



**ANTIMICROBIAL ACTIVITY AND EFFECTS OF SIREH (*Piper betle* L.)  
EXTRACT ON CONTROLLING BACTERIAL LEAF BLIGHT DISEASE IN  
RICE PLANT**

**By**

**NOR UMAIRA BINTI ABU ASAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
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Science**

**January 2022**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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**January 2022**

**Chair : Tan Geok Hun, PhD**  
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Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (XOO), which is a bacterial disease that leads to substantial losses of rice production. Several control measures have been evaluated by many researchers to reduce the incidence of this disease. However, some of the methods seem to have limited effectiveness. Thus, the application of natural products from *Piper betle* L. could be an alternative solution, as this plant is well-known in medicinal applications and contains a variety of bioactive compounds. This study was conducted to determine and identify the antibacterial activity and bioactive compounds of *P. betle* L. extract, respectively, as well as their effectiveness in disease suppression under glasshouse conditions. The antibacterial activity of the *P. betle* L extract (100, 50, 25, and 12.25 mg/ml) in four different solvents (methanol, ethyl acetate, hexane, and acetone) was evaluated using a disc diffusion assay, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values. The inhibition zone of methanolic extract appeared to have the highest diameter compared than those of other extracts, which is 32.67 mm at a concentration of 100 mg/ml, followed by 30.33 mm, 22.00 mm, and 20.30 mm for the concentrations of 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, respectively. The MIC and MBC values of the methanolic extract were 0.625 mg/ml suggested that the extract has a bactericidal effect of XOO. The time-kill studies revealed that the 0.625 mg/ml concentration of methanolic extract had a time and concentration-dependent killing effect on XOO. The phytochemical screening was examined for all the extracts and observed the methanolic extract has high content of flavonoids, phenols, and tannins. Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of methanol extract revealed the presence of eugenol acetate [29.53%], 4-allyl-1,2-diacetoxybenzene [29.51%], and 2,3-dimethyl benzoic acid [22.82] as major compounds. The *in vivo* study of the concentration of 0.625 mg/ml of methanolic *P. betle* L. extract in glasshouse conditions revealed that the disease reduction percentage was reduced to 52.63% (T2) and 67.02% (T3) as early as day 7 of

post-treatment compared with negative control T1. These results demonstrated the treatments with *P. betle* L. extract were capable of controlling BLB disease, especially with the application of the extract as prevention compared to control of the disease. The methanolic extract of *P. betle* L. was proven to have an effective inhibitory effect on *XOO*, and the observation of significant disease suppression in glasshouse conditions indicates that the methanolic extract of *P. betle* L. may have the potential to be used as an alternative in management strategies for controlling bacterial leaf blight. In the future study, development of a formulation from methanol extract is one of the recommendations to be applied in glasshouse and field trials.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains

**ANTIBAKTERIA AKTIVITI DAN KEBERKESANAN EKSTRAK DAUN SIREH  
(*Piper betle* L.) MENGAWAL PENYAKIT HAWAR DAUN UNTUK TANAMAN  
PADI**

Oleh

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Penyakit hawar daun (BLB) yang disebabkan oleh *Xanthomonas oryzae* pv. *oryzae* (XOO) adalah penyakit bakteria yang menyebabkan kehilangan hasil beras yang banyak. Beberapa langkah pengawalan telah dinilai oleh banyak penyelidik untuk mengurangkan kejadian penyakit ini. Walau bagaimanapun, sebilangan daripada cara tersebut mempunyai keberkesanan yang terhad. Oleh itu, penggunaan produk semula jadi daripada *Piper betle* L. dipilih kerana tumbuhan ini terkenal dan mengandungi pelbagai sebatian aktif. Kajian ini dilakukan untuk mengenal pasti aktiviti antibakteria dan sebatian bioaktif ekstrak *P. betle* L., serta keberkesanannya terhadap kerintangan penyakit di dalam rumah kaca. Kegiatan antibakteria ekstrak *P. betle* L. (100, 50, 25, dan 12.25 mg/ml) dengan empat pelarut yang berbeza (metanol, etil asetat, heksana, dan aseton) dinilai menggunakan ujian penyebaran cakera, di mana nilai kepekatan minimum menghambat (MIC) dan kepekatan minimum membunuh (MBC) kemudian ditentukan. Zon penghambatan ekstrak metanol mempunyai diameter tertinggi berbanding dengan ekstrak lain, iaitu 32.67 mm pada kepekatan 100 mg/ml, diikuti oleh 30.33 mm, 22.00 mm, dan 20.30 mm untuk kepekatan 50 mg/ml, 25 mg/ml, dan 12.5 mg/ml masing-masing. Sementara itu, nilai MIC dan MBC diperhatikan pada kepekatan terendah untuk ekstrak metanol dengan 0.625 mg/ml untuk kedua-duanya dan ini menunjukkan ekstrak mempunyai kesan pembunuhan terhadap XOO. Kajian pembunuhan mengikut masa dilakukan dan mendedahkan ekstrak metanol dengan kepekatan 0.625 mg/ml menunjukkan kesan pembunuhan bergantung pada masa dan kepekatan terhadap XOO. Pemeriksaan fitokimia diperiksa ke semua ekstrak dan diperhatikan ekstrak metanol sekali lagi menunjukkan kandungan flavonoid, fenol, dan tanin yang tinggi. Analisis GC-MS ekstrak metanol mendedahkan terdapat eugenol asetat [29.53%], 4-allyl-1,2-diacetoxybenzene [29.51%], 2,3-dimethyl benzoic acid [22.82] sebagai sebatian utama. Kajian in vivo ekstrak metanol *P. betle* L. dalam keadaan rumah kaca menunjukkan bahawa peratusan pengurangan penyakit dikurangkan kepada 52.63% (T2) dan 67.02% (T3)

seawal hari ke-7 selepas rawatan. Hasil ini menunjukkan rawatan dengan ekstrak *P. betle* L. mampu mengawal penyakit BLB, terutama pada penggunaan ekstrak sebagai pencegahan dibandingkan dengan pengendalian penyakit. Ekstrak metanol *P. betle* L. terbukti mempunyai kesan penghambatan yang berkesan terhadap *XOO* dan pemerhatian penekanan penyakit yang ketara pada keadaan rumah kaca menunjukkan bahawa ekstrak metanol *P. betle* L. berpotensi digunakan sebagai alternatif dalam strategi pengurusan untuk mengawal penyakit padi. Dalam kajian yang akan datang, pengembangan formulasi daripada ekstrak metanol adalah salah satu cadangan yang boleh diterapkan di rumah kaca dan percubaan di lapangan.



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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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## TABLE OF CONTENTS

	Page
<b>ABSTRACT</b>	i
<b>ABSTRAK</b>	iii
<b>ACKNOWLEDGEMENTS</b>	v
<b>APPROVAL</b>	vi
<b>DECLARATION</b>	viii
<b>LIST OF TABLES</b>	xiii
<b>LIST OF FIGURES</b>	xiv
<b>LIST OF ABBREVIATIONS</b>	xv

### CHAPTER

<b>1</b>	<b>INTRODUCTION</b>	1
	1.1 Background of the Study	1
	1.2 Problem Statement	2
	1.2 Objective	2
	1.3 Hypothesis	3
<b>2</b>	<b>LITERATURE REVIEW</b>	4
	2.1 Rice	4
	2.1.1 Rice industry	4
	2.1.2 Bacterial leaf blight disease	4
	2.1.3 Mode of infection of bacterial leaf blight disease	4
	2.1.4 Symptoms of bacterial leaf blight diseases	5
	2.1.5 Control methods of bacterial leaf blight diseases	6
	2.2 Plant Extract	7
	2.2.1 Extraction using maceration method	7
	2.2.2 Plant extract in plant diseases management	7
	2.2.3 Impact of Plant Extract on Controlling Plant Diseases and Crop Growth	8
	2.3 <i>Piper betle</i> L.	11
	2.3.1 The origin of <i>Piper betle</i> L.	11
	2.3.2 Taxonomy of <i>Piper betle</i> L.	12
	2.3.3 Traditional uses of <i>Piper betle</i> L.	12
	2.3.4 Beneficial of <i>Piper betle</i> L.	12
	2.3.5 Phytochemical and Bioactive Compound in <i>Piper betle</i> L.	13
	2.3.6 Antibacterial Effect	15
	2.3.6.1 Principle of disc diffusion Assay	15
	2.3.6.2 Principle of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)	15

	2.3.6.3	Principle of time-kill kinetics Study	15
	2.3.6.4	Impact of <i>Piper betle</i> L. extract on microbial activity	16
	2.3.6.5	The activity of the extract to the bacteria cells	17
<b>3</b>		<b>ANTIBACTERIAL PROPERTIES AGAINST THE <i>Xanthomonas oryzae</i> pv. <i>oryzae</i>, PHYTOCHEMICAL ANALYSIS AND DETERMINATION OF BIOACTIVE COMPOUND</b>	<b>18</b>
	3.1	Introduction	18
	3.2	Materials and Methods	18
	3.2.1	Collection and preparation of plant materials	18
	3.2.2	Extract preparation	19
	3.2.3	Preparation of test pathogen	19
	3.2.4	Determination of antibacterial activity of <i>Piper betle</i> L. extract against <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> using disc diffusion assay	20
	3.2.5	Determination of Minimum Inhibitory Concentration (MIC)	20
	3.2.6	Determination of Minimum Bactericidal Concentration (MBC)	20
	3.2.7	Time killing kinetics of methanolic <i>P. betle</i> L.	20
	3.2.8	Data analysis	21
	3.2.9	Phytochemical analysis	21
	3.2.9.1	Alkaloids	21
	3.2.9.2	Phenols	22
	3.2.9.3	Flavonoids	22
	3.2.9.4	Tannins	22
	3.2.9.5	Saponins	22
	3.2.9.6	Glycosides	22
	3.2.9.7	Terpenoids	23
	3.2.9.8	Steroids	23
	3.2.10	Gas Chromatography-Mass Spectroscopy (GC-MS) analysis	23
	3.3	Results	24
	3.3.1	Percentage yield of extract	24
	3.3.2	Disc diffusion assay	24
	3.3.3	Minimum Inhibitory Concentration (MIC)	27
	3.3.4	Minimum Bactericidal Concentration (MBC)	28
	3.3.5	Time-kill kinetics of methanolic <i>P. betle</i> L. extract	28
	3.3.6	Phytoconstituents in <i>P. betle</i> L. extract	29
	3.3.7	Chemical constituents of <i>P. betle</i> L. extract using GC-MS	30

3.4	Discussion	36
3.5	Conclusion	41
<b>4</b>	<b>IN VIVO STUDY OF <i>P. betle</i> L. METHANOLIC EXTRACT ON BACTERIAL LEAF BLIGHT DISEASE UNDER GLASSHOUSE CONDITION</b>	<b>42</b>
4.1	Introduction	42
4.2	Materials and Methods	43
4.2.1	Experimental location	43
4.2.2	Preparation of pre-germinated seeds for sowing	43
4.2.3	Preparation of bacterial host	43
4.2.4	Plant extract suspension preparation	43
4.2.5	Glasshouse study	43
4.2.6	Data collection	45
4.2.7	Experimental design and statistical analysis	47
4.3	Results	47
4.3.1	Plant physiology (height, width, and chlorophyll) of rice plant	47
4.3.2	Disease scoring of bacterial leaf blight on rice plants	49
4.3.3	Plant weight and yield of rice grains	53
4.4	Discussion	55
4.5	Conclusion	57
<b>5</b>	<b>SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH</b>	<b>58</b>
5.1	Summary	58
5.2	Conclusion	59
5.3	Recommendation for Future Research	59
	<b>REFERENCES</b>	<b>60</b>
	<b>APPENDICES</b>	<b>75</b>
	<b>BIODATA OF STUDENT</b>	<b>89</b>
	<b>LIST OF PUBLICATIONS</b>	<b>90</b>

## LIST OF TABLES

Table		Page
2.1	The list of plant extracts in the management of plant diseases	10
3.1	Percentage yield of crude extract of <i>P. betle</i> L. with different solvent	24
3.2	Antibacterial activity of <i>P. betle</i> L. extracts against	27
3.3	Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)	28
3.4	Phytoconstituents in <i>P. betle</i> L. extract with four different solvents	30
3.5	Composition of chemical constituents in the methanol extract of <i>P. betle</i> L. leaves	32
3.6	Composition of chemical constituents in the acetone extract of <i>P. betle</i> L. leaves	33
3.7	Composition of chemical constituents in the ethyl acetate extract of <i>P. betle</i> L. leaves	34
3.8	Composition of chemical constituents in the hexane extract of <i>P. betle</i> L. leaves	35
4.1	The list of treatment used in the glasshouse study	44
4.2	Disease severity scale for evaluation of bacterial leaf blight	47

## LIST OF FIGURES

Figure		Page
2.1	Three main phases of the disease	6
2.2	The <i>Piper betle</i> L.	11
2.3	Major bioactive compound in <i>P. betle</i> L. extract	14
3.1	The inhibition zone of <i>P. betle</i> L. extract against XOO with different solvents	26
3.2	The killing kinetics curve of <i>P. betle</i> L. methanolic extract against XOO	29
3.3	GC-MS chromatogram of methanolic extract of <i>P. betle</i> L.	32
3.4	GC-MS chromatogram of acetone extract of <i>P. betle</i> L.	33
3.5	GC-MS chromatogram of ethyl acetate extract of <i>P. betle</i> L.	34
3.6	GC-MS chromatogram of hexane extract of <i>P. betle</i> L.	35
4.1	A disease severity (score) diagram to estimate symptoms of bacterial blight disease on rice	46
4.2	The average (a) plant height and (b) width of rice plants treated with treatment (T1-T6).	48
4.3	The average chlorophyll content of the rice plants with and without treatment	49
4.4	The characteristic bacterial leaf blight disease symptoms on 7 days after post-treatment	51
4.5	The average (a) plant disease severity percentage (b) disease reduction percentage of rice plant inoculated with <i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	52
4.6	The average (a) biomass (fresh weight and dry weight) and (b) yield of rice grains (fresh weight and dry weight)	54

## LIST OF ABBREVIATIONS

UPM	Universiti Putra Malaysia
°C	degree celsius
ANOVA	One-Way Analysis of Variance
UPM	Universiti Putra Malaysia
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
LB	Luria Bertani
BLB	Bacterial Leaf Blight
XOO	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>
OD	Optical density
CLSI	Clinical and Laboratory Standard Institute
CFU	Colony forming unit
NIST	National Institute of Standard and Technology
DMSO	dimethyl sulfoxide
DAT	Day after treatment
CRD	Completely randomised design



## CHAPTER 1

### INTRODUCTION

#### 1.1 Background of the Study

Rice is one of the primary food crops for a third of the global population and plays an important role in nutrition (Khan et al., 2015). In terms of consumers and producers in Asia, it ranks second after wheat (Rajamoorthy et al., 2015). The most common rice disease is bacterial leaf blight (BLB), which is caused by the Gram-negative bacteria, *Xanthomonas oryzae* pv. *oryzae* (XOO) has a significant impact on rice productivity (Wang et al., 2017). Plant extracts possess potent activity against rice pathogenic microorganisms (Chanprapai et al., 2017). Piper betle L., often known as Sireh in Malaysia and betel in English, belongs to the Piperaceae family of plants (Venkateswarlu et al., 2014). The extract of *P. betle* L. also has the best antibacterial effect on both Gram-negative and Gram-positive bacteria (Nguyen et al., 2020). The therapeutic profile reveals *P. betle* L. to have high potential for treating many diseases (Umar et al., 2018). Because of their potential, the extract, or compounds of *P. betle* L. leaves could be a highly promising antibacterial agents for treating diseases. (Syahidah et al., 2017).

*P. betle* L. leaf exhibits antibacterial properties against a variety of microorganisms (Gram-negative and Gram-positive bacteria) (Jesonbabu et al., 2011). *P. betle* L. extract also inhibits the growth of *Staphylococcus aureus* in conjunctivitis patients and has potential use as an antibacterial agent (Lubis et al., 2020). The methanol extract was considered the most effective, with a maximum inhibition zone up to 40 mm (Jayalakshmi et al., 2013). The *P. betle* L. leaf extract has antibacterial effect on bacteria damage plasma cell membrane and produces nucleoid coagulation (Jesonbabu et al., 2011).

A phytochemical study on *P. betle* L. methanolic extract revealed the existence of terpenoids, phenols, saponins, alkaloids, glycosides, flavonoids, steroids, and tannins (Syahidah et al., 2017). The extract with a high concentration of active phenolics and flavonoids may provide highly inhibitory effects against microorganisms (Nouri et al., 2017, Muruganandam et al., 2017). Plant extracts have the effect of promoting rice plant growth. The crude chloroform extract of *Piper betle* L. improves tomato plant growth and reduces *Fusarium oxysporum* f. sp. *Lycopersici* causes *Fusarium* wilt (Manoj et al., 2011). Furthermore, *G. linearis* leaf extract has the potential to be employed as a biostimulant to enhance yield and growth of maize (Aulya et al., 2018). The shoot length of the rice variety was enhanced using aqueous extracts of *Zingiber officinale*, *Azadirachta indica*, and *Allium sativum* (Archana et al., 2017).

Moreover, under glasshouse conditions, plant extracts of *Piper sarmentosum* increased root dry weight, root length, and seedling height of rice plant compared

to the control (Syed et al., 2019). In addition, the disease severity of a BLB was reduced by 80.89% with the application of a *Piper sarmentosum* extract formulation (Syed et al., 2020). The extracts of *Chromolaena odorata* can also reduce the disease severity up to 50% of BLB diseases using a foliar spray and seed treatment method (Nguyen et al., 2011). Due to the geographic area and weather factors, XOO can cause yield losses of up to 70% in irrigated and lowland rice (Wonni et al., 2016). The loss of yield during the BLB outbreak has also been estimated at around 20-50%, depending on the rice variety, location, and weather. However, the yield losses are worst in tropical countries, as reported in the Philippines, India, and Indonesia. In Malaysia, the estimated yield loss due to BLB infection was 50-80% (Ramli, 2017).

Given the above finding, it is important to take a look the potential antibacterial effect of *P. betle* L. leaf extracts on rice pathogens. From the previous study revealed by Syahidah et al., (2017), eugenol and hydroxychavicol were the active compounds in charge for the antibacterial activity of the methanolic extract of *P. betle* L. Phytochemical investigation of *P. betle* L. extract revealed the appearance of saponins, flavonoids, phytosterols, phenols, carbohydrates, proteins, alkaloids, tannins, and volatile oil (Saini et al., 2016).

## 1.2 Problem Statement

*P. betle* L. research has exploded in popularity over the past, with numerous studies demonstrating plants' antimicrobial potential. Plant-based antibiotics, bactericides, and biopesticides have been the focus of recent studies and research. Plant extracts, which contain a variety of phytochemicals, have been shown to have antimicrobial effects. However, there are few reports *in vitro* and *in vivo* on the antibacterial effect of *P. betle* L. extracts on the rice bacterial pathogen XOO that causes BLB diseases.

## 1.3 Objective

The objectives of this study are:

1. To determine the antibacterial effect of *Piper betle* L. extracts against *Xanthomonas oryzae* pv. *oryzae*.
2. To determine the phytochemical constituents and active compounds responsible for antibacterial activities by GCMS analysis.
3. To see how effective *Piper betle* L. extract on disease suppression and the growth of rice plants.

#### 1.4 Hypothesis

The hypotheses of this study are:

1. The methanolic extract of *Piper betle* L. will show greater activity compared to other extracts.
2. The active component responsible for the antibacterial activities will be revealed by GCMS analysis.
3. The *Piper betle* L. extracts will have an effect on disease suppression and the growth of rice plant



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