UNIVERSITI PUTRA MALAYSIA

ANTIBACTERIAL ACTIVITY OF *Dillenia suffruticosa* LEAF EXTRACT AGAINST *Xanthomonas oryzae pv. oryzae*

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BY

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

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ENDORSEMENT

This project report entitled Antibacterial activity of *Dillenia suffruticosa* leaf extract against *Xanthomonas oryzae* pv. *oryzae* is prepared by Mohd Fardzryn Bin Sani and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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LIST OF ABBREVIATIONS

^{o}C	Degree Celsius
%	Percentage
Ha	Hectare
cm	Centimetre
mm	Millimetre
g	Gram
L	Litre
ml	Millilitre
µl	Microliter
rpm	Revolution per minute
Mol. weight	Molecular weight
ANOVA	Source of variance
SAS	Statistical analysis system
LSD	Least significance difference
CRD	Completely randomized design
Xoo	*Xanthomonas oryzae* pv. *oryzae*

ABSTRACT

Bacterial leaf blight caused by seed-borne bacteria *Xanthomonas oryzae* pv. *oryzae* (Xoo) is an important rice disease which caused yield losses of up to 50% of actual yield. Recently, chemical control method used to suppress the disease, but it causes harmful impact to human and environment. Natural plant extract offers safe and eco-friendly way to control plant disease. *Dillenia suffruticosa* has been reported as a medicine for various diseases but, the use of *D. suffruticosa* extracts to control the growth of any plant pathogen has not been reported. The objective of this study was to determine the antibacterial effect of *D. suffruticosa* leaf extracts against Xoo *in vitro*. Agar-well diffusion method was used to screen the antibacterial activity of *D. suffruticosa* leaf extracts against Xoo. Methanol, ethanol and water leaf extracts with different concentrations (0, 25, 50, 100 and 200 mg/mL) were tested. Methanol and ethanol leaf extracts possess significant inhibitor effect against Xoo with the inhibition zones ranges from 16.03-21.55 mm and 12.65-18.98 mm, respectively. The minimum inhibitory concentration and minimum bactericidal concentration of the methanol extract was 1.56 mg/mL and 6.25 mg/mL, respectively, obtained with macro-broth dilution technique. The Gas chromatography–Mass spectrum analysis identified the presence of sixteen bioactive compounds in the extracts; 2-Propanone, 1-hydroxy-, Propanoic acid, 2-Propenoic acid, 2-Propanone, 1,1-dichloro-, 2-Pentanone, 4-hydroxy-4-methyl-, 1,3-Butadiene-1-carboxylic acid, 1,1,2-Triacetoxyethane, Tridecane, 2,5-dimethyl-, Neophytadiene, 7-Octadecyne, 2-methyl-, Trichloroacetic acid, undec-2-enyl ester, n-Hexadecanoic acid, 2- Phytol, 9,12-Octadecadienoyl chloride, (Z, Z)-, Octadecanoic, acid and Vitamin E. The results of this study inferred that extract from *D. suffruticosa* leaf can provide an alternative for antibacterial option.

ABSTRAK

Bakteria Hawar daun disebabkan oleh bakteria bawaan benih *Xanthomonas oryzae* pv. *oryzae* (Xoo) merupakan penyakit penting tanaman padi yang menyebabkan kerugian hasil sehingga 50% daripada hasil sebenar. Pada masa kini, kaedah kawalan kimia telah digunakan untuk menyekat penyakit ini, tetapi ianya memberikan kesan berbahaya kepada manusia dan alam sekitar. Ekstrak tumbuhan semulajadi menawarkan cara yang selamat dan mesra alam untuk mengawal penyakit tumbuhan. *Dillenia suffruticosa* telah dilaporkan sebagai ubat untuk pelbagai penyakit tetapi, penggunaan ektrak *D. suffruticosa* untuk mengawal patogen pertumbuhan belum dilaporkan. Objektif kajian ini adalah untuk menentukan kesan antibakteria ekstrak daun *D. suffruticosa* terhadap Xoo *in vitro*. Kaedah resapan agar digunakan untuk mengenalpasti aktiviti antibakteria ekstrak daun *D. suffruticosa* terhadap Xoo. Ekstrak daun menggunakan metanol, etanol dan air dengan kepekatan yang berbeza (0, 25, 50, 100 dan 200 mg/mL) telah diuji. Ekstrak daun metanol dan etanol mempunyai kesan perencat ketara terhadap Xoo dengan zon perencatan antara 16.03 – .55 mm dan 12.65 – 18.98 mm. Kepekatan perencatan minimum dan kepekatan bakteria minimum ekstrak metanol adalah 1.562 mg/mL dan 6.25 mg/mL, yang diperolehi dengan teknik *macro-broth dilution*. Analisis *Gas chromatography–Mass spectrum* mengenal pasti kehadiran enam belas sebatian bioaktif dalam ekstrak; *2-Propanone, 1-hydroxy-, Propanoic acid, 2-Propenoic acid, 2-Propanone, 1,1-dichloro-, 2-Pentanone, 4-hydroxy-4-methyl-, 1,3-Butadiene-1-carboxylic acid, 1,1,2-Triacetoxyethane, Tridecane, 2,5-dimethyl-, Neophytadiene, 7-Octadecyne, 2-methyl-, Trichloroacetic acid, undec-2-enyl ester, n-Hexadecanoic acid, 2- Phytol, 9,12-Octadecadienoyl chloride, (Z,Z)-, Octadecanoic, acid dan Vitamin E.* Keputusan kajian ini menyimpulkan bahawa ekstrak dari daun *D. suffruticosa* boleh memberikan alternatif sebagai pilihan antibakteria.

CHAPTER 1: INTRODUCTION

1.1 Justification of the study

Rice (*Oryza sativa* L.) is important crops in Asia. Rice is a staple food for Malaysians and its was importance to increase the production of rice due to the demand for food is growing every year. The total area of paddy field in Malaysia was about 688,207 ha in 2013 and the national average yield is about 3.8 t/ha (DOA, 2013). Cooperation between paddy agencies with the farmers are important in achieving high rice production. Effective management of rice disease is important due to crop pest and diseases is a major problem with the quantity and quality of rice production.

The main constraint on the production of paddy rice is the rice disease that can strike at any stage of rice growth and post-harvest stage. Bacterial leaf blight (BLB) disease caused by seed-borne bacteria *Xanthomonas oryzae pv. Oryzae* (Xoo) is one of important disease of rice. The disease was considered as the one most serious disease affecting the rice industry, which has led to declining the rice production and its quality then brings losses to farmers. The disease has been potential for causing yield losses up to 50%, intensity of crop loss depends on stage of the crop (Yugander *et al*., 2015). Bacterial leaf blight gives large impact in yield loss and cause death of premature plant.

The pathogen is known to be seed-borne and it was hard to control due to Xoo can infect every phase of plant grow (Suparyono *et al*., 2004). Several methods of the disease control are used to control bacterial leaf blight in rice such as chemical, cultural

and physical method. Bacterial leaf blight can be controlled by several broad spectrum bactericides but the chemical is expensive and can affect the beneficial microorganisms (Govindappa *et al.*, 2011). Besides, there is no suitable bactericide that available to suppress the disease development and its effect (Saad and Habibudin, 2010). The worldwide trend was focused on control disease by using biocontrol agents as an alternative method than synthetic chemical that causes harmful impact to human and environment.

Natural plant extract offers an alternative for control disease due to awareness towards health hazards, environmental pollution and negative effect on non-target organisms. Various of the plant has been used in order to control plant disease because of their antimicrobial traits. The use of some selected plant extract against bacterial leaf blight has been reported by several researchers (Kagale *et al*., 2004; Govindappa *et al*., 2011).

Dillenia suffruticosa or Simpoh air is a shrub tree and possess a medicinal plant that has been traditionally used to treat cancerous growth (Ahmad and Holdsworth, 1995) and promote wound healing (Mat Salleh and Latiff, 2002). Besides, *D. suffruticosa* has the ability to staunch bleeding (Ahmad and Holdsworth, 1995), anti-fungal (Wiart *et al.*, 2004) and Phyto- remediation properties. Wiart *et al*. (2004) also reported that this plant has antibacterial and antifungal activities. However, the use of *D. suffruticosa* to control rice pathogenic bacteria, Xoo has not been reported.

1.2 Objectives

The specific objectives of the study are:

1. To determine the antibacterial effect of *D. suffruticosa* leaf extract on growth of plant pathogenic bacteria, *Xanthomonas oryzae* pv. *oryzae in vitro*.
2. To examine the effect of different solvent extraction of *D. suffruticosa* leaf against Xoo.

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