UNIVERSITI TEKNOLOGI MARA

ANTIBACTERIAL ACTIVITY OF Asystasia gangetica LEAF CRUDE EXTRACTS AGAINST Erwinia chrysanthemi, PATHOGEN OF FRUIT COLLAPSE DISEASE IN JOSAPINE VARIETY OF PINEAPPLE

HAMIMAH BINTI MAZLAN

Thesis submitted in fulfillment of the requirements for the degree of Master of Science (Crop Protection)

Faculty of Plantation and Agrotechnology

March 2018

ABSTRACT

This study was carried out to evaluate the antibacterial activities of Asystasia gangetica leaf extracts against pathogen of pineapple fruit collapse disease Erwinia chrysanthemi also known as Dickeya zeae. The dried leaves of A. gangetica were extracted by sequential extraction of increasing polarity solvents (hexane, chloroform, methanol, aqueous). The screening of antibacterial activity via in vitro assays against E. chrysanthemi showed that methanol leaf extracts gave the greater growth inhibition zone (17.6 mm) compared to hexane, chloroform and aqueous extracts with 5 mm, 11.9 mm, and 14.4 mm zone of inhibition respectively. Furthermore, methanol extracts also showed a greater inhibition zone compared to positive control (11 mm zone of inhibition) at concentration of 10 000 ppm. The phytochemical screening showed that saponin, alkaloid, flavanoid, glycoside, tannins and terpenoid present in A. gangetica leaf extracts. The active crude extracts (methanol) were further purified by Flash Vacuum Chromatography (FVC) fractionation, resulted in total of 11 fractions. The fraction (F5) eluted with ethyl acetate: methanol in the ratio of 5: 5 showed the most active fraction to show the antibacterial activity against E. chrysanthemi. The determination of major compound in methanol crude extract by Gas Chromatography Mass Spectrometry (GCMS) analysis as done to confirm the presence of Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester as the major compound. The effectiveness of active crude extract in vivo assays was conducted and found that the preventive control was more effective with the lower severity than curative control. The results proven that A. gangetica leaf potentially can be used as biocontrol sources for bacterial fruit collapse disease of pineapple.

ACKNOWLEDGEMENT

Alhamdulillah and praise be to Allah the almighty with His blessed, I am finally complete my research project. During the process in completing this Final Year Project, I share meaningful experiences with many people who directly or indirectly contribute to the success of this report. Sincerely I would like to express my deepest appreciation to two important people in this thesis, my supervisor. Dr. Neni Kartini Bt Che Mohd Ramli and Associate Professor Dr. Nor Yuziah Binti Mohd Yunus from UiTM Pahang for her supervisions, guidance, encouragement, and patience throughout the progress of completing this thesis for their endless guidance and encouragement throughout the projects.

This research project brought me the opportunity to cooperate with several of personnel from various agencies, who cooperate, and share the information for this project. Greatest thanks to Mr. Rosdi, Mr. Fauzi also other UiTM Pahang staff.

A large gratitude with loves especially to my parent, and Mazlan Bin Ahmad that always prays for my success and my siblings which always concern about me. I would like thanks to my friend, Khairun Nur binti Ali that always give help and support. My thanks also to my members, Nadiahtul Afiqah binti Sulaiman, Nurnadirah Binti Mat Nawi and all my friends that I should mention their name here for helping and supporting me until I complete my thesis Master. Thank you for your endless du'a, patience and support. I deeply owe a favor to them for love and scarified.

TABLE OF CONTENTS

		Page			
CONFIRMATION BY PANEL OF EXAMINERS					
AUTHOR'S DECLARATION					
ABSTRACT ACKNOWLEDGEMENT TABLE OF CONTENTS					
			LIS	T OF TABLES	ix
			LIS	T OF FIGURES	x
LIS	T OF PLATES	xi			
LIS	LIST OF SYMBOLS				
LIS	T OF ABBREVIATIONS	xiii			
СН	APTER ONE: INTRODUCTION	1			
1.1	Background	1			
1.2	Problem Statement	4			
1.3	Research Objectives	7			
1.4					
1.5	Significant of Study	7			
1.6	Scope of Study	8			
СН	APTER TWO: LITERATURE RIVIEW	9			
2.1	Pineapple Fruit collapse disease	9			
2.2	Erwinia chrysanthemi	10			
2.3	Symptom of Pineapple Fruit Collapse Disease	11			
	2.3.1 Epidemiology of Pineapple Fruit Collapse Disease	13			
2.4	Control Measure of Pineapple Fruit Collapse Disease of Pineapple	12			
	2.4.1 Cultural Control	14			
	2.4.2 Chemical Control	16			
	2.4.3 Biological Control	22			

2.5	Potential Use of Plant Extracts as Biopesticide			
2.6	Potent	ial of A. gangetica as Biopesticide	24	
CH	APTEI	R THREE: METHODOLOGY	27	
3.1	Plant	Sample Collection for Plant Extract	27	
3.2	Disease Sample Collection			
3.3	Isolation of Pineapple Bacterial Fruit Collapse Disease			
3.4	Pathogenicity Test			
3.5	Identification of E. chrysanthemi			
	3.5.1	Preliminary Identification	29	
	3.5.2	Gram Staining	29	
	3.5.3	Potassium Hydroxide Test	29	
	3.5.4	Selective Medium Identification	30	
	3.5.5	PCR Identification	30	
3.6	Preparation of Plant Extracts			
	3.6.1	Solvent Extraction	31	
	3.6.2	Aqueous Extraction	33	
3.7	Antiba	acterial Activity Screening of A. gangetica against E. chrysanthemi	33	
3.8	Deterr	nination of Effective Inhibitory Concentration	35	
3.9	Phytochemical Test			
	3.9.1	Steroid	36	
	3.9.2	Alkaloids	36	
	3.9.3	Flavanoid	36	
	3.9.4	Tannins	36	
	3.9.5	Phenols	36	
	3.9.6	Glycosides	37	
3.10	Fracti	onation of Active Crude Extracts	37	
	3.10.1	Admixture Preparations	37	
	3.10.2	Column Packing	37	
	3.10.3	Thin Layer Chromatography	38	
	3.10.4	Bioasays of The Fractions against E. chrysanthemi	39	
3.11	3.11 Gas Chromatography Mass Spectrometry			
3.12	.12 <i>In-vivo</i> Test			