simpson, 2010), Marantaceae (Nursery, 2023), Cannaceae (Simpson, 2010), Costaceae (Hubert Schaller, 2010), and Lowiaceae (Syauqina, 2019). The Zingiberaceae family includes *K. parviflora*. Furthermore, *K. parviflora* are classified as Liliopsida (Liliopsida, 2019). This is because both plants employ the same technique. Antioxidants include

K. parviflora and. Antioxidants include flavonoids, which act as antioxidants to protect cells from free radical damage. Not only that, but there are additional anti-inflammatory characteristics that may aid in the reduction of inflammation and discomfort.

Antiviral compounds derived from natural sources are being used to study the plant *K. parviflora* (Sormpet et al., 2017). *K. parviflora* is a Thai herb of the Zingiberaceae family known for its antiviral protease activity (Singh et al., 2023). Zingiberaceae rhizomes have been shown to contain a variety of bioactive compounds, including curcuminoids, sesquiterpenes, and flavones (Alolga et al., 2022). *K. parviflora* is a health-promoting herb that is used in Thailand to treat colic, peptic ulcers, and duodenal disorders (Singh et al., 2023). As a result, it is used as a starting point for the identification of the active substance. These proteases are all required for the life cycle viruses from which they originate, making them all attractive targets for antiviral drug therapy (Mahajan et al., 2021).

2.2 *Curcuma caesia*

2.2.1 Taxonomy

Curcuma caesia is studied in the order Zingiberales (Arpit Singh, 2023). Zingiberales is a class in the liliopsida family (Ho Dinh Hai, 2015). The tropics are home to members of the Zingiberales. Taxonomists believe that the phylogeny of Zingiberales is the structural line of all so or monophyletic organizations (Chodon Sass, 2016). This tree is a developing plant with only 30cm offspring. If eaten, grows *C. caesia* from rhizomes and grass fragments and emits a strong and pungent

odor. The upper part of the leaf has a heart-shaped red stripe in the center, and the stem is dark at the base. *C. caesia* is popular due to its unique medicinal value, which has caused this plant to be hunted by traditional. However, the primary application of components from the black turmeric tree has always been bathing the body, which has always been popular in the past.

Musaceae (Qamar & Shaikh, 2018), Heliconiaceae (Leonardo Arias-Bernal, 2019), Strelitziaceae (Simpson, 2010), Marantaceae (Amelia Ralston Okabayashi, 2019), Cannaceae (Maas-van De Kamer & Maas, 2008), Costaceae (Maji et al., 2020), and Lowiaceae (Syauqina, 2019). *K. parviflora* is a member of the Zingiberaceae family. Following that, *K. parviflora* is classified as liliopsida (Liliopsida, 2019). This is because both plants employ the same technique. *C. caesia* is an antioxidant. Flavonoids, which act as antioxidants to protect cells from free radical damage, are examples of antioxidants (Asdedi et al., 2016). Not only that, but there are additional features that can help with inflammation and discomfort reduction.

Plant *C. caesia* is being studied using antiviral compounds derived from natural sources (Bhardwaj et al., 2023). *C. caesia* is a Thai herb that belongs to the Zingiberaceae family and is known for its antiviral protease curcuminoids, sesquiterpenes, and flavones have been found in Zingiberaceae Rhizomes (Ibrahim et al., 2023). *C. caesia* is a beneficial herb that is used to treat colic, peptic ulcers, and duodenal disorders in Thailand (Das et al., 2013). As a result, it is used to identify active ingredients as a starting point (Katiyar et al., 2012). Because these proteases are all required for the viral life cycle from which they originate, they are

all appealing targets for antiviral drug therapy. Figure 2.1: Phylogenetic tree of family Zingiberaceae.

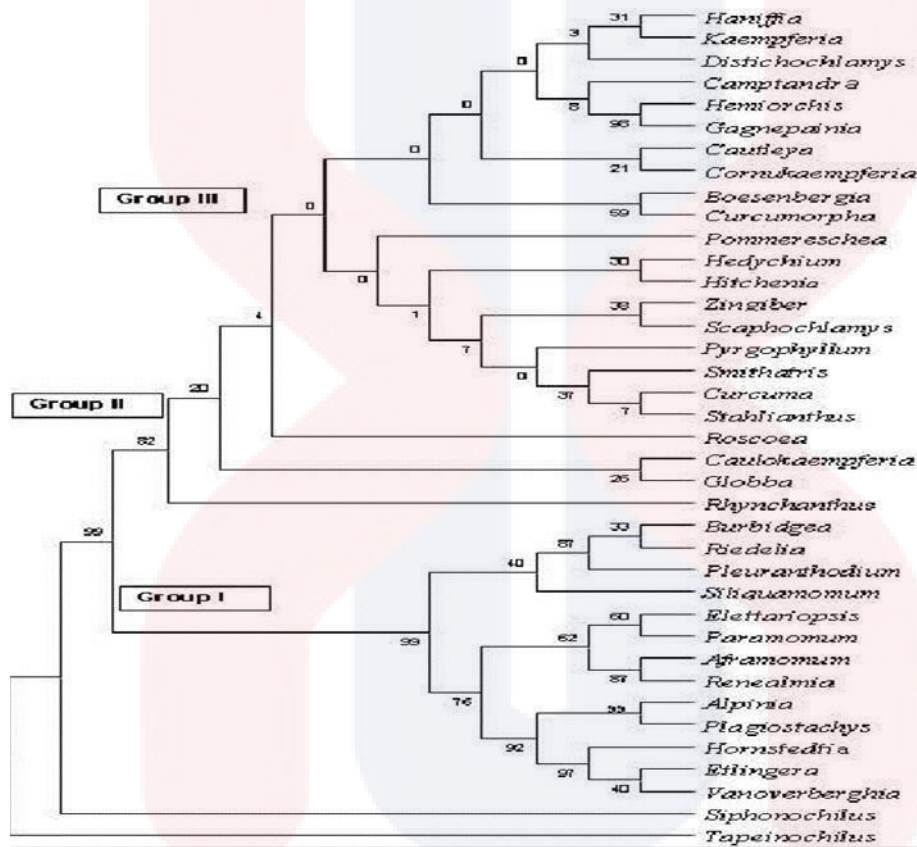


Figure 2.1: Phylogenetic tree of family Zingiberaceae (Selvaraj et al., 2008)

Keampferia and *Curcuma* are genera of the Zingiberaceae family (Figure 2.1).

Asians have been using it as a traditional medicine since centuries. *Kaempferia* contains more than 683 species and *Curcuma* contains 126 species that are cultivated in the forests of Thailand and India (Saokaew et al., 2016), (Ewon & Bhagya, 2019).

It can grow up to 30cm for *Kaempferia* and *Curcuma*. Directly, the genus *Kaempferia* and *Curcuma* will grow with leafy shoots and long rhizomes and have flowers with each shoot can have a length of 30 cm. The figure 2.2 and figure

2.3 show the pinpoint in the map area *K. parviflora* and *C. caesia*. *Kaempferia* and

Curcuma can be found predominantly around Malaysia but natively in Thailand, Malaysia, Indonesia, India, Bangladesh, Nepal, China, Vietnam and so on (figure 2.2 and figure 2.3). In Malaysia, there are *K. parviflora* and *C. caesia* which can be found in Selangor, Pahang and Perak.



Figure 2.2: Distribution *Kaempferia parviflora* at Thailand and Vietnam as shown in red box. (Botanic, 2018)

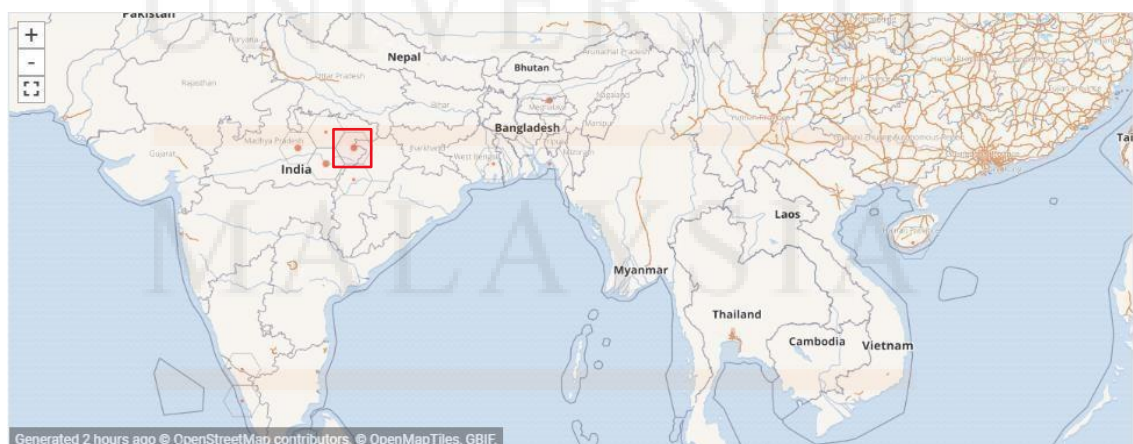


Figure 2.3: Distribution *Curcuma caesia* at India and Bangladesh as shown in red box (Pal et al., 2024).

2.3 Morphology of *K. parviflora* and *C. caesia*

In terms of previous history, *K. parviflora* research on Thai Ginseng was at a moderate level and began to receive human trials (Kamal Patel, 2022). The exam has been used by farmers in Thailand to be used as folk medicine for centuries (Surasak Saokaew, 2017), for the *C. caesia* plant has also been used by local communities for their own health because history reveals that plants have been a source of valuable natural products to always maintain human health (Tamang, 2022).

However, the color of these two plants is purple black (*Kaempferia parviflora*) and blue-black (*Curcuma caesia*) (MARDI, 2019). In addition, *K. parviflora* has yellowish-purple rhizomes and turns blackish-purple when mature (Dawn Series, 2014). *C. caesia*, on the other hand, has a bluish-black rhizome-colored rhizome that darkens as it gets older, gives off a strong odor and tastes quite bitter if consumed (MARDI, 2019).

The main distinguishing characteristic of *K. parviflora* is its rhizome as in figure 2.5: *K. parviflora* rhizome. The rhizomes are cylindrical and fleshy, with a distinct color pattern (Ruangrunsi et al., 2018). When young, they are yellowish purple, but as they age, they turn blackish purple. The rhizomes are fragrant and contain essential oils, so they have a unique fragrance (Sharmeen et al., 2021). *C. caesia* rhizome is thicker and more branching than *K. parviflora* as in figure 2.6: comparison of *C. caesia*. The hue is a striking blue black that deepens as the rhizome grows. The rhizome has a strong camphor-like odor and a highly bitter taste. It also includes essential oils and other bioactive substances (Ibrahim et al., 2023).

Table 2.1: Morphological characteristics of *Kaempferia parviflora* sample collected in southern Thailand (Labrooy et al., 2018).

Cup.	Thai Name	Botanical Name	Use
1.	Wan Chao Noi Maha Phrom	<i>K. aff. Rotunda</i> L. (kariyil et al., 2020)	Medicinal & spiritual
2.	One Fire Nok- kum	<i>K. aff _ parviflora</i> Wall. (South Africa, 2020)	Medicinal & spiritual
3.	One Khum -down	<i>K. aff. roscoeana</i> Wall. (Saensouk et al., 2022)	Spiritual
4.	One Knock Khum	<i>K. elegans</i> Wall. Ex Baker (Singh et al., 2023)	Spiritual
5.	A Kam-bang	<i>K. aff _ elegans</i> Wall. ex-Baker (Pham Ngoc Khanh,2021)	Spiritual
6.	One Krachae Chan	<i>K aff galanga</i> L. (Bungorn Sripanidkulchai, 2011)	Medicinal & spiritual
7.	A Mai-deed	<i>K. rotunda</i> L. (J Nat Prod. 2014)	Medicinal & spiritual
8.	Want to Dam	<i>K. rotunda</i> L. (J Nat Prod. 2014)	Spiritual
9.	One Pro Home	<i>K. galanga</i> L. (Si - Yu Wang,2021)	Medicinal

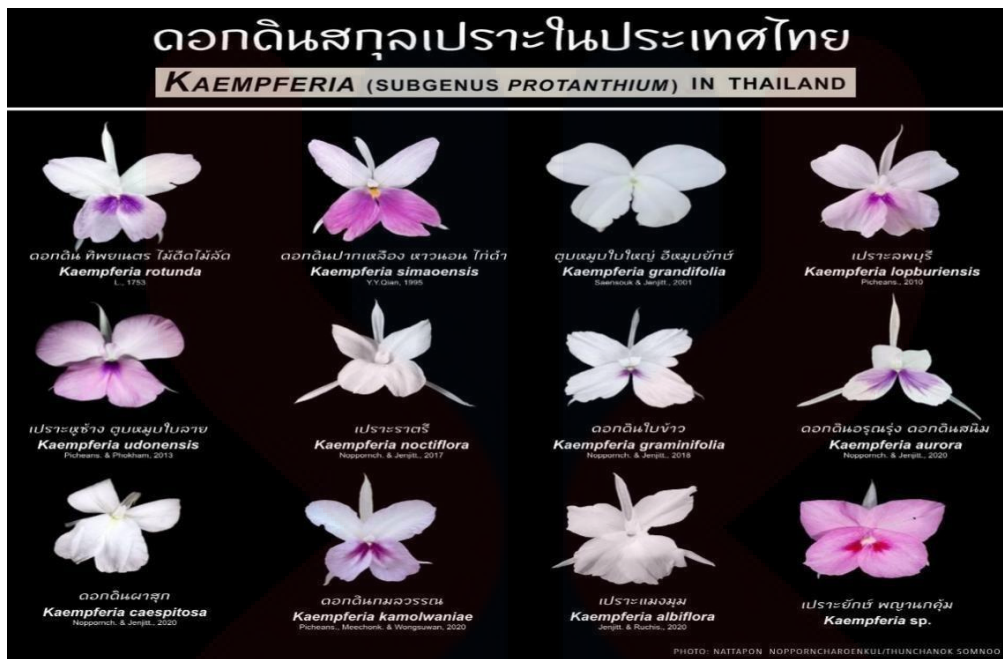


Figure 2.4: Comparison of *Kaempferia parviflora* with other species. (Labrooy et al., 2018)



Figure 2.5: *Kaempferia parviflora* rhizomes. (DQ Farm, 2018)

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Table 2.2: Morphological characteristics of *Curcuma caesia* sample that was collected in southern India. (Saha et al., 2016).

Species and Populations	Place of collection
<i>Curcuma amada</i>	Suryamaninagar, West Tripura
<i>Curcuma longa</i> 1 (Wild)	Jampui Hill, North Tripura
<i>Curcuma longa</i> 2 (Cultivated Pop-I)	Madhupur, Sepahijala
<i>Curcuma longa</i> 3 (Cultivated Pop-II)	Suryamaninagar, West Tripura
<i>Curcuma caesia</i>	Suryamaninagar, West Tripura
<i>Curcuma zedoaria</i>	Baramura Hill, West Tripura








<p><i>C. alismatifolia</i></p> <ul style="list-style-type: none"> ▪ Antiinflammatory ▪ Antioxidant ▪ Wound healing 	<p><i>C. amada</i></p> <ul style="list-style-type: none"> ▪ Antipyretic ▪ Laxative ▪ Antiallergic ▪ Analgesic 	<p><i>C. aromatica</i></p> <ul style="list-style-type: none"> ▪ Wound healing ▪ Antimicrobial ▪ Antioxidant 
<p><i>C. roscoeana</i></p> <ul style="list-style-type: none"> ▪ Antiproliferative against colon cancer cell lines 	<p><i>C. caesia</i></p> <ul style="list-style-type: none"> ▪ Antivenom ▪ Emollient 	<p><i>C. prakasha</i></p> <ul style="list-style-type: none"> ▪ Treatment of bruises and wounds 
<p><i>C. longa</i></p> <ul style="list-style-type: none"> ▪ Antiinflammatory ▪ Inhibition of TNF ▪ Antiproliferative 	<p><i>C. zedoaria</i></p> <ul style="list-style-type: none"> ▪ Carminative ▪ Neuroprotective ▪ Antitumor ▪ Antiinflammatory 	<p><i>C. xanthorrhiza</i></p> <ul style="list-style-type: none"> ▪ Antioxidant ▪ Antibacterial ▪ Hepatoprotective ▪ Digestive stimulant 
<p><i>C. australasica</i></p> <ul style="list-style-type: none"> ▪ Inhibition of prostaglandin and nitric oxide ▪ Antiinflammatory 	<p><i>C. petiolata</i></p> <ul style="list-style-type: none"> ▪ Cytotoxic against cholangiocarcinoma 	<p><i>C. comosa</i></p> <ul style="list-style-type: none"> ▪ Reduction of post-partum inflammation ▪ Treatment of neuro-disorders 

Figure 2.6: Comparison of *Curcuma Caesia* with other species. (Asakawa, 2010)

Especially, the color of *K. parviflora* flowers is white with purple spots in the middle (Labrooy et al., 2016) while *C. caesia* flowers are white with some shades of purplish or pink in the middle (Adam Harith, 2019) compared to other species (Figure 2.4 and Figure 2.6). Pure length is about 30cm. For the average width of the middle labellum is 8-16cm. In addition, the stigma of the *K. parviflora* tree is formed with a ciliated rim (Chayan Picheansoonthon, 2008). Not only that, but the *C. caesia* tree also has an average length of 30cm like *K. parviflora*. The size of the rhizomes on the *C. caesia* tree is 2-6 cm in diameter and often variable in size. And for the tree *C. caesia* scalpel Oval wide and glabrous (Tamang, 2022).

2.4 Distribution of *K. parviflora* and *C. caesia*

K. parviflora distributes widely in southern Thailand, Peninsular Malaysia, Indonesia and as (Techaprasan et al., 2010). Figure 4 shows a map distribution of *K. parviflora* through the southern provinces of Thailand. *K. parviflora* is found in the upper parts of the Straits of Thailand such as, Kanchanaburi and Phetchabun. *K. parviflora* is widely distributed in the lower parts of southern Thailand such as Kanchanaburi and Phetchabun. It shows the breakdown in the middle of the Thai Strait consisting of the provinces of Kanchanaburi and Phetchabun. *C. caesia* is widely distributed in southern India, Bangladesh, Nepal, and Vietnam (Lal et al., 2020). *C. caesia* is found in the lower parts of the Indian subcontinent such as Tamil Nadu, Andhra Pradesh and so on. It shows the split at the end of the Indian Straits comprising the provinces of Tamil Nadu and Andhra Pradesh.

There are other species of *Kaempferia* and *Curcuma* that are spread throughout southern Thailand and southeastern India. For example, the widely distributed *K. pulchra* in Nakhan Si Thammarat and Prachuap Khiri Khan. *C. aeruginosa* is widespread in Kerala and Karnataka. It grows together with other species of the genus *Kaempferia* and *Curcuma*. *K. parviflora* can grow in humid shaded areas. For *C. caesia* also grows in shady areas of the valley. In primary forests that have low to high altitudes, it is less to be able to see these species.

3.0 MATERIALS AND METHODS

3.1 Preparation of stock solution *Kaempferia parviflora* and *Curcuma caesia* from Rhizome part.

The texture of *Kaempferia parviflora* and *Curcuma caesia* rhizome stock varies depending on the plant. For example, table 3.1: characteristics of the extract of *K. parviflora* and *C. caesia*. *K. parviflora* has a slightly viscous and watery consistency, but *C. caesia* has a waterier and less mucousy texture. These distinctions are critical for recognizing and employing each plant in a variety of applications. Furthermore, the color of the stock varies according on the plant *K. parviflora* rhizomes generate a blackish-purple tint, whilst *C. caesia* rhizomes provide a bluish-black color. These contrasting colors are important in identifying and distinguishing the two plants in both traditional and modern settings. Furthermore, the rhizome stocks differ in smell. *K. parviflora* has a sweet and spicy flavor, whilst *C. caesia* has a pungent and strong scent.

Parameter	Rhizome <i>K. parviflora</i>	Rhizome <i>C. caesia</i>
Texture	Viscosity and watery consistency	More watery and less mucilaginous
Colour	Blackish purple	Bluish black
Odor	Sweet spicy aroma	Pungent and sharp aroma

Table 3.1: Characteristics of the extract of *K. parviflora* and *C. caesia*

K. parviflora was selected for studying antimicrobial activity against both gram-positive bacteria (Sizar et al., 2023) and gram-negative bacteria (Oliveira & Reygaert, 2023) to assess the plant's potential against microorganisms that may cause diseases in humans or plants. The plant material was manually collected from Ginger and Herbs Nursery, Institute of Food Security and Sustainable Agriculture, Universiti Malaysia Kelantan, UMK Jeli campus. Additionally, the research site was chosen as a *K. parviflora* agricultural park due to its soil pH of 5.5 (Subaryanti et al., 2020) and temperature ranging from 20°C to 30°C (Karyati et al., 2018). Furthermore, several characteristics were utilized for identifying *K. parviflora* in the field, including leaf shape, leaf pattern, rhizome color, and rhizome odor. The rhizome of *K. parviflora* will be used to extract a natural essence without chemicals. Subsequently, the *K. parviflora* extract exhibited varying degrees of antibacterial activity when assessed using the paper disc diffusion method.

Furthermore, the effectiveness of *K. parviflora* extract against gram negative bacteria is equally significant (Krongrawa et al., 2022), especially given the challenges posed by gram-negative bacteria such as *E. coli* (Oliveira & Reygaert, 2023) and *S. aureus* (Sizar et al., 2023), which are known for their antibiotic resistance. This highlights the significance of environmental factors influencing the bioactivity of plant extracts (Liu et al., 2016).

The use of chemical-free extraction methods to produce natural essence from *K. parviflora* rhizomes highlights the importance of environmentally friendly and sustainable extraction methods in herbal medicine development (Zhang et al., 2018). Overall, these findings provide important insights into the antimicrobial potential of *K. parviflora* extracts, paving the way for future research aimed at elucidating the

underlying mechanism of action and exploring their potential use in the development of new antimicrobial agents (Jin & Lee, 2018).

C. caesia was chosen to investigate antimicrobial activity against both gram-positive (Sizar et al., 2023) and gram-negative bacteria (Oliveira & Reygaert, 2023) in order to assess the plant's potential against microorganisms that can cause disease in humans or plants. The plant material was manually collected from the Ginger and Herbs Nursery, Institute of Food Security and Sustainable Agriculture, UMK Jeli campus. Furthermore, the research site was selected as a *C. caesia* plant agricultural park because the soil has a pH of 5.5 (Subaryanti et al., 2020) and a temperature range of 20°C to 30°C (Karyati et al., 2018), which is suitable. In addition, several features were used to identify *C. caesia* in the field, including leaf shape, leaf pattern, rhizome color, and rhizome smell.

Furthermore, the effectiveness of *C. caesia* extract against gram-negative bacteria is equally significant (Krongrawa et al., 2022), especially given the challenges posed by gram-negative bacteria such as *E. coli* (Oliveira & Reygaert, 2023) and *S. aureus* (Sizar et al., 2023), which are known for their antibiotic resistance. This highlights the significance of environmental factors in influencing the bioactivity of plant extracts (Liu et al., 2016).

When compared to the standard, *C. caesia* was found to be a stronger growth inhibitor against both bacterial strains (Ibrahim et al., 2023). Medicinal plants exhibit antimicrobial activity with different mechanisms (Vaou et al., 2021). This can be achieved by inhibition of cell wall synthesis, disruption of cell membrane permeability, causing membrane disruption, modifying cellular constituents, and inducing cell damage or mutation. Most solvents, such as ethanol, hexane, and methanol, when used for plant extracts, exhibit inhibitory effects on both Gram-

positive and Gram-negative bacteria (Borges et al., 2020). The present findings demonstrate its antimicrobial properties, and its rhizome can be exploited as a source of bioactive compounds for medicinal use (Vaou et al., 2021).



3.2 Preparation of Nutrient Agar

The Oxoid nutrient agar used in this project is a well-known and accepted brand worldwide in the field of microbiology. Autoclaving is an important method of sterilization in cleaning the agar solution from contaminants. A feature of the autoclave process is the creation of high steam temperatures, all microorganisms, including bacteria, viruses and fungi, are killed. Cooling the agar solution at 40-45°C in a laminar flow cabinet helps reduce the risk of contamination and ensures that the agar solution is free of any contaminants. Laminar flow cabinets will ensure a controlled environment with unidirectional air flow, thereby reducing pollution. Solidification of the agar solution at approximately 27°C in a laminar flow cabinet ensures that the agar medium is uniform and homogeneous. Storing nutrient agar plates at a certain temperature range of 2°C to 8°C is usually typical, as it helps slow down bacterial growth and, in this case, contamination. This ensures that the agar plate remains alive and can be used for a longer period of time, whenever needed.

3.3 Preparation of Stock Culture of Bacteria

The preparation of stock cultures is a crucial stage in microbiological research, allowing the maintenance of bacterial cultures for extended periods. *Staphylococcus aureus* is a Gram-positive bacterium commonly found on human skin and mucous membranes. Its main characteristics include the ability to produce toxins and cause skin infections. *Escherichia coli* is a Gram-negative bacterium

often found in the human gastrointestinal tract. Its main features include the production of beta-lactamase and the ability to cause urinary tract infections. Nutrient agar plates provide a suitable medium for the growth and reproduction of bacteria, allowing confirmation of the purity of bacterial cultures. The freeze-drying process preserves bacterial cultures by removing water and preventing contamination. Storage conditions of -20°C or -80°C are typical for the storage of lyophilized stock cultures, as they prevent degradation and contamination, thereby ensuring the longer viability of stock cultures and making them accessible for use when needed. The preparation of stock cultures is one of the most critical stages in microbiological research and requires meticulous attention to detail to ensure that bacterial cultures are pure, viable, and well-preserved. The continuous streaking method, 100 μl of bacteria was transferred from the main culture to nutrient agar.



Figure 3.1: The continuous streak method for microorganisms culture

(Qi et al., 2018)

3.4 Preparation of *K. parviflora* and *C. caesia* plant extraction

3.4.1 Disc diffusion assay to evaluate the efficiency of antimicrobial agents

The present study is all about the determination of the antimicrobial properties of *K. parviflora* and *C. caesia* rhizomes. Dried rhizomes were ground into a fine powder, and various concentrations of extracts, ranging from 2 to 10 mg/ml, were prepared and then diluted using methanol for test purposes. The extract solutions were then taken for an antibacterial study against *Escherichia coli* and *Staphylococcus aureus* by disc diffusion assay methods. The entire agar plate was smeared with bacteria inoculum, and sterile discs were impregnated with the plant extracts and placed on the surface of the agar. After incubation at 37°C for 24-48 hours, the inhibition zones around the discs were then measured for antibacterial effectiveness. The results of the test indicate that larger inhibition zones correlated with higher antibacterial activity and served to determine the minimum inhibitory concentration (MIC) of the extracts. This study shall identify *K. parviflora* and *C. caesia* extracts as natural antimicrobial agents.

3.5 Determination of Bacteria Growth, Inhibition Zone, and Antimicrobial Activity of *K. parviflora* and *C. caesia* at Different Concentrations.

3.5.1 Antimicrobial Assay

Antimicrobial experiments were conducted to evaluate the efficacy of extracts from *K. parviflora* and *C. caesia* against various bacterial strains. Bacterial cultures from both gram-positive and gram-negative groups were obtained from the

Microbiology and Biochemistry Laboratory of the Faculty of Earth Sciences (FSB), Universiti Malaysia Kelantan (UMK), Jeli Campus. Nutrient agar (Oxoid, England) was used as the culture medium. Streptomycin (Oxoid, England) served as a reference antibiotic to compare the antibacterial activity of the extracts. Positive controls included extracts from the rhizomes of *K. parviflora* and *C. caesia* at various concentrations. The extracts were prepared by dissolving the rhizome powder in distilled water or another appropriate solvent.

1-2 mL of bacterial culture was inoculated onto nutrient agar plates. An antibiotic disc (streptomycin) and an extract disc were placed on the surface of the inoculated agar plate. The agar plate was then incubated at 37°C for 24 hours. After incubation, the agar plate was examined for any zones of inhibition around the antibiotic and extract discs. The diameter of the inhibitory zone (diz) surrounding each disc was measured using a digital ruler or caliper.

The presence of an inhibitory zone around the antibiotic and extract discs indicates antibacterial activity. The diameter of the inhibition zone (diz) is directly proportional to the extract's antibacterial activity. The results of this investigation were compared to assess the antibacterial activity of the extracts and their potential as antimicrobial agents.

Next, using a micropipette, each filter paper disc was individually soaked with plant extracts at concentrations ranging from 0 mg/ml to 10 mg/ml. Streptomycin (Oxoid, England) was used as a positive control, while methanol served as the negative control. Bacteria were inoculated onto agar plates and

incubated for 18 to 24 hours at 37°C. Following incubation, an inhibition zone formed around the filter paper containing the antimicrobial agent. Each nutrient agarplate was divided into eight sections, each containing filter paper discs with negative and positive control agents. The permeation test was conducted in triplicate for each concentration and for three extracts of *K. parviflora* and *C. caesia*.

Following the incubation period, the zones of inhibition around the filter paper discs soaked in plant extracts were measured to determine the antimicrobial effectiveness of each extract at different concentrations. The results of this test will provide valuable information regarding the antibacterial properties of *K. parviflora* and *C. caesia* extracts. To ensure the accuracy and reliability of the data, each test was repeated three times. This will enable researchers to better understand the effectiveness of these plant extracts in inhibiting bacterial growth and to compare the efficacy of the two plant species.

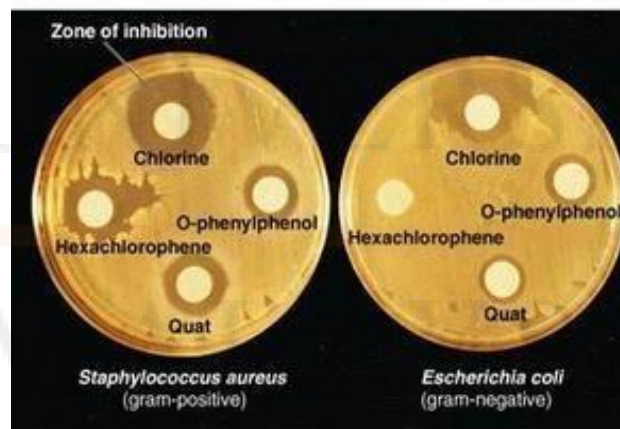


Figure 3.2: The presence of the inhibition zone around the disk (Horvath et al., 2016)

3.5.2 Measurement of Inhibition Zone in *K. parviflora* and *C. caesia*

To quantify the inhibition zones of *K. parviflora* and *C. caesia* extracts, a Digital Vernier Caliper was used to accurately measure the diameter of the inhibition zone and the diameter of the disk in millimeters. This equipment, provided by the Laboratory of Microbiology and Biochemistry at the Faculty of Earth Sciences, Universiti Malaysia Kelantan (UMK), Jeli Campus, ensures precise measurement of antimicrobial activity. The measurement taken encompassed the entire inhibition zone the clear area around the antimicrobial agent which indicates the effectiveness of the extract in inhibiting microbial growth.



Figure 3.3: Digital Vernier Caliper (Norman et al., 2016)

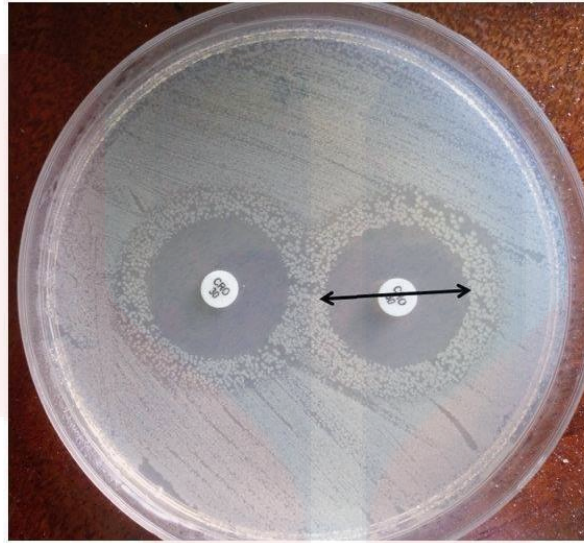


Figure 3.4: Measurements for the normalized width of the inhibition zone (Byakika et al., 2019)

3.6 Statistical analysis of *K. parviflora* and *C. caesia*

The measurement of the zone of inhibition was expressed as the mean and analyzed using Two-Way ANOVA (analysis of variance). Statistical analysis was conducted to compare the inhibitory activity of *K. parviflora* and *C. caesia* extracts against different selective bacteria. The test was performed in triplicates for each concentration, and the mean value was calculated to evaluate the results, especially when variability was large. Significant differences between different concentrations of *Macrotermes carbonarius* defense fluid extract on the inhibition zone were determined using two-way ANOVA analysis, as described (Arghya Ghosh, 2018). The two-way analysis of variance (ANOVA) was conducted using IBM® Software (SPSS®) Statistics Version 21 to compare differences in inhibition zones resulting from the parts of plant extracts, specifically involving the rhizomes of *K. parviflora* and *C. caesia* (Rashid et al., 2022). Data were considered significantly different when $P < 0.05$, which was statistically significant in both cases.

4. RESULT AND DISCUSSION

4.1 Microbial growth of *Escherichia coli* and *Staphylococcus aureus*

The spread and streak plate methods were used in this study to analyze the growth of gram-positive and gram-negative bacteria. The bacteria were then cultivated in vitro with nutrient agar as the growth medium. Furthermore, the bacteria were moved to universal bottles for storage (Vidal - Verdú et al., 2022). For example, Figure 4.1 depicts the microorganisms contained in the universal bottle. Furthermore, the diagram below displays the bacterial agar plates of *E. coli* (Oliveira & Reygaert, 2023) and *S. aureus* (Sizar et al., 2023) that were cultivated at 35°C on nutritional agar using the spread and streak plate technique.

In this work, a serial dilution approach was used to decrease the bacteria to levels that were easier to count when plated on agar plates. Serial dilution is used to aid in the detection of bacteria (colony forming units, CFU) (Ben-David & Davidson, 2014). Following dilution, the bacteria were disseminated onto nutrient agar using the spread plate technique (Sanders, 2012). After up to 24 hours of incubation at 35°C, bacterial colonies appeared on the agar plates, and the number of colonies was determined using the conventional plate count method. Only plates with a colony count of 30 to 300 bacteria were judged statistically significant (O'Toole, 2016) and appropriate for the next phase, which involved isolating pure bacteria using the streak plate technique (Sanders, 2012).

After an incubation period of up to 24 hours, Figure 4.2 illustrates the growth of bacteria on an agar plate with more than 30 colonies visible (O'Toole, 2016). These two agar plates were selected because they allowed for the presence of two species of bacteria on each plate.

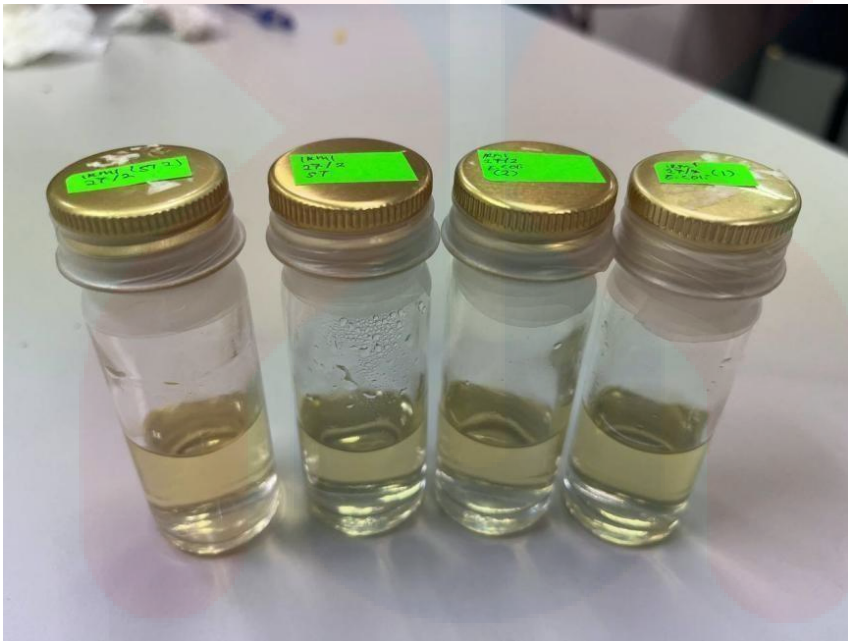


Figure 4.1: Depicts the microorganisms contained in the universal bottle.

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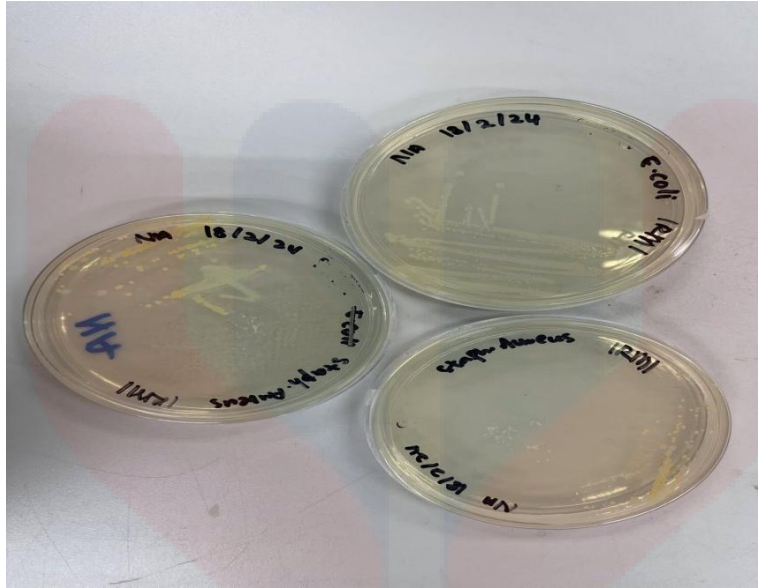


Figure 4.2: illustrates the growth of bacteria on an agar plate.

4.2 Preparation the extract of *K. parviflora* and *C. caesia*

To prepare the *K. parviflora* and *C. caesia* extracts, 0.05 grams of each extract were weighed separately and placed in individual test tubes. Next, 1000 μ l of methanol was added to each test tube containing the extracts (Pawar & Surana, 2010). The test tubes were then placed on a vortex mixer (Karki, 2022) to thoroughly combine the *K. parviflora* and *C. caesia* extracts with the methanol solution. Vortex mixing meant that the extracts were evenly distributed in the solvent (Awad et al., 2021), allowing for more efficient extraction of bioactive chemicals. Following vortex mixing, the extract-solvent mixture was submitted to additional processing or analysis, depending on the experimental methodology or intended application. This produced extract can be used for numerous applications, such as antimicrobial testing, phytochemical analysis, or the creation of herbal products (Sasidharan et al., 2010).

Depending on the intended purpose, the extract-solvent mixture may require further stages after vortex mixing (Xu et al., 2023). For example, if the extracts are being prepared for antimicrobial testing, the mixture may be diluted further to obtain the assay's necessary concentration. If the extracts are intended for phytochemical analysis, they may be subjected to filtration or centrifugation to remove any insoluble particles or detritus prior to analysis. Furthermore, if the extracts are to be used for formulation, additional substances or excipients may be added to produce the final product (Markl & Zeitler, 2017). This could include combining the extracts with additional herbal extracts, oils, or additives to improve the formulation's stability, bioavailability, or other desirable features. Overall, the produced extracts can be used as valuable starting materials for a variety of applications in research, pharmacology, cosmetics, and herbal medicine (Bolouri et al., 2022). Each stage in the preparation process is carefully carried out to assure the final extract product's purity, uniformity, and efficacy.

The combined extract will be aliquoted into microcentrifuge tubes at concentrations of 0mg/ml, 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml, and 10mg/ml, with volumes of 200 μ l, 400 μ l, 600 μ l, 800 μ l, and 1000 μ l, respectively, as illustrated in figure 4.3. Following that, each tube was supplemented with methanol in the amount of 1mL. To adjust the concentrations, 800 μ l of methanol were added to the 2mg/ml concentration, 600 μ l to the 4mg/ml concentration, 400 μ l to the 6mg/ml concentration, 200 μ l to the 8mg/ml concentration, and the 10mg/ml concentration will remain constant. This process guarantees that each tube has a total capacity of 1mL, with the right extract and methanol concentrations for further testing or analysis. In addition, prepare 1 mL of unmixed methanol to be placed over the 0mg/ml region. Figure 4.5: show 1mL methanol into a Microcentrifuge tube.

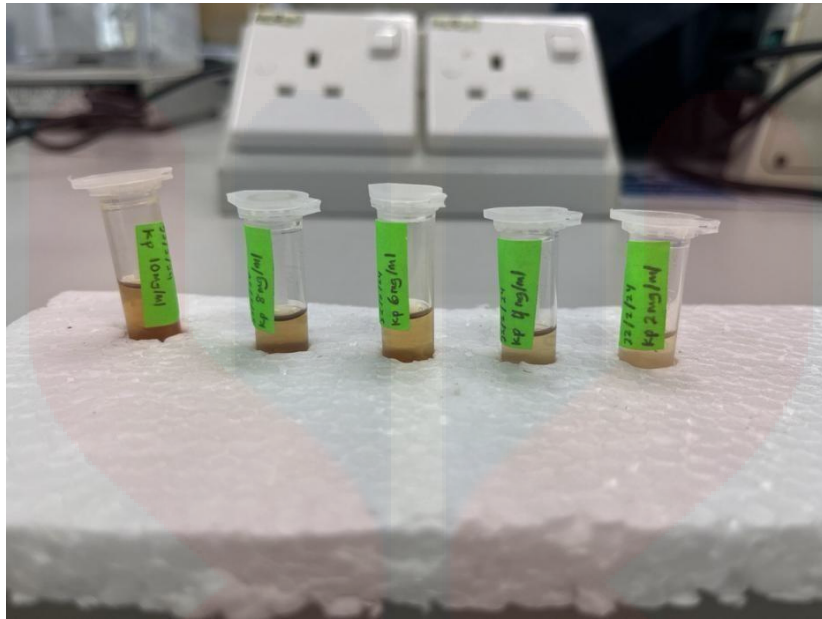


Figure 4.3: The extract *K. parviflora* that has been mixed into a microcentrifuge tube.

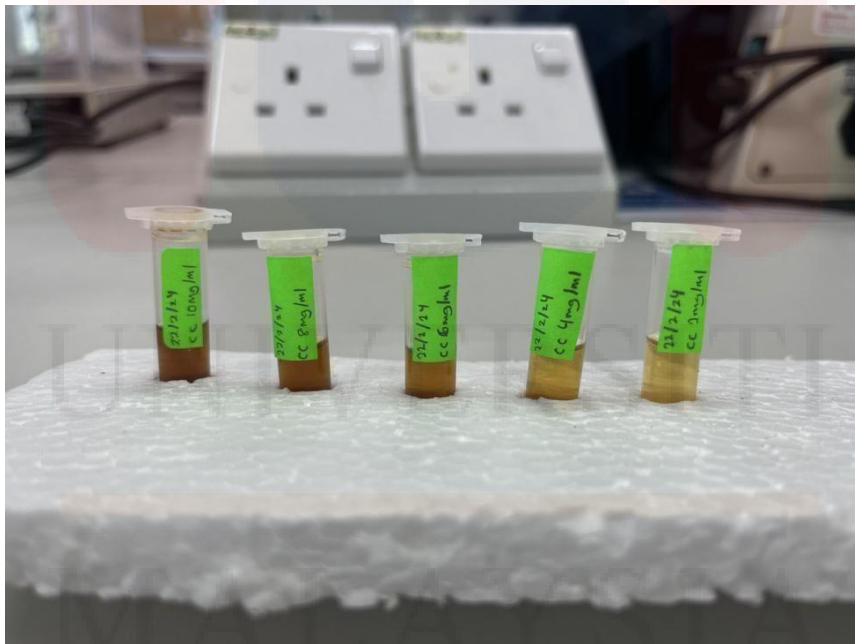


Figure 4.4: The extract *C. caesia* that has been mixed into a microcentrifuge tube.

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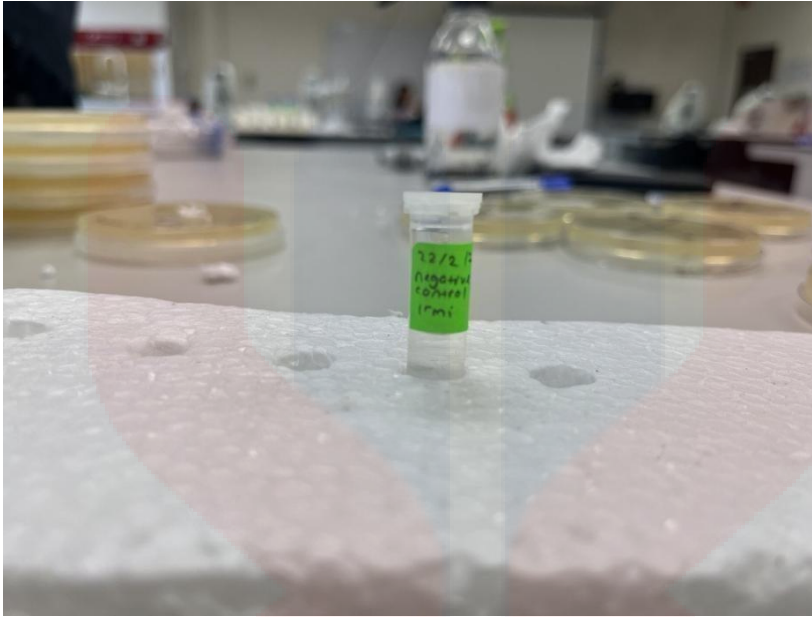


Figure 4.5: 1mL methanol into a microcentrifuge tube.

4.3 Antimicrobial activity of *K. parviflora* and *C. caesia*.

The disc diffusion technique was used to determine antibacterial activity against both gram-positive and gram-negative microorganisms (Balouiri et al., 2016). Antimicrobial activity against gram-positive and gram-negative bacteria is assessed using simple, practical, and well-standardized procedures (Balouiri et al., 2016). The diffusion technique is used in the current investigation because it is widely recognized and continues to be used in many clinical microbiology settings for monitoring the antibacterial performance of diverse liquids. The antibacterial activity of the defense liquid against *K. parviflora* and *C. caesia* was evaluated as possible antimicrobial agents in this study (Bhaskaracharya et al., 2023).

The defensive liquid from *K. parviflora* and *C. caesia* was extracted using methanol as the solvent. Initially, the sample was defatted with methanol and kept at room temperature for up to 2 hours to aid in dissolution (Abu et al., 2017). Methanol

was chosen as the solvent because of its known ability to extract lower molecular weight polyphenols. Furthermore, its simple evaporation allows for separation from the liquid extract during the evaporation process. Furthermore, methanol's amphiphilicity allows it to remove both polar and non-polar compounds from the sample (Primorac et al., 2018). Methanol is often regarded as the purest solvent for extracting antioxidant chemicals.

In this investigation, the screening microorganism was a gram-negative bacteria called *E. coli*. *S. aureus* is one of the gram-positive bacteria employed in this investigation (Bannerman et al., 2004). This study examined antimicrobial activity using recognized or standard bacteria such as *E. coli* and *S. aureus* in order to assess the efficiency of antibiotics or experimental groups against specific germs. The least inhibitory concentration was determined by testing several concentrations of defensive liquid extract (0mg/ml, 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml, and 10mg/ml). Gentamicin was utilized to identify any difficulties with the minimal inhibitory concentration. The disc diffusion method was used to investigate the antibacterial activity of liquid extracts of *K. parviflora* and *C. caesia* against a total of two bacteria. The diameter of the clear zone of inhibition was evaluated in millimeters.

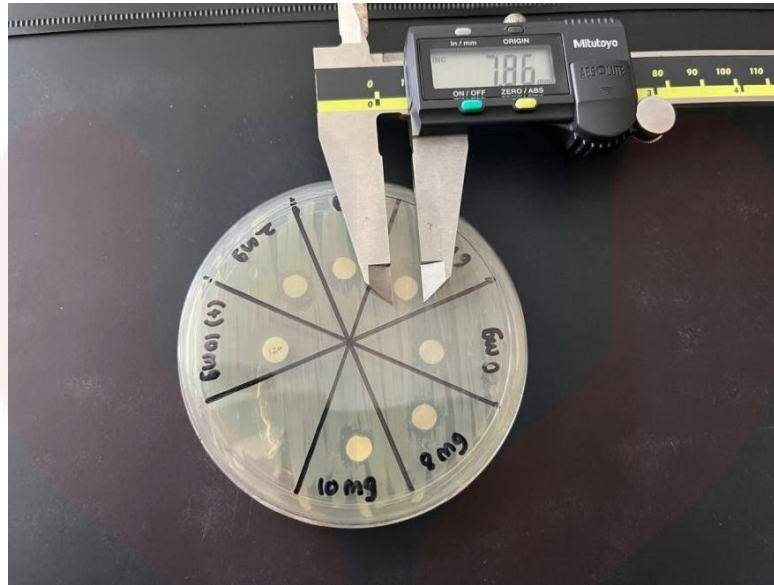


Figure 4.6: Disk diffusion plate of *K. parviflora* and *C. caesia*.

The bacterial inhibition zone was measured three times at each precision, and the overall mean and standard deviation were computed (Zhang et al., 2017). Figure 4.6 shows the effectiveness of *K. parviflora* and *C. caesia* antibacterial activities. The findings of this investigation demonstrate the antibacterial activity of the defense liquid derived from *K. parviflora* and *C. caesia* against gram-negative and gram-positive bacteria, as shown by percentage zone results. Table 4.1 shows the zones of inhibition for both gram-negative and gram-positive bacteria. As the efficiency of the defensive liquid extract increases, so do its antimicrobial capabilities, resulting in a clearer and bigger bacterial suppression zone (Balouiri et al., 2016).

The defense fluid extract also displayed antibacterial efficacy against the typical gram-negative and gram-positive bacterium, *E. coli* and *S. aureus*, with obvious inhibition zone findings at all doses tested (Skwarczynski et al., 2022).

The Inhibition zone (mm)			
Species of plant	Concentration (mg/ml)	<i>E. coli</i>	<i>S. aureus</i>
		Mean±SD	Mean±SD
Rhizome <i>K. parviflora</i>	0	6.84±0.262	6.33±0.320
	2	6.84±0.262	7.84±0.850
	4	9.33±0.255	7.30±0.130
	6	9.16±0.389	8.95±0.100
	8	10.32±0.453	11.33±0.200
	10	9.28±0.250	7.40±0.134
	10 (Streptomycin)	10.57±0.311	13.83±0.100

Table 4.2: The difference in the width of the inhibition zone of the liquid *K. parviflora* extract.

The Inhibition zone (mm)			
Species of plant	Concentration (mg/ml)	<i>E. coli</i>	<i>S. aureus</i>
		Mean±SD	Mean±SD
Rhizome <i>C. caesia</i>	0	8.60±0.544	10.94±0.100
	2	11.30±0.100	11.30±0.134
	4	11.63±0.200	8.72±0.100
	6	10.97±0.120	9.87±0.150
	8	11.30±0.100	7.70±0.100
	10	9.30±0.100	11.90±0.100
	10 (Streptomycin)	9.30±0.100	16.50±2.200

Table 4.3: The difference in the width of the inhibition zone of the liquid *C. caesia* extract.

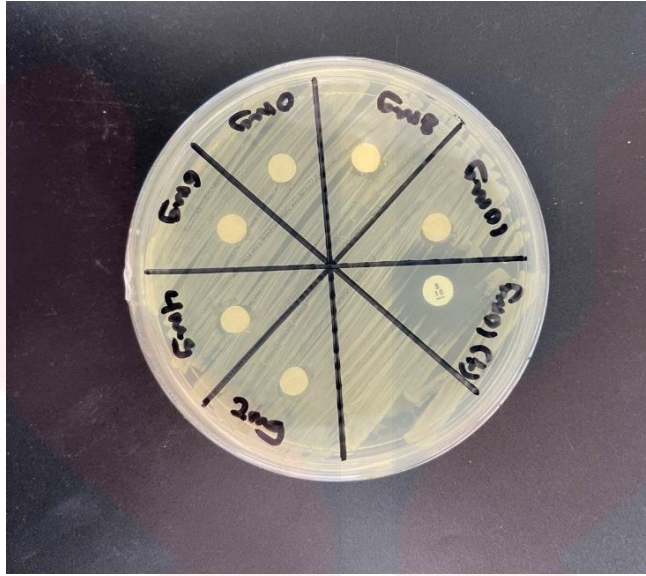


Figure 4.7: Disk diffusion plate of *K. parviflora*

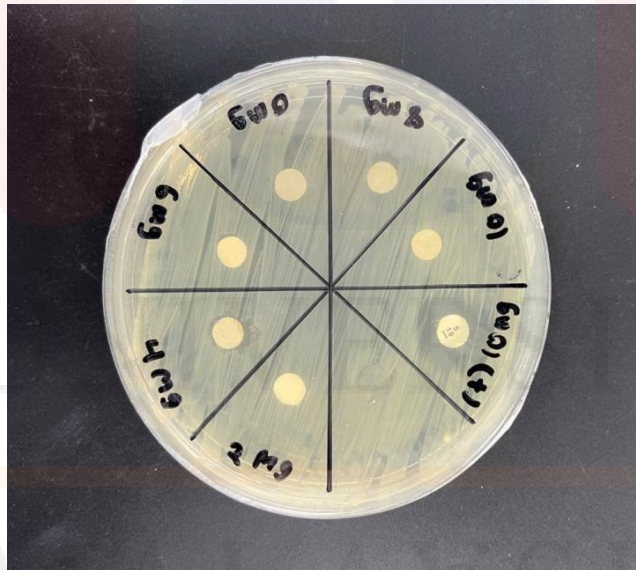


Figure 4.8: Disk diffusion plate of *C. caesia*

The percentage zone of bacterial inhibition was determined for two concentrations of both gram-positive and gram-negative bacteria, and the average results are shown in table 4.2 The difference in the width of the inhibition zone of the liquid *K. parviflora* extract and table 4.3 The difference in the width of the inhibition zone of the liquid *C. caesia* extract. Furthermore, the width of the inhibitory zone steadily expanded in proportion to the concentration of the protective liquid extract. The rise in inhibition zone width with isolate dilution suggests that bacteria grow more resistant to higher isolate concentrations.

In theory, the size of the inhibition zone is generally proportional to the degree of antimicrobial activity detected in the sample. A bigger zone of inhibition suggests more antibacterial activity and effectiveness. This notion is backed by the findings of this investigation. Table 4.2 The difference in the width of the inhibition zone of the liquid *K. parviflora* extract and table 4.3 the difference in the width of the inhibition zone of the liquid *C. caesia* extract.

Table 4.2 displays the values for the extracts of *K. parviflora* and *C. caesia* at concentrations (mg/ml) that exhibit significant antibacterial activity. *K. parviflora* has a smaller inhibition zone against *E. coli*, measuring 6.84 ± 0.262 mm, compared to *C. caesia* at the same dose, which has an inhibition zone of 8.60 ± 0.544 mm, indicating that black turmeric is more effective against these bacteria. Conversely, *K. parviflora* has a narrower inhibition zone of 6.33 ± 0.320 mm against *S. aureus*, compared to *C. caesia* at the same dose, which has an inhibition zone of 10.94 ± 0.100 mm, indicating that *C. caesia* may be more effective against these bacteria. These findings illustrate the variable potential of extracts depending on the type of bacteria and emphasize the potential of both plants as natural antibacterial agents.

The variance in mean concentration between different concentrations of *K. parviflora* and *C. caesia* rhizomes indicates that the two species have distinct biochemical compositions or metabolic activities. Understanding these distinctions may have consequences for a variety of fields, including herbal medicine and phytochemical studies. Further research into the precise chemicals responsible for these changes, as well as their potential biological activity, may shed light on the therapeutic or physiological qualities of these plants.

4.4 Determination of Minimum Inhibition (MIC) of *K. parviflora* and *C. caesia*

The results of the antimicrobial assay revealed that *K. parviflora* exhibited a MIC of 8 mg/mL, with average inhibition zones of 10.64 mm against *E. coli* and 11.46 mm against *S. aureus*. At a lower concentration of 2 mg/mL, the inhibition zones measured 9.70 mm against *E. coli* and 7.90 mm against *S. aureus*. *C. caesia* exhibited a MIC of 2 mg/mL against both *E. coli* and *S. aureus*, with inhibition zones measuring 11.37 mm and 11.38 mm, respectively. However, at higher concentrations (4 mg/mL), the inhibition zones were 11.63 ± 0.200 mm against *E. coli* and 8.72 ± 0.100 mm against *S. aureus*. The data indicate that the antimicrobial activity of *K. parviflora* was most pronounced at 8 mg/mL and almost equally effective at 2 mg/mL, whereas *C. caesia* antimicrobial activity was most pronounced at 2 mg/mL, with almost equal effectiveness at 4 mg/mL. Overall, the study establishes that both plant extracts possess significant antimicrobial activity against *E. coli* and *S. aureus*, with *K. parviflora* exhibiting stronger activity at higher concentrations and *C. caesia* showing stronger activity at lower concentrations.

5. CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In conclusion, based on the results obtained, the entire objective of this study was achieved. The plants *Kaempferia parviflora* and *Curcuma caesia* at the Ginger and Herbs Nursery, Institute of Food Security and Sustainable Agriculture, Universiti Malaysia Kelantan, were successfully identified. Gram-positive and gram-negative bacteria were identified through the gram staining method *Staphylococcus aureus* for gram-positive bacteria, and *Escherichia coli* for gram-negative bacteria. Furthermore, the antimicrobial activity of liquid raw extracts of *K. parviflora* and *C. caesia* selected for study was successfully examined. The results showed a significant difference in the efficiency of extracts from *K. parviflora* and *C. caesia* compared to commercial antibiotics such as gentamicin. Both *Staphylococcus aureus* and *Escherichia coli* bacteria exhibited inhibition zone diameters. These findings indicate that the antimicrobial properties of *K. parviflora* and *C. caesia* liquids can be a source of natural products that provide new solutions to the problem of bacterial resistance to antibiotics. They also serve as a reference for further studies on the antimicrobials of *K. parviflora* and *C. caesia*.

5.2 RECOMMENDATION

Based on the study's findings, several recommendations are made to improve the use of *K. parviflora* and *C. caesia* as antibacterial agents. Further study should be conducted to isolate and identify the individual active components that are responsible for their antibacterial properties. Clinical studies are required to determine its safety and efficacy in people. Understanding the mechanisms of action and investigating synergistic effects with conventional antibiotics might improve their efficacy. Standardizing the extraction process and finding suitable doses are critical for maintaining consistent potency. It is advised to do broader spectrum testing against numerous microorganisms, such as fungi and viruses. Creating practical therapeutic formulations, such as topical creams and oral supplements, will make them easier to utilize. Sustainable sourcing and cultivation procedures should be followed to avoid depletion of natural resources. It is critical to raise public and healthcare professional knowledge of the advantages of these extracts, as well as to encourage legislative support for future medicinal plant research and development. Implementing these guidelines would considerably improve the use of *K. parviflora* and *C. caesia* to battle bacterial illnesses, as well as contribute to the larger field of natural antimicrobial agents.

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APPENDIX

value	Descriptives								Between-Component Variance
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
E.coli_0mg/ml_Rhizome_K.p	2	6.8350	.26163	.18500	4.4844	9.1856	6.65	7.02	
E.coli_2mg/ml_Rhizome_K.p	2	9.3500	.49497	.35000	4.9028	13.7972	9.00	9.70	
E.coli_4mg/ml_Rhizome_K.p	2	9.3300	.25456	.18000	7.0429	11.6171	9.15	9.51	
E.coli_6mg/ml_Rhizome_K.p	2	9.0550	.38891	.27500	5.5608	12.5492	8.78	9.33	
E.coli_8mg/ml_Rhizome_K.p	2	10.3200	.45255	.32000	6.2540	14.3860	10.00	10.64	
E.coli_10mg/ml_Rhizome_K.p	2	9.2800	.22627	.16000	7.2470	11.3130	9.12	9.44	
E.coli_strepto10mg/ml_Rhizome_K.p	2	10.5700	.31113	.22000	7.7746	13.3654	10.35	10.79	
E.coli_0mg/ml_Rhizome_C.c	2	8.5850	.54447	.38500	3.6931	13.4769	8.20	8.97	
E.coli_2mg/ml_Rhizome_C.c	2	11.3000	.09899	.07000	10.4106	12.1894	11.23	11.37	
E.coli_4mg/ml_Rhizome_C.c	2	11.6300	.18385	.13000	9.9782	13.2818	11.50	11.76	
E.coli_6mg/ml_Rhizome_C.c	2	10.9750	.12021	.08500	9.8950	12.0550	10.89	11.06	
E.coli_8mg/ml_Rhizome_C.c	2	11.2550	.07778	.05500	10.5562	11.9538	11.20	11.31	
E.coli_10mg/ml_Rhizome_C.c	2	9.2650	.06364	.04500	8.6932	9.8368	9.22	9.31	
E.coli_strepto10mg/ml_Rhizome_C.c	2	9.2550	.07778	.05500	8.5562	9.9538	9.20	9.31	
St_0mg/ml_Rhizome_K.p	2	6.3250	.31820	.22500	3.4661	9.1839	6.10	6.55	
St_2mg/ml_Rhizome_K.p	2	7.8400	.08485	.06000	7.0776	8.6024	7.78	7.90	
St_4mg/ml_Rhizome_K.p	2	7.2900	.12728	.09000	6.1464	8.4336	7.20	7.38	
St_6mg/ml_Rhizome_K.p	2	8.9450	.07778	.05500	8.2462	9.6438	8.89	9.00	
St_8mg/ml_Rhizome_K.p	2	11.3250	.19092	.13500	9.6097	13.0403	11.19	11.46	
St_10mg/ml_Rhizome_K.p	2	7.3950	.13435	.09500	6.1879	8.6021	7.30	7.49	
St_strepto 10mg/ml_Rhizome_K.p	2	13.8300	.09899	.07000	12.9406	14.7194	13.76	13.90	
St_0mg/ml_Rhizome_C.c	2	10.9450	.09192	.06500	10.1191	11.7709	10.88	11.01	
St_2mg/ml_Rhizome_C.c	2	11.2850	.13435	.09500	10.0779	12.4921	11.19	11.38	
St_4mg/ml_Rhizome_C.c	2	8.7250	.07778	.05500	8.0262	9.4238	8.67	8.78	
St_6mg/ml_Rhizome_C.c	2	9.8750	.14849	.10500	8.5408	11.2092	9.77	9.98	
St_8mg/ml_Rhizome_C.c	2	7.6650	.06364	.04500	7.0932	8.2368	7.62	7.71	
St_10mg/ml_Rhizome_C.c	2	11.8900	.05657	.04000	11.3818	12.3982	11.85	11.93	
St_strepto 10mg/ml_Rhizome_C.c	2	16.4900	.08485	.06000	15.7276	17.2524	16.43	16.55	
Total	56	9.8868	2.15968	.28860	9.3084	10.4652	6.10	16.55	
Model	Fixed Effects		.23276	.03110	9.8231	9.9505			
	Random Effects			.41068	9.0441	10.7294			4.69540

Test of Homogeneity of Variances

value	Levene Statistic	df1	df2	Sig.
Based on Mean	2.552E+28	27	28	.000
Based on Median	2.552E+28	27	28	.000
Based on Median and with adjusted df	2.552E+28	27	7.506	.000
Based on trimmed mean	1.010E+28	27	28	.000

ANOVA

value

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	255.015	27	9.445	174.331	.000
Within Groups	1.517	28	.054		
Total	256.532	55			

Robust Tests of Equality of Means

value

	Statistic ^a	df1	df2	Sig.
Welch	351.950	27	10.012	.000
Brown-Forsythe	174.331	27	9.261	.000

a. Asymptotically F distributed.



value

Tukey HSD^a

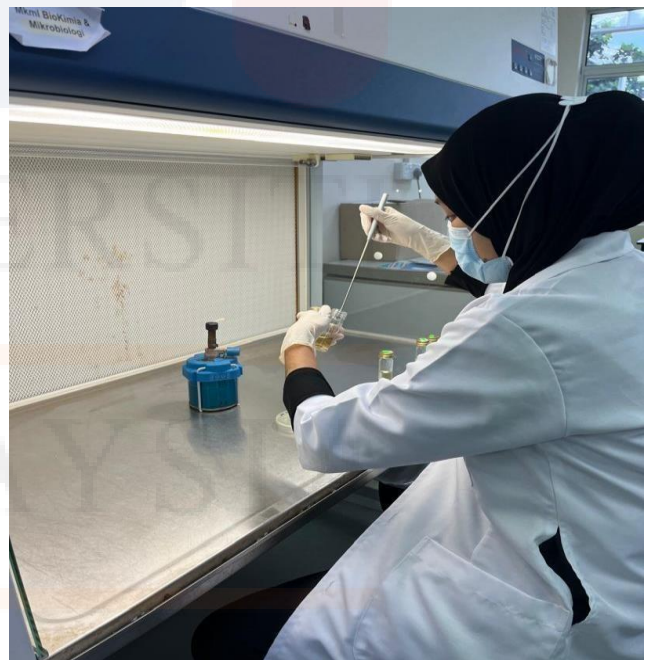
Concentration (mg/ml)	N	Subset for alpha = 0.05															
		1	2	3	4	5	6	7	8	9	10	11	12	13			
St_0mg/ml_Rhizome_K.p	2	6.3250															
E. coli_0mg/ml_Rhizome_K.p	2	6.8350	6.8350														
St_4mg/ml_Rhizome_K.p	2		7.2900	7.2900													
St_10mg/ml_Rhizome_K.p	2		7.3950	7.3950													
St_8mg/ml_Rhizome_C.c	2		7.6650	7.6650	7.6650												
St_2mg/ml_Rhizome_K.p	2			7.8400	7.8400	7.8400	7.8400										
E. coli_0mg/ml_Rhizome_C.c	2				8.5850	8.5850	8.5850										
St_4mg/ml_Rhizome_C.c	2					8.7250	8.7250										
St_6mg/ml_Rhizome_K.p	2						8.9450	8.9450									
E. coli_6mg/ml_Rhizome_K.p	2						9.0550	9.0550									
E. coli_strepto10mg/ml_Rhizome_C.c	2							9.2550	9.2550								
E. coli_10mg/ml_Rhizome_C.c	2							9.2650	9.2650								
E. coli_10mg/ml_Rhizome_K.p	2							9.2800	9.2800								
E. coli_4mg/ml_Rhizome_K.p	2							9.3300	9.3300								
E. coli_2mg/ml_Rhizome_K.p	2							9.3500	9.3500								
St_6mg/ml_Rhizome_C.c	2								9.8750	9.8750							
E. coli_8mg/ml_Rhizome_K.p	2									10.3200	10.3200						
E. coli_strepto10mg/ml_Rhizome_K.p	2									10.5700	10.5700	10.5700					
St_0mg/ml_Rhizome_C.c	2										10.9450	10.9450	10.9450				
E. coli_6mg/ml_Rhizome_C.c	2										10.9750	10.9750	10.9750				
E. coli_8mg/ml_Rhizome_C.c	2										11.2550	11.2550	11.2550				
St_2mg/ml_Rhizome_C.c	2											11.2850	11.2850				
E. coli_2mg/ml_Rhizome_C.c	2											11.3000	11.3000				
St_8mg/ml_Rhizome_K.p	2												11.3250	11.3250			
E. coli_4mg/ml_Rhizome_C.c	2													11.6300			
St_10mg/ml_Rhizome_C.c	2														11.8900		
St_strepto 10mg/ml_Rhizome_K.p	2															13.8300	
St_strepto 10mg/ml_Rhizome_C.c	2																16.4900
Sig.		.886	.157	.806	.071	.097	.261	.064	.419	.061	.281	.056	1.000	1.000			

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

FYP FSB





KELANTAN