



UNIVERSITI PUTRA MALAYSIA

***ANTIBACTERIAL ACTIVITY OF PSIDIUM GUAJAVA (GUAVA) LEAF
EXTRACT AGAINST XANTHOMONAS ORYZAE PV. ORYZAE AND
PSEUDOMONAS FUSCOVAGINAE***

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CERTIFICATION FORM

This project report entitled “Antibacterial Activity of *Psidium guajava* (Guava) Leaf Extract Against *Xanthomonas oryzae pv. oryzae* And *Pseudomonas fuscovaginae*” is prepared by Nur Afiqah Syahirah Binti Paisol and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the reward of the degree of Bachelor of Agricultural Science.

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ABSTRACT

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most important in disease of rice. The symptom usually established mainly on leaf blades, leaf sheath and sometimes on grains. Yellow water soaked lesions will appear on an infected leaves margin of its leaf blade. The lesion will appear parallel along the leaf and then will join together covering the whole leaf. Early in the morning, milky dew drop bacterial discharge will emerge on young lesion. The leaf then dries up with white lesions as the disease progress and the margin of the leaf blade will becomes wavy. Bacterial sheath brown rot shows symptom such as discoloration of the leaves and then spreaded to midrib or vein of the leaves. Brown necrotic lesion will be also visible from small to large. Usually, these diseases was managed through application of copper-based fungicide or bactericide. However, these chemical control has created environmental issues as well as problems to human health. Therefore, biological control is now emphasized as another strategy to control pathogens. This study was conducted to determine the antagonistic activity of guava leaf extract against *Xanthomonas oryzae* pv. *oryzae* and *Pseudomonas fuscovaginae*, determination of minimum inhibitory concentration and to determine the minimum bactericidal concentration of guava against *Xanthomonas oryzae* pv. *oryzae* and *Pseudomonas fuscovaginae*. Guava leaves were collected and extracted with two different solvents with different concentration to get the crude extract. Leaf extract was screened with *Xanthomonas oryzae* pv. *oryzae* and *Pseudomonas fuscovaginae* by using agar well diffusion method and result shows that, guava leaf extract can inhibit the growth of *Xanthomonas oryzae* pv. *oryzae* at minimum concentration of 50 mg/ml with 2.3833 means diameter of inhibition zone but cannot inhibit *Pseudomonas fuscovaginae*. Further experiment with minimum inhibitory concentration and minimum bactericidal concentration found out that the leaf extract can only make the *Xanthomonas oryzae* pv. *oryzae* in bacteriostatic state

instead of inhibit the bacterial growth. Thus, guava extract have potential to be used as biological control to control bacterial leaf blight, but cannot be used to control brown sheath rot.



ABSTRAK

Penyakit hawar daun yang disebabkan oleh bakteria *Xanthomonas oryzae* pv. *oryzae* adalah salah satu penyakit yang paling penting dalam keluarga rumput, Poaceae. Gejala penyakit hawar daun ini biasanya ditunjukkan pada bilah daun, sarung daun dan kadang-kadang pada bijirin. Lecur berair yang berwarna kuning akan muncul pada margin daun dimana luka ini akan kelihatan selari bersama-sama daun dan seterusnya meliputi seluruh daun. Daun kemudiannya menjadi kering dimana margin bilah daun akan menjadi berombak dengan luka-luka putih sebagai tanda penyakit yang telah merebak. Penyakit sarung reput coklat menunjukkan simptom seperti perubahan warna daun dan kemudiannya akan merebak ke tulang daun atau urat daun. Luka coklat nekrotik juga akan dilihat dari kecil ke besar. Biasanya, penyakit-penyakit ini diatasi melalui penggunaan racun kulat berasaskan tembaga atau racun anti-bakteria. Walau bagaimanapun, kawalan penyakit menggunakan bahan kimia ini telah menimbulkan isu berkaitan alam sekitar dan juga masalah kepada kesihatan manusia. Oleh itu, kawalan biologi kini diberi penekanan sebagai satu lagi strategi untuk mengawal patogen dan penyakit. Kajian ini bertujuan untuk menentukan aktiviti antagonistik ekstrak daun jambu batu terhadap *Xanthomonas oryzae* pv. *oryzae* dan *Pseudomonas fuscovaginae*, penentuan kepekatan perencatan minimum dan untuk menentukan kepekatan bakteria minimum jambu batu terhadap *Xanthomonas oryzae* pv. *oryzae* dan *Pseudomonas fuscovaginae*. Daun jambu batu telah dikumpulkan dan diekstrak dengan dua pelarut yang berbeza dengan kepekatan yang berbeza untuk mendapatkan ekstrak mentah. Ekstrak daun telah ditemukan dengan *Xanthomonas oryzae* pv. *oryzae* dan *Pseudomonas fuscovaginae* dengan menggunakan agar baik kaedah penyebaran dan hasil menunjukkan bahawa, jambu batu ekstrak daun boleh menghalang pertumbuhan *Xanthomonas oryzae* pv. *oryzae* dengan 50mg/ml kepekatan minima dengan 2.3833 bererti diameter zon perencatan tetapi tidak boleh

merencanakan pertumbuhan *Pseudomonas fuscovaginae*. Eksperimen diteruskan lagi dengan kepekatan perencatan minimum dan kepekatan bakteria minimum mendapati bahawa ekstrak daun hanya boleh membuat *Xanthomonas oryzae* pv. *oryzae* dalam bentuk bakteriostatik dan bukannya menghalang pertumbuhan bakteria tersebut. Oleh itu, ekstrak jambu batu hanya mempunyai potensi untuk digunakan sebagai kawalan biologi untuk mengawal hawar daun bakteria berbanding penyakit reput sarung coklat.



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LIST OF ABBREVIATIONS

CFU	colony-forming units/millimeter
BLB	Bacterial leaf blight
<i>Xoo</i>	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>
RTD	Rice tungro disease
EPS	Extracellular polysaccharide
SBR	Sheath brown rot
NA	Nutrient agar
rpm	Round per minutes
mg/ml	Milligram per millilitre
KBS	King's B media
PSA	Peptone Sucrose Agar
OD	Optical density
MHA	Mueller Hinton Agar
μ l	Microliter
cm	Centimetres
MIC	MIC
MHB	Mueller Hinton Broth
TTC	Triphenyl Tetrazolium Chloride
MBC	Minimum Bactericidal Concentration
SAS	Statistical Analysis Software
ANOVA	Analysis Of Variance

CHAPTER 1

INTRODUCTION

1.1 Overview

For many years ago, our ancestors have been using plants as their source of medicinal agents, either to cure themselves or towards their crops. They use the plants as medicine in crude extract form as they does not develop any technology yet to enhance the crude form. As years passed by in line with technology evolution, researchers enhance the plant based medicine into modern drugs that have various medicinal properties. The World Health Organization (WHO) has stated that various medicinal plants have been used as source of medicine in daily life to treat numerous kind of disease all over the world as medicinal plants are the best source to acquire a vast amount of drugs (Alo *et al.*, 2012). Main advantage using these natural derived products include safe to human health, does not have adverse effect to environment as well as easy accessibility and affordability of plants (Al-Zubaydi *et al.*, 2009).

In agriculture sector, pest and disease management are well known for its usage of chemical to get rid of the unwanted species from destroying the precious crops. These chemical usages have been creating awareness towards peoples who are concern about food safety and if the chemical usage is overused, it will lead to pollution and contamination to the environment as well as harmful effect to the ecosystem. Moreover, overuse of these chemical would lead to emergence of resistance varieties towards particular chemical. Either use of copper based chemical, antibiotics or bactericide to control bacterial leaf blight disease, these chemical are not effective anymore to control the pathogen. Regarding to this issue,

researchers have been trying to find alternative ways to manage the pest and disease instead of using chemical which have an easy access at the markets. One of the ways is by using natural based products, the plant itself. Secondary metabolites produced by plants that contain a lot of bioactive molecules (Dash *et al.*, 2011) are the important components that have the healing power to cure diseases and inhibit the growth of pathogen (Mahesh and Satish, 2008).

Despite being immobile, plants have boundless ability to defend itself from predation by insects, microorganisms and herbivores which also known as defense mechanism. These defense mechanisms contain secondary metabolites and compounds with antimicrobial properties (Gulluce *et al.*, 2007). Discovery of these antimicrobial properties of the plants lead to research to identify active compound that have potential to be developed as natural anti-bacterial agents (Bhardwaj and Laura, 2009, Dash *et al.*, 2011). Thus, plant-based product can be produced and used as biocontrol agent to control pest and pathogen. Usage of biocontrol to control pathogen is a trending nowadays as they put not only environment, but also human, animals and crops in a secured condition. They are also biodegradable and non-polluting components.

1.2 Problem statement

Psidium guajava or locally known as common guava, is a tropical fruits that grown annually and cultivated and commonly found in many tropical and subtropical regions. These annual crops are well known due to its leaves and stem that have many medicinal properties as well as the sweetness of its fruit. *Psidium guajava* has been reported in the literature to have various antibacterial activity, but this substance have no yet investigated against *Xanthomonas oryzae* pv. *oryzae* and *Pseudomonas fuscovaginae* using the leaf extract.

1.3 The objectives of the study

Thus, the specific objectives of the study were:

1. To determine the antagonistic activity of guava leaf extract against *Xanthomonas oryzae* pv. *oryzae* and *Pseudomonas fuscovaginae*.
2. To determine the minimum inhibitory concentration of guava against *Xanthomonas oryzae* pv. *oryzae* and *Pseudomonas fuscovaginae*.
3. To determine the minimum bactericidal concentration of guava leaf extract against *Xanthomonas oryzae* pv. *oryzae* and *Pseudomonas fuscovaginae*.

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