EXAMINING MICROFLORA DYNAMIC LINKED TO FEMALE *RAFFLESIA KERRI* IN LOJING HIGHLAND, KELANTAN

by

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DECLARATION

I declare that this entitled "Examining Microflora Dynamic Linked to Female Rafflesia Kerri in Lojing Highland, Kelantan" is the result of my own research except as cited in the reference. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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LIST OF ABBREVIATIONS

F1S1	Flower 1 Stage 1
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F2S1	Flower 2 Stage 1
F2S2	Flower 2 Stage 2
F2S3	Flower 2 Stage 3
F3S1	Flower 3 Stage 1
F3S2	Flower 3 Stage 2
F3S3	Flower 3 Stage 3
ml	milimeter
L	Liter
LB	Luria Broth
SIM	Sulfur Indole Motility
NaCI	Sodium Chloride

LIST OF SYMBOLS

%	Percentage
℃	Temperature (Degree Celcius)

MEMERIKSA DINAMIK MIKROFLORA YANG BERKAITAN DENGAN RAFFLESIA KERRI BETINA DI LOJING HIGHLAND, KELANTAN

ABSTRAK

Genus Rafflesia, yang terdapat dalam keluarga *Rafflesiaceae* di hutan hujan tropika Asia Tenggara, adalah bunga terbesar di dunia. *Rafflesia kerri* Meijer, ditemui di Tanah Tinggi Lojing, adalah spesies holoparastik yang bergantung pada pokok *Tetrastigma* untuk kelangsungan hidup, perlindungan, dan khasiat, sama seperti spesies lain dalam genus ini. Penghuni bakteria dalam *Rafflesia kerri* telah diambil sampel untuk menilai ketumpatan mikroflora sebagai tindak balas yang berbeza-beza kepada keadaan peringkat mekar debunga *Rafflesia kerri*. Kajian yang dijalankan bertujuan untuk menilai kepadatan mikroflora dan aktiviti biokimia sebagai tindak balas kepada pelbagai peringkat pembungaan *Rafflesia kerri*. Analisis tiga bunga betina pada peringkat mekar yang berbeza mendedahkan variasi dalam komposisi bakteria sebanyak 55.56% gram-positif dan 44.44% hram-negatif. Kajian itu menyimpulkan bahawa aktiviti mikrobiologi bakteria mempengaruhi baunya, membantu menarik lalat keluarga *Diptera* untuk proses pendebungaan. Selain itu, bunga *Rafflesia*, yang terkenal dengan ciri uniknya, adalah bunga individu terbesar di dunia, dengan beberapa spesies mencapai diameter lebih tiga kaki. Ia terkenal dengan bau busuknya, sering disamakan dengan bau daging busuk, yang menarik lalat untuk pendebungaan. Bunga *Rafflesia* tidak mempunyai batang, daun dan akar, bergantung pada tumbuhan perumahnya untuk nutrien dan sokongan. Ciri-ciri tersendiri ini menjadikan bunga *Rafflesia* sebagai subjek kajian yang menarik dalam dunia botani.

EXAMINING MICROFLORA DYNAMIC LINKED TO FEMALE RAFFLESIA KERRI IN LOJING HIGHLAND, KELANTAN

ABSTRACT

The *Rafflesia* genus, found in the *Rafflesiaceae* family in the tropical rainforests of Southeast Asia, is the largest flower in the world. *Rafflesia kerri* Meijer, found in the Lojing Highlands, is a holoparastic species that depends on *Tetrastigma* trees for survival, protection, and nutrition, just like other species in this genus. The bacterial population in *Rafflesia kerri* was sampled to assess microflora density in response to varying conditions of the *Rafflesia kerri* pollen bloom stage. The study was conducted to evaluate microflora density and biochemical activity in response to various stages of *Rafflesia kerri* flowering. Analysis of three female flowers at different blooming stages revealed a variation in bacterial composition of 55.56% gram-positive and 44.44% gram-negative. The study concluded that the microbiological activity of the bacteria affects its smell, helping to attract flies of the *Diptera* family for the pollination process. Additionally, the *Rafflesia* flower, known for its unique characteristics, is the largest individual flower in the world, with some species reaching over three feet in diameter. It is known for its foul smell, often likened to the smell of rotting meat, which attracts flies for pollination. *Rafflesia* flowers have no stems, leaves and roots, relying on their host plants for nutrients and support. These distinctive features make the *Rafflesia* flower an interesting subject of study in the botanical world.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Rafflesia kerri is only distributing within the mountain range of Southern Thailand to Kelantan and Perak, Peninsular Malaysia (Norhazlini et al., 2020; Kedri et al., 2018; Nasihah et al., 2016). The occurrence of *R. kerri* in Lojing Highlands, Kelantan, Peninsular Malaysia has attracted many local tourists as well as those from abroad. In Peninsular Malaysia, the species was initially discovered by Forest Gan and Matthew Wong in 2002 on the Main Range at the border of Kelantan and Perak. Then, further explorations were discovered new populations at Lojing Highlands (Wong & Gan, 2003).

Nais (2001) explained *Rafflesia* flower blooms only occur for the duration of eight to twelve days, and thus pollination process essential to be happened within this short period. High visitation frequency and high number of pollinators to visit the flower will increase the pollination success rate. The foul scent emitted believed to play the major role in attracting the pollinators to visit the flower. Therefore, albeit *Rafflesia* grown deep in the forested area, a strong deceitful smell acts as chemical cues release to the environment during the fresh blooming stage, favour the pollinators to locate the flower even from far distance. An update by Norhazlini et al. (2020) on floral scent emission of *R. kerri* in Lojing Highlands, listed the floral volatile constituents were from various chemical classes such as, long chain hydrocarbons, organosilicon compounds, primary alcohols, aromatic acids as well as miscellaneous compounds.

1.2 Problem Statement

The problem statement draws attention to a substantial knowledge vacuum regarding the microfloral dynamics of female *Rafflesia kerri* in Lojing Highland, Kelantan. There is a worrying drop in the population of *Rafflesia,* with a particular mention of *R. kerri,* despite the area's recognized high species diversity of the genus *Rafflesia,* especially in the Lojing Highland. This decrease emphasizes how critical it is to look into and comprehend the variables affecting *R. kerri's* overall health and ability to reproduce.

The current knowledge gap is exacerbated by the lack of previous research on the microflora dynamics associated with female *R. kerri* in the Lojing Highland. *Rafflesia* plant microflora is vital to many ecological processes, such as pollination and nutrient cycling, making it an integral part of the plant's reproductive ecology.

The aim of this study is to close this gap by measuring the density of microflora in response to different environmental factors surrounding female *R. kerri*. This involves investigating how environmental factors affect the *R. kerri*-associated microflora community. It is important to comprehend the complex relationships between *R. kerri* and its associated microflora, as highlighted by the recognition of the potential influence of these factors on the decline in *Rafflesia* populations.

Therefore, the study's objectives are to present an exhaustive list of microflora that have been quantified and evaluate the biochemical activity of particular microflora species linked to female *R. kerri*. This multidisciplinary

approach will help identify the microflora species that are present and provide insight into their interactions and functional roles within the *R. kerri* ecosystem. The result obtained from this study could play a crucial role in guiding management and conservation strategies aimed at mitigating the decline of *Rafflesia* populations in the Lojing Highlands, with a particular focus on *R. kerri.*

1.3 Objective of the Study

The aim of the study is to examine the microflora dynamic linked to female *Rafflesia kerri* in Lojing Highland, Kelantan. Meanwhile, the specific objectives are as follows:

i) To quantify microflora density in response to the blooming stages of female *Rafflesia kerri*.

ii) To determine the biochemical activity of selected microflora species associated with female *Rafflesia kerri.*

1.4 Scope of the Study

A field survey was conducted in the Rafflesia Conservation Park, Lojing Highland, Kelantan, during of 14 February 2024 to 3 March 2024. The focus of this study is on the female flowers of *Rafflesia kerri*. The initial phase involves the identification of female flowers. To facilitate sample collection, a process was followed where the sample was swabbed using a sterile swab and then stored in Luria Broth (LB). Sampling was carried out across various flower stages to assess variable levels of density. The collected samples were then transferred to the laboratory for further analysis. The process was requiring the use of a cast plate method to effectively isolate microbial colonies, followed by quantification which involves counting colony forming units (CFU). Afterwards, dominant colonies

were selected for comprehensive biochemical testing, allowing accurate identification of the various microbial species associated with female *R. kerri*.

1.5 Significant of the Study

An important knowledge gap in our comprehension of the ecological interactions within this distinct ecosystem is revealed by the analysis of the microflora dynamics associated with female *R. kerri* in the Lojing Highland, Kelantan. Research on this particular topic is conspicuously lacking, despite the region's known diversity of *Rafflesia* species.

It is difficult to understand the complex interactions between *R. kerri* and its microbial environment because of this lack of information. Numerous bacteria, fungi, and other microorganisms collectively known as microflora are essential to ecological processes like nutrient cycling, symbiotic relationships, and plant health. Lack of a focused investigation on the microflora dynamics associated with female *R. kerri* restricts our comprehension of the elements affecting the reproductive success and general well-being of this distinct plant species.

The populations of *Rafflesia* in the Lojing Highlands are currently exhibiting a declining trend. This raises questions about possible ecological imbalances or environmental stressors that may be affecting this iconic plant. Determining the underlying ecological dynamics causing the observed population decline requires examining the microflora linked to female *R. kerri.*

Therefore, by investigating the microflora dynamics associated with female *R. kerri* in the Lojing Highland, Kelantan, this study seeks to close this knowledge gap. In order to shed light on the composition, abundance, and possible interactions between the microbial community and the plant, the study aims to quantify and characterize the microflora associated with *R. kerri*. The findings are expected to contribute valuable insights into the ecological requirements of *R. kerri*, facilitating informed conservation efforts and enhancing our understanding of the broader ecosystem dynamics in the Lojing Highland.

CHAPTER 2

LITERATURE REVIEW

2.1 *Rafflesia kerri* in General

Rafflesia, a parasitic flowering plant genus in *Rafflesiaceae*, is one of the most astonishing flowers ever discovered in the botanical world. The reason why the Rafflesia plant is so popular as a tourist attraction is that it is rare, gorgeous, and exclusive to Southeast Asia, making it an attractive sight for visitors. (Peters & Ting, 2016). According to Siti Munirah et al. (2021), these are 14 species of Rafflesia discovered in Malaysia. This unique flower is only available in five countries and only in the Southeast Asia which are Malaysia, Indonesia, the Philippines, Brunei and Thailand. Thailand has only species which is *R. kerri* unlike other that has more than one species. *R. kerri* was initially described from Ban Lam Lieng in Surat Thani, Thailand (Meijer, 1984).

R. kerri is a member of genus Rafflesiaceae and it is a family of holoparasite flowering plant for being one of the world's largest flowers after *R. arnoldii*. The smallestRafflesia in average among its genus is *R. verrucosa* that found in the Philippines (Balete et al., 2010). It lives as a holoparasite plant depending entirely on a host plant as liana due to lack of root, stem and leaf (Rinrampai et al., 2014).

According to Essays (2013), when the flower buds of *R. kerri* begin to bloom, the dimensions of the flower opening are in the range of 50-70 cm in diameter. The length of the perigone inner lobe is 13-20 cm and the width is around 19-24 cm. Petals of *R. kerri* are dull red with brownish brown color and have numerous pf warts with 3-4 mm slots between them.

R. kerri has the smallest warts among the Rafflesia family. In addition, the opening of the flower diaphragm is about 12-17 cm wide and the upper face of the flower is 3-4concentric rings of white spots surrounded by a dark red border (Essay, 2013).

Another characteristic of *R. kerri* flowers are the mature buds which is about 16-25 cm in diameter. Ramenta flowers are often unbranched and with a slight bulge at the apex. The upper type close to the diaphragm is about 10 mm long while the lower type close to the flower tube is about 5 mm long. The total number of *R. kerri* anthers is approximately 26-31 (Essay, 2013).

According to the natives in Thailand, they believe that *R. kerri* flower buds are one of the traditional medicines that can be used to heal the womb of women after giving birth and to strengthen their energy of men. Some natives sell it because most of them still believe that *R. kerri* has medicinal value and even now the buds can be seen being sold at a very cheap price to the local people themselves in the market. The survival of *R. kerri* is threatened due to excessive collection by local people including the destruction of its habitat (Meijer & Elliott, 1990).

2.2 Distribution and Ecology of *Rafflesia*

Rafflesia can only be found in Southeast Asian tropical rainforests. According to Bendiksby et al. (2010). An estimated of 40 species of *Rafflesia* are recorded in Peninsular Malaysia, Sumatra, Java, Borneo, and the Philippines. The following table shows the distribution of *Rafflesia* species in Southeast Asia (Table 2.2)

Table 2.2 Distribution of *Rafflesia* species in Southeast Asia (Norhazlini et al., 2022)

Localities	Species
Borneo	*R. arnoldii* R. Br., *R. borneensis* Koorders, *R. ciliata* Koorders, *R. keithii* Meijer, *R. pricei* Meijer, *R. Tengku-adlinii* Mat Salleh & Latiff, *R. tuan-mudae* Beccari, *R. witkampii* Koorders
Java	*R. patma* Blume, *R. rochussenii* Teijs. & Binn., *R. zollingeriana* Koorders
Peninsular Malaysia	*R. cantleyi* Solms, *R. kerrii* Meijer, *R. azlanii* Latiff & Wong, *R. su-meiae* Wong, Nais, & Gan, *R. Sharifah-hapsahiae* Adam, Mohamed, Aizat-Juhari & Wan, *R. parvimaculata* Sofiyanti, Mat-Salleh, Khairil, Zuhailah, Mohd. Ros & Burslem, *R. tuanku-halimii* Adam, Aizat - Juhari, Azilah & Wan, *R. tiomanensis* Siti-Munirah, Salamah & Razelan, *R. tunku-azizahiae*
The Philippines	*R. balatei* Barcelona & Cajano, *R. leonardi* Barcelona & PeIser, *R. lobata* Galang & Madulid, *R. manillana* Tescehemacher, *R. mira* Fernando & Ong, *R. philippensis* Blanco, *R.schadenbergiana* Geoppert, *R. speciosa* Bercelona & Fernando, *R. verrucosa* Balete, PeIser, Nickrent & Barcelona, *R. lagascae* Blanco, *R. aurantia* Barcelona, Co & Balete, *R. mixta* Barcelona, Manting, Arbolonio, Caballero & Pelser, *R. consueloae* Galindon, Ong & Fernando
Sumantra	*R. arnoldii* R. Br., *R. gadutensis* Meijer, *R. hasseltii* Suringar, *R. micropylora* Meijer, *R. kemumu* Susatya, Hidayati & Riki, *R. atjehensis* Koorders., *R. bengkuluensis* Susatya, Arianto & Mat-Salleh, *R. lawangensis* Mat-Salleh, Mahyuni & Susatya, *R. meijer* Wiriadinata

& Sari

Thailand *R. kerri* Meijer

2.3 Life Cycle of *Rafflesia*

Figure 2.3 shows the life cycle undertaken by Rafflesia to reach completion. The life cycle of Rafflesia comprises eight stages, initiating with the host organ swelling and culminating in the fully opened flower at Stage 5. Subsequently, the cycle progresses through the maturation of the fruit, spanning from Stage 6 to Stage 8, wherein the seed germinates and inoculates the host plant. The entire process of the Rafflesia life cycle requires approximately two years for completion (Nais, 2004).

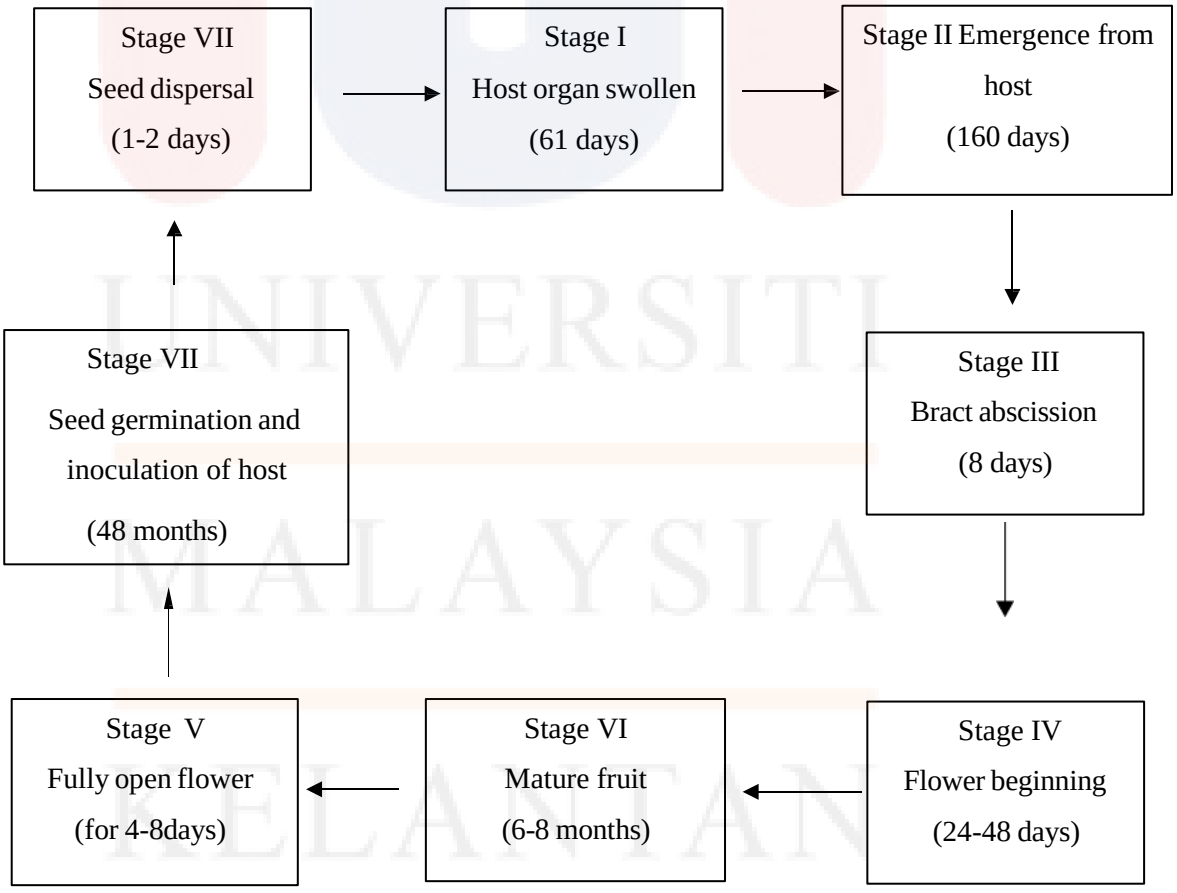

Figure 2.3 The Life Cycle of *Rafflesia* redrawn from Nais (2004).

2.4 Morphology of *Rafflesia*

Not only the size, the morphology structure of *Rafflesia* is very attractive. One of the things that draws tourists to these blooms is their typical colour, which is either orange or red. In contrast to the surrounding forest, which is replete with shades of green and brown from the leaves and tree trunks, the colour of this bloom is very striking. Every *Rafflesia* species has unique traits that might represent it. Even though the species appear to be nearly identical, there are structural differences that allow the species to be distinguished from one another. Examples of these differences include aperture size (Sofiyanti et al., 2007), white spot size (Nais, 2001), flower size, and flower shape (Mat Salleh, 1991). Furthermore, the morphology of the internal structure that is not easily seen without careful observation is one of the distinguishing characteristics of *Rafflesia* species.

Rafflesia flowers typically have five petals. Petals from Rafflesia flowers are thicker and have more white bumps on their surface than petals from other flowers. The *Rafflesia* flower has a sizable hole in the middle that is referred to as the aperture. A column is the structure that is located beneath the aperture (Mat Salleh, 1991). The column's surface is populated by discs and processes. There are windows and ramenta on the side of the column wall (Sofiyanti et al., 2007). Sofiyanti et al., (2007) also stated that the morphology inside the perigon tube is known as the internal morphology. The diaphragm is the structure on the surface of *Rafflesia* that lies between the hole and the petals. In addition to flower petals, white nodules are present on the diaphragm's surface (Nais, 2001).

Rafflesia's morphological parts that are visible to the unaided eye only consist of the flower portion; *Tetrastigma,* the part connecting the host tree, is hidden. The component enters the host's tissue directly and resembles a fine hair (Nais, 2001; Mat Salleh, 1991). The danger that this fine hair poses to its host tree is not instantaneous, according to Mat Salleh (1991). The parasitic flower poses a constant threat to its host tree because it slowly erodes it as it absorbs nutrients and water from *Tetrastigma* (Akhriadi et al., 2010; Nais, 2001).

Safiyanti et al. (2012) claimed that before they mature, *Rafflesia* seeds typically have a brown, chestnut-shaped shape. The ovule's shape is nearly identical to that of the seed, but its size varies according to the flower's diameter. The size of the ovules and pollen increases with the diameter of the *Rafflesia* flower. *Rafflesia* ovules are white, shaped like a J, and have an uneven attachment to the placenta before they mature (Sofiyanti et al., 2012). Typically, the smooth surface of pollen contains a single pore. Despite originating from fine, powdered grains, pollen disperses both individually and in clusters. The presence of a yellow mush adhering to the powder grains is the contributing factor to this dispersal behaviour.

2.5 The Host Plant of *Rafflesia*

In tropical and subtropical regions of Asia, the species *Tetrastigma* (Liana) serves as the host plant for *Rafflesia* (Barcelona et al., 2006). *Rafflesia* is well known for lacking any kind of vegetative structure, including a stem and leaves. Its thick, long, and woody stem and tail, which are largely visible at higher elevations, are other reasons why it is also said to be a giant vine. It can be found in trees, bushes, forest margins, and riverbanks alongside roadways (Latiff,

1984). It takes up all the minerals and nutrients required from its host plant. Table 2.5 provides an overview of the updated host plant species that are parasitized by *Rafflesia* in Malaysia.

Table 2.5. The updated of host plant species parasitized by *Rafflesia* in Malaysia.

Host plant species	**Rafflesia species**
T. diepenhorstii	*R. tuan-mudae*
T. rafflesiae	*R. azlanii, R. cantleyi, R. keithii, R. kerri, R. parvimaculata, R. pricei, R. sharifahhapsahiae, R.su-meiae, R. tengku-adlinii, R. tuan mudae, R. tuanku-halimii*
T. hookeri, T. quadrangulum	*R. kerri*
T. papillosum	*R. pricei*

Sources: Ahmad Puad et al. (2020); Kedri et al. (2018); Wan Zakaria et al. (2016); Nasihah et al. (2016); Veldkamp (2009); Nais (2001); Latiff (1984).

Three hierarchical levels can be used to describe the relationship between *Rafflesia* and its host plant: internal (individual level), macroevolutionary (species level), and microevolutionary (population level), which includes host-parasite co- evolution. According to Nais (2001), the host is involved in how the tissue is twisted around and the nutrients can be accessed. The first level, or internal relationship, is connected to the troops of the internal tissue of the parasite flower *(Rafflesia)*. *Rafflesia* and *Tetrastigma* are connected by the haustorium. Haustoria can form from the roots, storage, absorption and anchoring. Endophyte is the term for this haustoria fragment (Nais, 2001).

The host-plant relationship progresses to the population level, as highlighted by Nais (2001). This level is connected to both the *Rafflesia* bud's relationship with its host plant and the distribution of the level population. Within the population, variables such as bud abortion, flower set, growth rate, and flower size can be identified. This host plant's distribution pattern is dispersed randomly. In the meantime, the evolution of the flower on different host plant species may be the cause of the co-evolution of the host and parasite. Through the use of molecular techniques, the co-evolution of the parasite and the host plant may be resolved.

2.6 Conservation of *Rafflesia*

The natural habitat for *Rafflesia* is tropical rainforest. Nevertheless, Malaysia is experiencing swift changes in land use, with numerous natural forest areas being transformed for development purposes, including large-scale plantations, residential zones, urbanization, and the establishment of road networks This will eventually have a detrimental effect on this iconic species. Rafflesia is characterized by a significant gender imbalance, a limited distribution range, elevated bud mortality, a low rate of pollination success, a diminished fruit set rate, and a dependence on specific host plants. Given these distinctive traits, it becomes imperative for the government to sustain and strengthen its conservation endeavors for the preservation of this unique flower (Hidayati & Walck, 2016).

There are two methods for conserving or protecting the Rafflesia species, these are *ex-situ* and *in-situ* conservations. According to Nais (2001), *in-situ* conservation refers to the preservation of a species within its natural habitat, whereas *ex-situ* conservation involves relocating the species to an off-site location

and planting it away from its natural surroundings. In addition to employing laboratory techniques like tissue culture for their propagation and preservation, this strategy involves conserving the entire plant or a portion of it in botanical gardens and gene banks. However, there have been numerous failed attempts to use the tissue culture method with *Rafflesia* species.

According to Peters & Ting (2016), Sabah Parks were undertaken three Rafflesia conservation initiatives in Sabah. In Sabah, three *Rafflesia* conservation initiatives are being implemented by Sabah Parks, according to Peters & Ting (2016). These initiatives include the creation of a *Rafflesia* reserve, an information centre, and a conservation incentive programme. In order to encourage indigenous people (*Dusun*) to participate in the programme, the government is also involved in the creation of the Rafflesia Conservation Incentive Scheme.

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of the Study Area

The study area is located between 4º32' and $4^0$47' N in latitude and between 101º20' and 101º34' E in longitude (Qayyum, 2015; Nasihah et al., 2013). at the *Rafflesia* Conservation Parks, near to Kg. Jedip in Lojing Highland, Kelantan as shown in Figures 3.1a and 3.1b (Dony et al., 2015. Sg. Dawai (N 04 38’02.8”, E 101 30’16.3”) and Sg. Dekong (N 04 38’02.8”, E 101 30’16.3”) are the two main rivers in Lojing Highland (Sharifah et al., 2014).

Covering 1,817 square kilometers, Lojing Highland is situated in the southwest of the State of Kelantan. This highland has an approximate elevation of 610–1,500 meters above sealevel with the temperature range of 18^OC–25^OC degrees Celsius (Dony et al., 2014).

Lojing Highland is a naturally occurring hilly forest with breathtaking views between 1,000 and 2,300 meters above sea level. The area is situated near the Pahang border in Cameron Highlands. Some of the forest areas in Lojing Highland were cleared for agricultural purposes (Dony & et al., 2014).

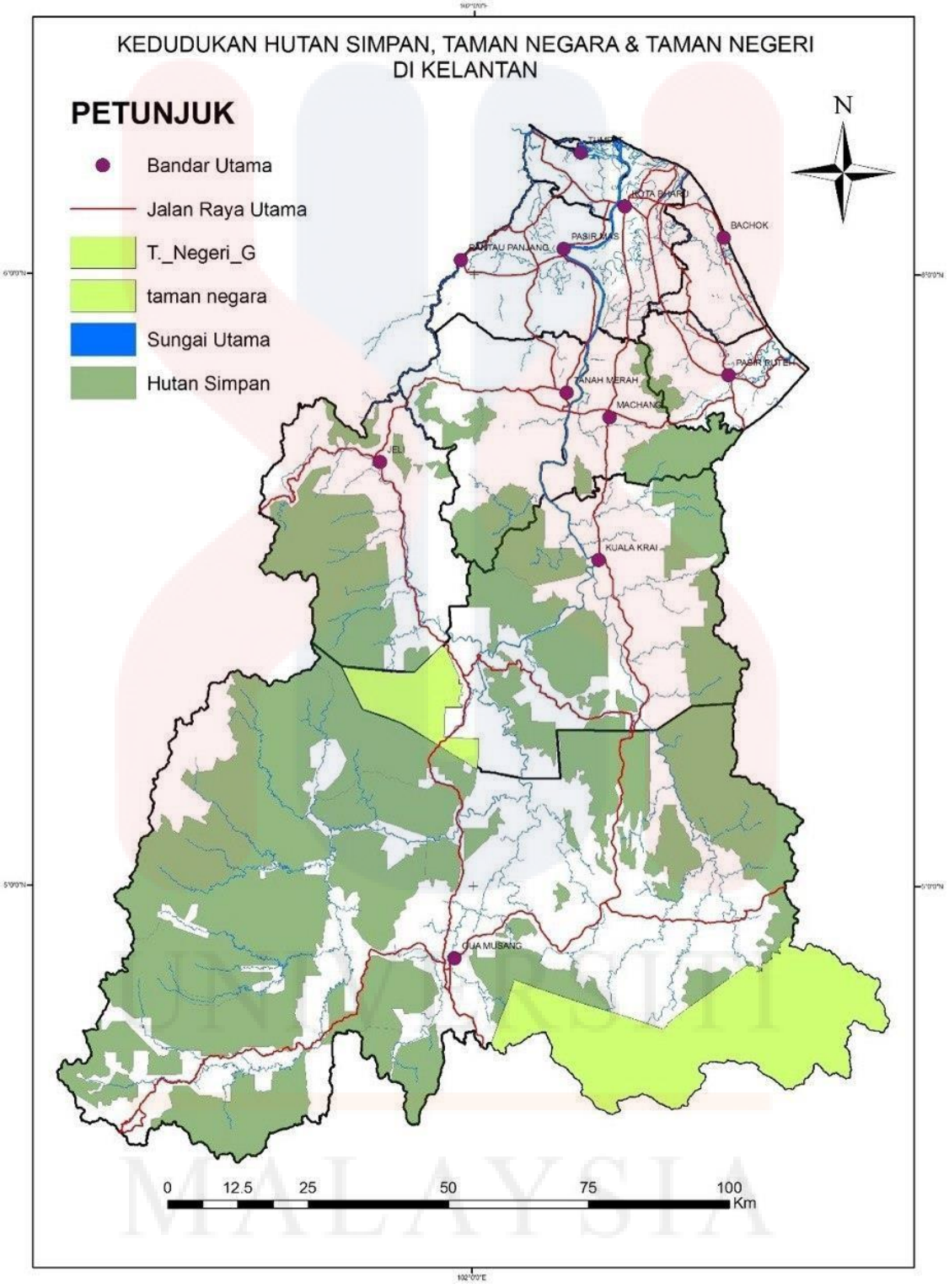

Figure 3.1a. Locations of study area, Forest Reserves, National Parks, State Parks in Kelantan Malaysia

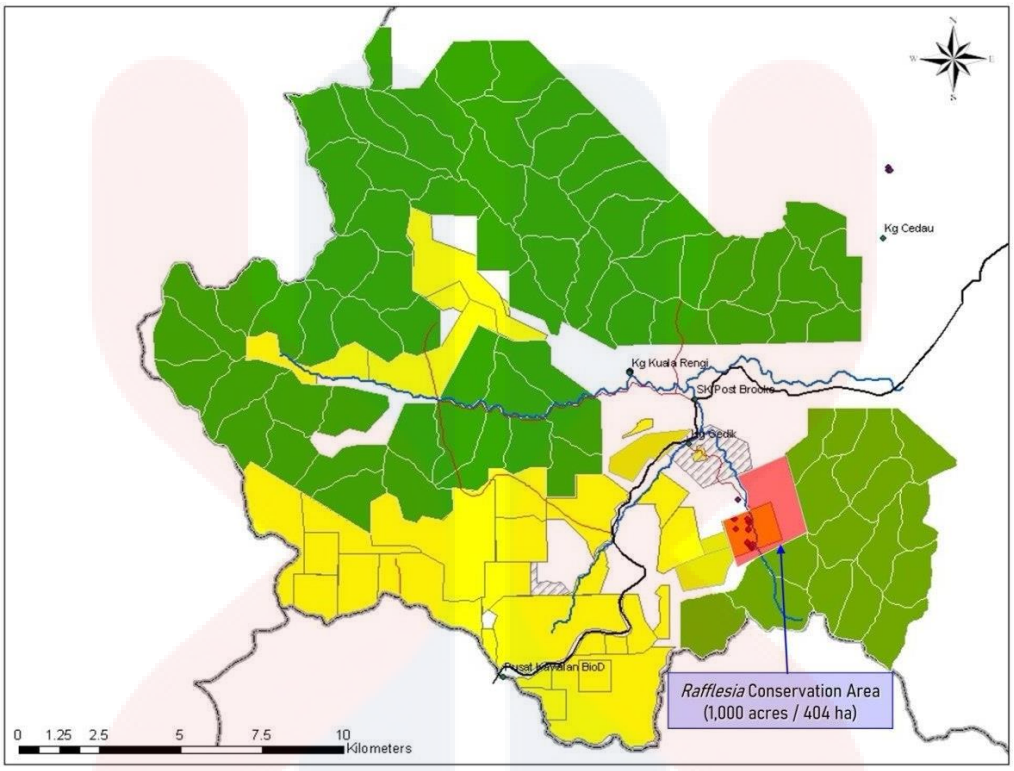

Figure 3.1b. Close up view of study area; Rafflesia Conservation, Area Lojing Highlands Kelantan, Malaysia

3.2 Materials

The materials utilized in this study are present in Table 3.2 below

Table 3.2. The material used and their functions

No	INSTRUMENT AND CHEMICAL USED	FUNCTIONS
1	GPS Garmin Montana	To map the location of the collected samples
2	Sterilized wooden swab stick	To collect the sample
3	Centrifuge tube	To contain liquid during centrifuge
4	Distilled water	To sterilization
5	Ethanol (80%)	To disinfect and prevent the cross contamination while collecting the sample
6	Glove	To reduce the cross contamination while collecting the sample
7	Tissue paper	To wipe the tool used
8	Ziploc bag 11'x16'	To store the sample collected in a centrifuge tube
9	Blue cap bottle (1L)	To collect the filtered solution

10	Petri dish	To culture different type of cells
11	Stirrer	To agitate the liquid and mixtures
12	Distilled water	To sterilization
13	Laminar Flow	To prevent airborne contamination
14	Hirayama HV-10 Autoclave Machine	To kill harmful bacteria
15	Bunsen burner	To sterilize objects on high heat
16	Weighing boat	To weighing, evaporating and dispensing
17	Electronic balance	To determine an objective mass
18	1000µL micropipette	To measures and transfer liquid solution
19	Forcep	To hold the filter paper
20	Parafilm	To sealing if container
21	Incubator	To grow and maintain cell culture
22	Glove	To reduce the cross contamination while collecting the sample
23	Inoculation loop	To transfer and spreading sample
24	Rubber bulb	To vacuum for filling reagents
25	Sterile Pasteur pipette	To transfer liquid and sample
26	Light microscope	To visualize bacteria in great detail
27	Slide cover	To hold and protect specimen from contamination environment
28	Inoculating needles	To transfer and spreading sample
29	Glass slide	To support sample which need to examined under microscope
30	Kovac's reagent	To determine the ability bacteria to split indole
31	MR-VP broth	To qualitative procedure for MR-VP test
32	Methyl red	to determine pH transition
33	Barritt's reagent A and B	To detection of acetoin production by bacterial
34	Crystal violet	To stain the nuclei of adherent cells
35	Methylene blue	To help make cell show up against their background
36	Glycerol stock	To preventing damage to the sample
37	Urea Broth	To test for rapid urease-positive bacteria
38	Phenylalanine agar slant	To determine whether the microbe can use the amino acid phenylalanine
39	Nutrient Agar powder	For the cultivation of bacteria and enumeration od bacteria
40	Ethanol (80%)	To disinfect and prevent the cross contamination while collecting the sample
41	Sucrose broth	To determination of fermentation of sucrose in the

		differentiation of bacteria
42	Safranin	To staining technique utilize it as a counterstain
43	Glucose broth	To cultivation and fermentation of bacteria
44	Phenol red	To provide a quick check for the health of the culture media
45	Lactose broth	To detect of coliform bacteria

3.3 Methods

The method used in this study was designed in-line with the objectives of the study as illustrated in flowchart of the study (Figure 3.3) and were elaborated in sub-topics below.

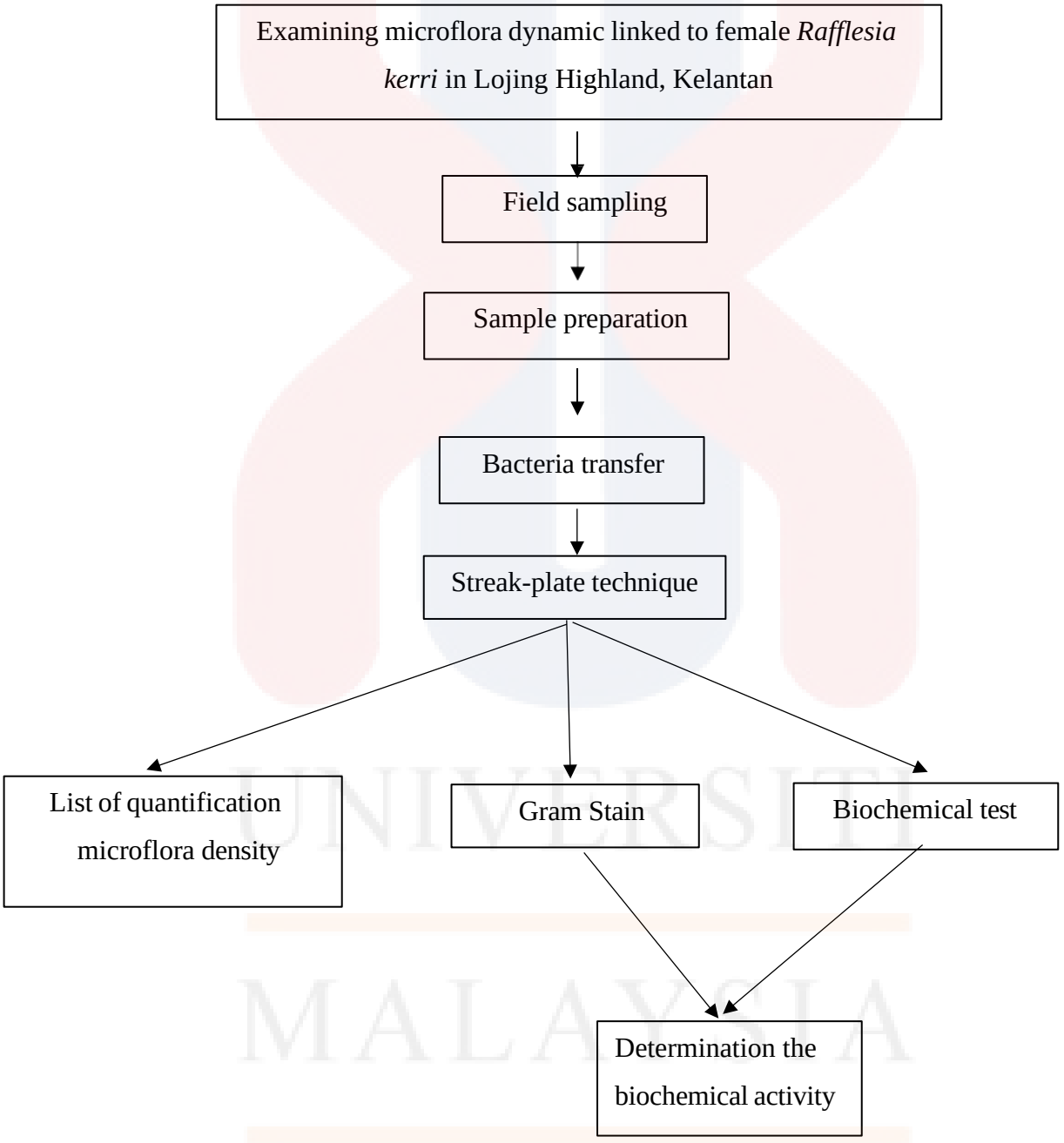

Figure 3.3a. Flowchart of the Study

3.3.1 Media Preparation

3.3.1.1 Luria Broth

For making 1L of LB broth, 25 grams of pre-mixed broth powder were used. This powder contained 10- grams of tryptone, 5 grams of yeast extract, and 10- grams of NaCI. All the powders were mixed with 1L of distilled H20. The bottle was swirled in a circular motion to mix the contents. Then, it was autoclaved at 121℃ for 30 minutes. After that, it was allowed to cool before being poured into sterile test tubes with lids.

3.3.1.2 Nutrient Agar

For 1L of nutrient media, 28 grammes of nutrient agar were used in the preparation process. The Schott bottle was filled with 500ml of water after being cleaned with distilled water and 28 grammes of nutritional agar powder were added using an electronic balance on a weighing boat. The bottle was rotated until the powder dissolved completely. Distilled water was then poured until it reached the calibration point. To obtain a clear solution, the mixture was heated to boiling and then autoclaved, with the caps being loosened. The media in Schott bottles were autoclaved at 121°C for 15 minutes. After the autoclaved media had cooled for a few minutes, it was poured into petri dishes in a laminar manner. To prevent condensation water from falling, the plates needed to be placed in an inverted position.

3.3.2 Field Sampling

The specific areas under the disc of *R. kerri* were swabbed several times using sterile wooden swab sticks. The swabs were then placed inside sterile sampling bottles containing Luria broth solution. The swabbing process was replicated three times under three different conditions: fresh bloom, mid-bloom, and late-bloom.

A marker was used to label each bottle with the corresponding sample information. Subsequently, the bottles were placed on a table at room temperature and left overnight.

3.3.3 Quantification of Microflora Density

3.3.3.1 Bacteria transfer

Spread plating was used in this study using nine test tubes, labeled 10^{-1} to 10^{-9}. Each test tube contained 9ml of Luria broth solution water and 1ml of the sample was put into the first tube, resulting in a dilution solution of 10-ml (10^{-1}). One milliliter of the diluted sample was then taken from the first test tube and transferred to the second test tube. This procedure was repeated until reaching the final test tube (10^{-9}). Using a sterile hockey stick, 1ml of the diluted samples from test tubes 10^{-3}, 10^{-6}, and 10^{-9} were injected into the appropriate nutrient agar and spread on a plate. The plates were incubated at room temperature overnight.

3.3.3.2 Streak-plate Technique

Sterilizing the wire loop in a flame was the first step in the process. The sterilized loop was then allowed to stand for a few seconds to cool. The loop was inserted into the sample containing the bacterial mixture. Using the inoculum, the edge of the plate was wiped flat. The inoculating loop was kept in constant contact with the surface of the agar while the sides of the plate were scraped. A streak was made from the smear, and the loop was sterilized again. During incubation, the bacteria multiplied and formed colonies. The plate was securely covered to prevent contamination.

3.3.4 Determination of Biochemical Activity

3.3.4.1 Gram Stain

Simple staining was used for staining. A smear loop was transferred onto a slide from a plate containing a pure culture of bacteria. After that, air drying occurred. The slide was fixed by passing it through the flame and setting it on the stain tray. Crystal violet was used for 60 seconds of staining, followed by methylene blue for another 60 seconds. To remove excess stain, the slides were tilted. Next, bibulous paper was used for wiping. A light microscope was then used to examine the slide and identify any gram-positive or gram-negative bacteria.

3.3.4.2 Indole Production

To inoculate a SIM tube, a needle was sterilized and allowed to cool. The needle was then touched to a pure colony. The needle was stabbed straight down into the SIM agar and then removed by lifting it straight up along the same stab path. All tubes were incubated at 37°C for 24 hours. After incubation, to perform the indole test, an ampule of Kovac's reagent was used. Seven to eight drops were added to the incubated SIM tube.

3.3.4.3 Methyl-Red (MR)

The loop was sterilized, allowed to cool, and then touched to a pure colony. Using sterile technique, each selected sample was inoculated into appropriately labelled medium by means of loop inoculation. The last sample served as a control to inject each tube from the selected sample. The tubes were incubated at 37°C for 24 hours. After incubation, the MR test was performed to test the strain's ability to perform mixed acid fermentation. Five to six drops of methyl-red were added to the

tube.

3.3.4.4 Voges- Proskauer (VP) reaction

The loop was sterilized, allowed to cool, and then touched to a pure colony. Using sterile technique, each selected sample was inoculated into appropriately labelled medium by means of loop inoculation. The last sample served as a control to inject each tube from the selected sample. The tubes were incubated at 37℃ for 24 hours. After incubation, the VP test was performed to test for the 2,3-butanediol fermentation pathway. Fifteen drops of Barritt's reagent A and five drops of Barritt's reagent B were added. The tube was capped and the contents were mixed.

3.3.4.5 Citrate test

A slant of Simmon's citrate agar was used. A needle was sterilized and allowed to cool. It was touched to a pure colony, and then the surface of the slant was streaked with the needle. When replacing the cap on the tube, it was screwed on enough to prevent it from falling off, but left loose. The test would not have been accurate if the cap was on tight. The tubes were incubated at 37℃ for 24 hours.

3.3.4.6 Hydrogen Sulfide Test

Using sulfure indole motility (SIM) medium, the bacteria were inoculated into labeled tubes by means of stab inoculation. The tubes were incubated at 37℃ for 24 hours. The medium was observed for the formation of black precipitate.

3.3.4.7 Urease Test

Pure colonies of bacteria were streaked onto the surface of a urea agar slant. The bacteria were inoculated into urea broth. The tube was incubated at 37℃ for 24 hours.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Microflora density

In this section, the discussion is focused on the list of microflora quantification in response to the flowering stages of female *R. kerri*. Bacteria is swabbed using sterile wood at under disc of rafflesia. Three replications were successfully done. Therefore, the data obtained throughout this study including the list of microflora quantification has been compiled and provided in Table 4.1 below.

Table 4.1: List quantification microflora density

Plate	Tube Dilution	ml of dilution plate	Final dilution on plate	Number of colonies	Bacterial count per ml of sample (CFU/ml)
Early Blooming					
F1S1	10^{-3}	1ml	1:1000	308	308,000
F1S1	10^{-6}	1ml	1:1,000,000	154	154,000,000
F1S1	10^{-9}	1ml	1:1,000,000,000	15	15,000,000,000
F2S1	10^{-3}	1ml	1:1000	10	10,000
F2S1	10^{-6}	1ml	1:1,000,000	8	8,000,000
F2S1	10^{-9}	1ml	1:1,000,000,000	5	5,000,000,000
F3S1	10^{-3}	1ml	1:1000	201	201,000
F3S1	10^{-6}	1ml	1:1,000,000	88	88,000,000
F3S1	10^{-9}	1ml	1:1,000,000,000	28	28,000,000,000
Middle Blooming					
F1S2	10^{-3}	1ml	1:1000	278	278,000
F1S2	10^{-6}	1ml	1:1,000,000	21	21,000,000
F1S2	10^{-9}	1ml	1:1,000,000,000	13	13,000,000,000
F2S2	10^{-3}	1ml	1:1000	10	10,000
F2S2	10^{-6}	1ml	1:1,000,000	8	8,000,000
F2S2	10^{-9}	1ml	1:1,000,000,000	20	20,000,000,000
F3S2	10^{-3}	1ml	1:1000	103	103,000
F3S2	10^{-6}	1ml	1:1,000,000	109	109,000,000

F3S2	10^{-9}	1ml	1:1,000,000,000	10-	10-,000,000,000
Late Blooming					
F1S3	10^{-3}	1ml	1:10-00	330	330,000
F1S3	10^{-6}	1ml	1:1,000,000	120	120,000,000
F1S3	10^{-9}	1ml	1:1,000,000,000	10-6	10-6,000,000,000
F2S3	10^{-3}	1ml	1:10-00	98	98,000
F2S3	10^{-6}	1ml	1:1,000,000	85	85,000,000
F2S3	10^{-9}	1ml	1:1,000,000,000	16	16,000,000,000
F3S3	10^{-3}	1ml	1:10-00	203	203,000
F3S3	10^{-6}	1ml	1:1,000,000	298	298,000,000
F3S3	10^{-9}	1ml	1:1,000,000,000	42	42,000,000,000

Based on Table 4.1, it shows the difference in the quantification of microflora density in response to the flowering stage of female *R. kerri* where in the early stages it shows a high density in flower 1. This is because a high microflora density may indicate a healthy and balanced community of microorganisms. According to observations, the presence of bacteria on the surface of plants can be caused by the odors emitted by plants that contribute to the direction of plant signals (Shiojiri et al., 2006). According to Bringel & Couee (2015), stating the presence of microbial communities between plants, bacteria and the atmosphere has influenced the smell released. In the middle stage, the density of microflora shows a decrease because the flower has shown a change in the physical structure of the flower cell that shows the process of decay occurs causing the presence of the microorganism community to decrease. This also happens because the smell produced by the flowers is reduced which is a factor in the reduction of microflora density. However, at a late stage, it shows a high density of microflora. While the bacteria that grow in the final stages occur as a result of the decomposition of the flower tissue This happens This causes the structure and cells of the flower to become damaged and damp. Bacteria found in water, soil, and on the surface of flowers will attack damaged flower cells and will be involved in the

decomposition process (Grube et al., 2015). This event causes the presence of a large community of microorganisms.

According to Table 4.1, it can be seen where the density of flower 1 is higher than flowers 2 and 3. This can happen due to the location of the three flowers where flower 1 grows in an area close to the vegetable garden compared to flowers 2 and 3 that grow in the interior. It is possible that the smell produced by the agricultural area causes the density of microflora in flower 1 to be higher compared to flowers 2 and 3. This could be the cause of the difference in the density of microflora that occurs in flowers 1, 2 and 3.

4.1.1 Morphology Characteristic

Figure 4.1 showed petri dish containing bacteria swabbed at under disc point after being incubated for 3 days at 37°C a in figure 4.1 below

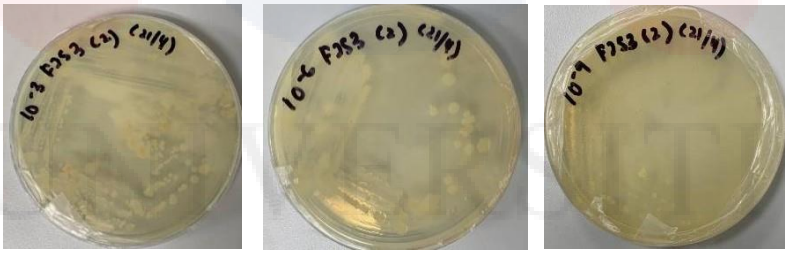

Figure 4.1: First, second and third replication of bacteria swabbed on disc of *Rafflesia kerri*

The edge margin, shape and color were determined through light microscope. At the point under disc of *R. kerri,* all color spotted on the plate is milky and cloudy, the shape of the bacteria consists of filamentous, irregular, and circular. The edge margin mainly builds up to undulate and entire.

Table 4.1.2: Morphology Characteristic of Under Disc on its edge margin, shape and color

Plate	Morphology Characteristic		
	Shape/Form	**Edge Margin**	**Color/ Opacity**
Flower 1			
F1S1 10--3	Filamentous	Filiform	Milky
F1S1 10--6	Irregular	Undulate	Milky
F1S1 10--9	Irregular	Undulate	Milky
F1S2 10--3	Circular	Undulate	Milky
F1S2 10--6	Irregular	Entire	Milky
F1S2 10--9	Filamentous	Entire	Milky
F1S3 10--3	Rhizoid	Filiform	Milky
F1S3 10--6	Irregular	Undulate	Milky
F1S3 10--9	Irregular	Lobate	Milky
Flower 2			
F2S1 10--3	Circular	Entire	Milky
F2S1 10--6	Irregular	Undulate	Milky
F2S1 10--9	Irregular	Undulate	Milky
F2S2 10--3	Circular	Undulate	Milky
F2S2 10--6	Irregular	Entire	Milky
F2S2 10--9	Filamentous	Entire	Milky
F2S3 10--3	Irregular	Lobate	Milky
F2S3 10--6	Irregular	Undulate	Milky
F2S3 10--9	Irregular	Undulate	Milky
Flower 3			
F3S1 10--3	Filamentous	Filiform	Milky
F3S1 10--6	Irregular	Undulate	Milky
F3S1 10--9	Circular	Entire	Milky
F3S2 10--3	Circular	Undulate	Milky
F3S2 10--6	Irregular	Entire	Milky
F3S2 10--9	Filamentous	Entire	Milky
F3S3 10--3	Filamentous	Filiform	Milky

F3S3 10--6	Irregular	Undulate	Milky
F3S3 10--9	Irregular	Undulate	Milky

4.2 Classification of Positive and Negative Bacteria

4.2.1 Gram Stained

The observation from light microscope consists of two types of common bacteria found in the diversity of bacteria upon swabbing on this specific point, which is Bacillus Gram Positive and Coccus Gram Negative bacteria as shown in Figures 4.2 and 4.3.

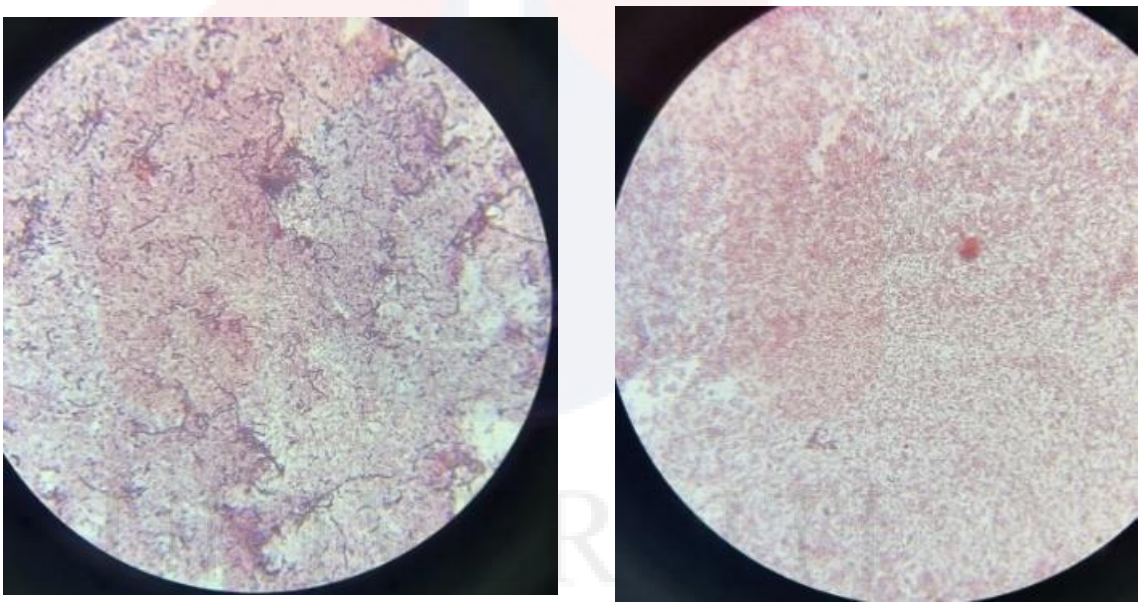

Figure 4.2: Bacillus Gram Positive and Coccus Gram Negative from under disc area, under 40x magnification light microscope

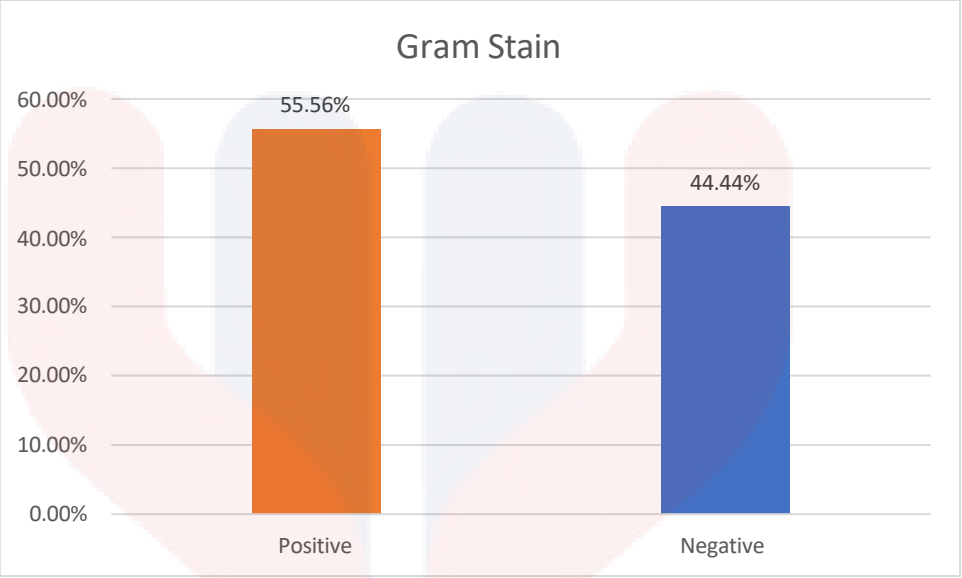

Figure 4.3: Classification of Gram Stained bacteria from different female *Rafflesia kerri* and replication in the petri dish.

Table 4.2: Classification of Gram Stained bacteria from different blooming stage and replication in petri dish

Source	Gram Stain		
	1st Replication	**2nd Replication**	**3rd Replication**
Early Blooming			
Female 1 Stage 1	Positive	Positive	Negative
Female 2 Stage 1	Positive	Positive	Negative
Female 3 Stage 1	Positive	Negative	Positive
Middle Blooming			
Female 1 Stage 2	Positive	Negative	Negative
Female 2 Stage 2	Positive	Negative	Negative
Female 3 Stage 2	Negative	Negative	Negative
Late Blooming			
Female 1 Stage 3	Positive	Positive	Negative
Female 2 Stage 3	Positive	Negative	Positive
Female 2 Stage 3	Positive	Positive	Positive

The results show that 55.56% of bacteria grown in *R. kerri* is gram positive. This growth shows a lot of bacteria in the early bloom. Gram-positive bacteria stain purple due to the presence of a thick layer of peptidoglycan in their cell walls,

which preserves the crystal violet stained by these cells. Possible bacteria present in gram positive are *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Clostridium*, and *Listeria* species (Sizar et al., 2023).

However, there's only 44.44% amount of percentage of gram-negative bacteria inhibition occur on the under disc of *R. kerri*. Because their peptidoglycan walls are thinner and do not hold onto crystal violet during the decolorization process, gram-negative bacteria are red. Possible bacteria present on gram negative are *Escherichia coli (E. coli*), *Pseudomonas*, *Xanthomonas* and *Rhizobium* species (Junio et al., 2023).

4.2.2 Biochemical Activity

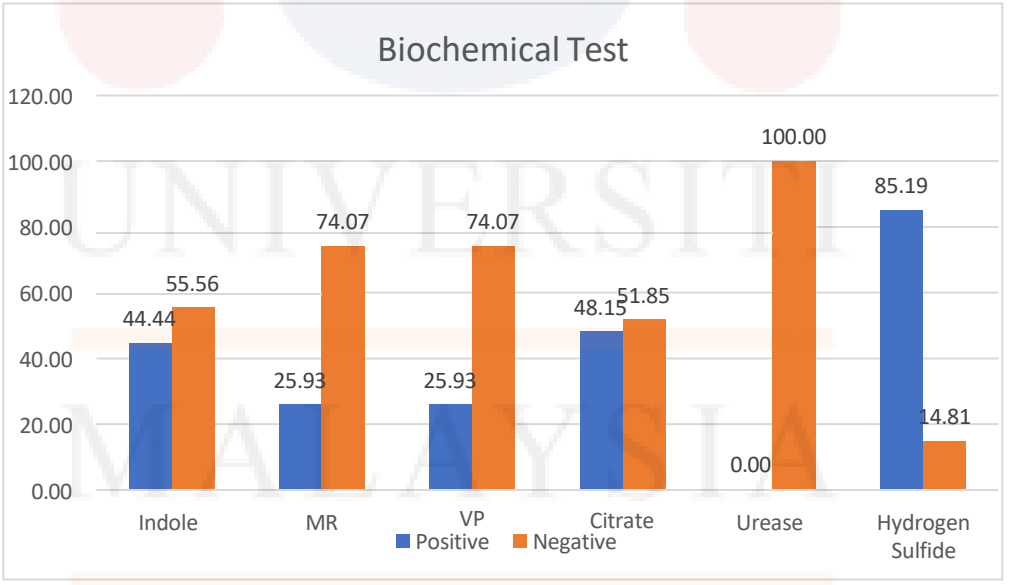

Figure 4.4: Classification of biochemical test bacteria from different female of *Rafflesia kerii* and replication in the test tube

The result from the biochemical test of Indole production indicated that 55.56% was responded in negative, while, 44.44% was reacted in positive as shown in Figure 4.4. Indole indicates the presence of tryptophanase enzyme in the organism that produces the foul smell to attract the pollinator to come sniff. Kovac's reagent is a chemical used to detect indole. When Kovac's reagent is added, indole forms a red ring, indicating that the bacterial species is tryptophanase positive and an indole producer. Indole negative bacterial species show no tryptophanase activity when Kovac's reagent will produce a dark yellow ring (Macfaddin, 2000).

For the Methyl-Red (MR) test, it shows that bacteria response more to the negative which is 74.07%compared to bacteria which react to the positive which is 25.93%. MR test detects sufficient acid production during glucose fermentation and maintains conditions so that the pH of the old culture is maintained below a value of about 4.5. A red-turned medium is positive, indicating the presence of a stable acidic end product. The medium remains yellow or turns orange, the test is negative, indicating the absence of significant acid production. If the bacterial culture does not have enough time to fully ferment glucose such as temperature or pH, is not optimal for the fermentation process, acid production may not be sufficient. This causes a negative MR test even though the bacteria are able to ferment mixed acids (Sagar, 2022).

For the Voges-Proskauer test, it shows that bacteria react to the negative more which is 74.07% compared to the bacteria which react to the positive which is 25.93%. The Voges-Proskauer (VP) test is a biochemical test that detects the ability of bacteria to metabolize pyruvate to a neutral intermediate product called

acetylmethylcarbinol or acetoin (Dahal, 2023). The reaction of the acetoin reagent and the additional reagent α-naphthol and potassium hydroxide will undergo a chemical reaction that produces a red complex, indicating a positive result. Whereas if acetoin is not present in a very low concentration, the reagent does not produce a color change, resulting in a negative test (Tilles and Forbes, 2014).

Figure 4.5: Citrate Test Results against selected microflora species

Referring to Citrate Test shows not much difference in bacterial response. The bacterial response to the positive citrate test was 48.13% while 51.85% was the bacterial response to the negative citrate test. Citrate agar is used to test an organism's ability to utilize citrate as a source of energy. Also provide valuable information about metabolic capabilities of bacteria in *R. kerri*. The medium

contains citrate as the sole carbon source and inorganic ammonium salts as the sole source of nitrogen (Simmons, 1926). Bacteria that use citrate convert it to an alkaline byproduct, raising the pH of the medium. This change in pH causes the bromothymol blue indicator to change from green to blue, indicating a positive result. However, bacteria that cannot use citrate do not change the pH of the medium, so the bromothymol blue indicator remains green, indicating a negative result (Macfaddin, 2000).

For the biochemical Urease test shown in the chart, the bacteria reacted completely negative. This is because some bacteria do not have the urease gene and cannot produce the urease enzyme. These bacteria cannot hydrolyze urea to ammonia and carbon dioxide, causing no change in the pH of the medium (Aryal, 2022).

Figure 4.6: Hydrogen Sulfide results against selected microflora species

Hydrogen sulfide is produced when sulfur compounds are reduced by bacterial strains. This test is used to determine whether microbes reduce sulfur- containing compounds to sulfide to produce hydrogen sulfide gas was important to scent emission and metabolism process to female *R. kerri* (Aryal, 2022). Bacteria that positively produce H_2S have the necessary genes encoding enzymes such as thiosulfate reductase. This enzyme allows bacteria to reduce sulfur compounds to H_2S. While negative bacteria lack enzymes and cannot produce hydrogen sulfide from sulfur-containing compounds (Tilles and Forbes, 2014).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, this study has recorded the composition of bacteria which revealed that 55.56% were Gram-positive and 44.44% were Gram-negative under microscopic examination of the three female flowers. Density of microflora in response to blooming stages of female *R. kerri* was related with composition scent, visitor and structure of flower. Biochemical activity of selected of microflora species associated with showing female *R. kerri* different positive and negative bacteria. The result showed that three female flowers at different blooming. The conclusion obtained from the results is that the smell of bacteria which is influenced by its microbiological activity plays a role in helping the pollination process by attracting flies of the Diptera family.

5.2 Recommendation

The Rafflesia is a widely recognized endangered species. Due to the overexploitation of the surrounding vegetative production, this species deserves special attention in Lojing Highland, Kelantan. The relationship between bacteria and pollination, which is crucial to the diversity of this organism, is covered in this study. On the other hand, there are still gaps in this research, particularly regarding the symbiotic relationship between the bacteria and the host. To learn more about this valuable flower, more specific research must be done. Investigating the behavior of specific pollinators attracted to Rafflesia, including the role of bacterial volatiles in attracting these pollinators, and assessing how bacterial communities on the flower influence pollination success rates and genetic diversity are also crucial. Additionally, studies on how habitat degradation and overexploitation in Lojing Highland affect the bacterial communities

associated with Rafflesia and its host, and how this impacts the flower's survival, are needed. Conservation strategies should include restoring and managing bacterial communities to support the health and reproduction of Rafflesia populations. Molecular and genetic research, such as sequencing the genomes of Rafflesia and its associated bacterial symbionts and using metagenomic approaches to explore microbial community diversity and function, will provide deeper insights. By focusing on these specific areas, researchers can gain a deeper understanding of the intricate relationships that sustain Rafflesia, ultimately aiding in the development of effective conservation strategies to protect this remarkable species in Lojing Highland, Kelantan.

REFERENCES

Ahmad Puad, A. S., Wan Zakaria, W. N. F., Geri, C., Zainudin, R. & Latiff, A. (2020). Notes on *Rafflesia hasseltii* Suringar (Rafflesiaceae), a New Record for Sarawak, Malaysia. *Malaysia Nature Journal*, 71(1), 19-25.

Akhriadi, P., Kiswanti, H. A., Taufiq, A., Alfajri, D. & Kardiman, R. (2010-). Assessment of Conservation Status of *Rafflesi*a in West Sumantra, Indonesia. Final Report to Rufford Small Grant (for Nature Conservation). *Rafflesia Monitoring Team (RMT) Padang*.

Amal-Najihah, M. N., Rabiatul-Adawiyah, I., Muhamad-Azhar, A., Nur-Hanisha, A. M., Nor-Hizami, H., Hasifah, A. A. & Sharifah-Aisyah, S. O. (2018) Landscape Ecological Assessment of Potential Ecotourism in Malaysia, *International Journal of Civil Engineering and Technology (IJCIET)*, 9(10-), 969-979.

Balete, D. S., Pelser, P. B., Nickrent, D. L., Barcelon, J. F. (2010-). *Rafflesia verrucosa* (*Rafflesiaceae*), A New Species of Small-Flowered *Rafflesia* from Eastern Mindanao, Philippines, 49-57.

Barcelona, J. F., Cajona, M O. & Hadsall, A. S. (2006). *Rafflesia baletei*, another new *Rafflesia (Rafflesiaceae*) from Philippines. *Kew Bulletin*, 231-237.

Bringel, F. & Couee, I. (2015). Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. Frontiers Microbiology, 6, 486.

Dony-Adriansyah, N., Nur-Syazwani, M. F. & Zurfarahin, Z. (2015) Geological Review of the *Rafflesia* Trail, Near Kampung Jedip, Lojing Highland: Inputs for a Nature-Based Tourism Site in Kelantan, Malaysia *J, Trop. Resour. Sustain. Sci*, 3, 86-97.

Dony-Adriansyah, N., Nur-Syazwani, M. F., Zurfarahin, Z., Sharifah-Aisyah, S. O. & Mohamad-Khidzir, M. I. (2014). Geological studies to support the tourism site: A case study in the *Rafflesia* Trail, near Kampung Jedip, Lojing Highland, Kelantan, Malaysia. *International Journal of Geoscience*, 5(08),835

Dony-Adriansyah, N., Zurfarahin, Z., Nur-Syazwani, M. F., Mohamad-Muqtada, A. K. & Arham-Muchtar, A. B. (2015). Study on geoheritage and water quality of Pos Hendrop hot spring, Lojing Highland, Kelantan, Malaysia. *J. Trop. Resour. Sustain. Sci*, 3, 222-233.

Essays, U. (2013). *Rafflesia* Species in Malaysia Identification. Retrieved from https://ukdiss.com/examples/unique-plant-types.php?vref=1.

Grube, M., Cernava, T., Soh, J., Fuchs, S., Aschenbrenner, I., Lassek, C., et al. (2015). Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative genomics. International Society for Microbial Ecology Journal, 9, 412-424.

Hidayati, S. N. & Walck, J. L. (2016). A review of the biology of *Rafflesia*: what do we know and what's next? *Botanic Garden Bulletin*, 19(2), 67-78.

Junio O., & Wanda C. R., (2023). Gram-Negative Bacteria https://www.ncbi.nlm.nih.gov/books/NBK538213/

Kedri, F. K., Zulhazman, H., Sukri, N S., Yaacob, S. H., Abd-Majid, N. K. S., Nasihah, M. & Amir. S. F. (2018). Distribution and ecology of *Rafflesia* in Royal Belum State Park, Perak, Malaysia. *International Journal of Engineering and Technology*, 7(2.29), 292-296.

Latiff, A. (1984). *Tetrastigma rafflesiae* and *hookeri.* Studies in Malesian Vitaceae VII. The genus *Tetrastigma* in the Malay Peninsula, 214.

MacFaddin JF. (2000). Biochemical Tests for the Identification of Medical Bacteria, 3rd ed, p 221–232. The Lippincott, Williams & Wilkins Co., Philadelphia, PA
.

Mat-Salleh, K. (1991). *Rafflesia* Magnificent Flower of Sabah. *Borneo Publishing Company Kota Kinabalu*, 1-9.

Meijer, W. (1984). New species of *Rafflesia*. *Blumea*, 30, 209-215.

Meijer, W. and Elliott, S. (1990). *Rafflesia* Taxonomy, Ecology and Conservation of *Rafflesia kerri* Meijer in Southern Thailand, 117-133.

Mjoberg E. & Barwell A. (1930). Forest Life and Adventures in the Malay Archipelago (210-p). London: George Allen & Unwin Ltd.

Nais, J. (2001). *Rafflesia* of the World. Borneo: *Natural History Publication Sdn. Bhd*. 243

Nais, J. (2004). *Rafflesia* Bunga Terbesar di Dunia: *Dewan Bahasa dan Pustaka, Kuala Lumpur*.

Nasihah, M., Zulhazman, H., Siti Munirah, M. Y., Qayyum Nadia, W. A. & A. Latiff. (2016). *Tetrastigma rafflesiae and hookeri*. *Tetrastigma hookeri* (Laws.) Planch. (Vitacaea), A Host Plant of *Rafflesia kerri,* 33-39.

Nasihah, M., Zulhazman, H., Siti-Munirah, M. Y., Razak, W., Fatimah, K. & Qayyum-Nadia, W. A. (2013). Soil nutrient dynamic at three defined elevation in relation to bud population of *Rafflesia kerri* in Lojing Highland of Kelantan, Malaysia. In U. M. Kelantan (Ed.).

Norhazlini, M. Z., Mailina, J., Shalini, M., Nor-Azah, M. A. & Zulhazman, H. (2020) Assessing the floral volatile constituents of male and female *Rafflesia kerri* Meijer from Lojing Highland, Penisular Malaysia. *IOP Conference Series: Earth & Enviroment Science*, 549, 012068.

Omeed S., Stephen W. L.; Chandrashekhar G. U. (2023) Gram-Positive Bacteria. https://www.ncbi.nlm.nih.gov/books/NBK470553/

Pelser, P. B., Nickrent, D. L., Gemmill, C. E. C., Barcelona, J. F. (2017). Genetic Diversity and Structure in the Philippine *Rafflesia lagascae* Complex *(Rafflesiaceae)* Inform its Taxonomic Delimitation and Conservation. *Systematic Botany*, *Volume* 42, 3, 543-553

Peters, R. F., & Ting, Y. Y. (2016). Protection of *Rafflesia* through the appreciation of the Dusun's indigenous knowledge; A preliminary case study at Poring-Sabah. *Journal of Tropical Biology & Conservation (JTBC), 13*

Pranshant Dahal (2023). VP Test: Principle, Reagents, Produce, Results and Use https://microbenotes.com/voges-proskauer-vp-test

Qayyum-Nadia, W. A. (2015). Assessment on growth and mortality rates of Rafflesia kerri Meijer (Rafflesiaceae) in Lojing Highland, Kelantan, Penisular Malaysia. Universiti Malaysia Kelantan.

Rao, P. V., Lee, L. H., Shamsul, M., Ikarastika, R. A. W. & Soon, J. M. (2016) Ethnomedicianl knowledge of Temiar ethnic tribe of Lojing Highland, Kelantan: a source for nutritional and antioxidant potential. *Paper presented at the Proceeding of the 5th World Conference on Applied Science, Engineering and Technology*, 2-4 June 2016, Vietnam.

Sagar Aryal (2022). Hydrogen Sulfide Test-Principle, Procedure Uses and Interpretation https://microbiologyinfo.com/hydrogen-sulfide-test/

Sagar Aryal (2022). Urease Test-Principle, Media, Produce and Result https://microbiologyinfo.com/urease-test-principle-media-procedure-and-result/

Sagar Aryan (2022). Methyl Red (MR) Test-Principle, Produce and Result Interpretation, https://microbiologyinfo.com/methyl-red-mr-test-principle-procedure-and-result-interpretation/

Sharifah Aisyah, S. O., Aweng, E. R., Ahmad-Fadli, A. S & Azrinaaini, M. Y. (2014) Benthic Macroinvertebrates Composition and Distribution at Sungai Dawai and Sungai Dekong in Lojing Highland, Gua Musang, Kelantan. *Jurnal Teknologi*, 68(3), 125-131.

Shiojiri, K., Kishimoto, K., Ozawa, R., Kugimiya, S., Urashimo, S., Arimura, G., et al. (2006). Changing green leaf volatile biosynthesis in plants: An approach for improving plant resistance against both herbivores and pathogens. Proceeding Natural Academy Science, USA, 10-3, 16672-16676.

Simmons JS. 1926. A culture medium for differentiating organisms of typhoid-colon-aerogenes groups and for isolation of certain fungi. J Infect Dis 39:209–214.

Sofiyangti, N. & Choong, C. Y. (2012). Morphology of Ovule, Seed and Pollen Grain of *Rafflesia R. BR. (Rafflesiaceae). Bangladesh J. Plant Taxon*. 19(2): 10-9-117.

Sofiyanti, N., Mat-Salleh, K., Purwanto, D. & Syahputra, E. (2007). The Note on Morphology of *Rafflesia hasseltii* Surigar from Bukit Tiga Puluh National Park, Riau. *Biodiversitas*. 9:257-261.

Tille, P. M., & Forbes, B. A. (2014). Bailey & Scott's diagnostic microbiology (Thirteenth edition.) P. 185 – 187. St. Louis, Missouri: Elsevier

Wan Zakaria, W. N. F., Ahmad Puad, A. S., Geri, C., Zainudin, R. & Latiff, A. (2016) *Tetrastigma diepenhorstii* (Miq.) Latiff (Vitaceae), a new host of *Rafflesia tuan-mudae*

Bec. *(Rafflesiaceae)* in Borneo. *Journal of Botany*, 2016,1-6.

Wong, M. & Gan, F. (2003). The elegant *Rafflesia kerri* of Kelantan. *Folio Malaysiana*,4(1). 53-62.

Zulhazman, H. (2015). Distribution of *Rafflesia* in Malaysia. Rafflesia of Kelantan, Peninsular Malaysia, 15-16