UNIVERSITI PUTRA MALAYSIA

IN-VITRO ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF BIOACTIVE COMPOUNDS FROM GUAVA (*Psidium guajava* L.) CRUDE LEAF EXTRACTS

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By

SITI NUR SARAH BINTI SHAFIEI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

August 2012

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

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August 2012

Chair: Associate Professor Kamaruzaman bin Sijam, PhD

Faculty: Agriculture

Plant pathogenic bacteria are known to be detrimental microbes able to reduce the quality and quantity of crop production around the world. *Psidium guajava* leaf was screened for its potential use as biological control agent against plant pathogenic bacteria. *P. guajava* leaf was successfully extracted using n-hexane, ethyl acetate and methanol by maceration. The yield percentage of crude extracts obtained for each extract was 0.93% for hexane, 1.28% for ethyl acetate and 1.70% for methanol leaf crude extracts. The higher yield obtained by methanol showed that methanol extracted more compounds that are readily soluble to methanol than hexane and ethyl acetate. For *in vitro* antibacterial activity, seven different species of plant pathogenic bacteria were used namely *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium chrysanthemi*, *Ralstonia solanacearum*, *Xanthomonas axonopodis* pv. *citri*, *Xanthomonas euvesicatoria*, *Xanthomonas oryzae* pv. *oryzae* and

Xanthomonas oryzae pv. *oryzicola.* For all crude extracts, four different concentrations 25, 50, 100 and 200 mg/ml were used in cup-plate agar diffusion method. Streptomycin sulfate at concentration of 30 µg/ml was used as positive control, while each respective solvent used for leaf extraction was used as negative control. The results obtained from *in vitro* studies showed only methanol extract possessed antibacterial activity tested on the plant pathogenic bacteria. Hexane and ethyl acetate did not show any antibacterial activity against plant pathogenic bacteria where no inhibition zones were recorded. *X. oryzae* pv. *oryzae* recorded the highest diameter of inhibition zones for all range of concentrations introduced followed by *X. oryzae* pv. *oryzicola, X. euvesicatoria, X. axonopodis* pv. *citri, P. chrysanthemi* and *P. carotovorum* subsp. *carotovorum.* For minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination, only methanol extract was subjected for the assay as only methanol extract exhibited antibacterial activity. The lowest concentration of methanol extract used in MIC assay was at 0.39 mg/ml which inhibited *X. oryzae* pv. *oryzae,* followed by *X. euvesicatoria* (0.78 mg/ml), and *X. oryzae* pv. *oryzicola, X. axonopodis* pv. *citri, P. chrysanthemi* and *P. carotovorum* subsp. *carotovorum* at concentration 1.56 mg/ml. This assay showed that methanol extract had bacteriostatic effects at concentration 0.39, 0.78 and 1.56 mg/ml for different species of plant pathogenic bacteria. The lowest MBC concentration recorded was at 0.78 mg/ml against *X. oryzae* pv. *oryzae*, 1.56 mg/ml against *X. euvesicatoria,* 3.13 mg/ml against *X. oryzae* pv. *oryzicola* and *X. axonopodis* pv. *citri,* and 6.25 mg/ml against *P. chrysanthemi* and *P. carotovorum* subsp. *carotovorum.* The phytochemical screening of *P. guajava* methanol leaf extracts showed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids and phenols. The thin layer chromatography (TLC) profiling of methanol extract

using hexane and ethyl acetate with ratio of 7:3 (v/v) gave 13 maximum colorful bands when visualized with 10% ethanolic sulfuric acid solution, with different retention factor, R_f values which proved the presence of various active secondary metabolites within methanol extract. The antibacterial activity of methanol extract was also screened through direct bioautography technique in order to detect the location of active bands on chromatograms developed in the same matter for TLC profiling. The most active R_f values which inhibited all tested plant pathogenic bacteria at the same R_f values location were at 0.63, 0.85, and 0.98. The R_f values obtained were compared to the previous studies performed using the same solvent system indicated that the R_f values recorded at >0.90 belongs to hydrocarbons, and 0.60-0.80 belongs to epoxides and esters which were found to fall within oxygenated terpenes group.

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

AKTIVITI ANTIBAKTERIA *IN-VITRO* DAN PENYARINGAN FITOKIMIA SEBATIAN AKTIF DARIPADA EKSTRAK MENTAH DAUN JAMBU BATU (*Psidium guajava* L.)

Oleh

SITI NUR SARAH BINTI SHAFIEI

Ogos 2012

Pengerusi: Profesor Madya Kamaruzaman bin Sijam, PhD

Fakulti: Pertanian

Bakteria penyakit tanaman dikenali sebagai mikrob pemusnah yang berupaya mengurangkan kualiti dan kuantiti penghasilan tanaman di seluruh dunia. Daun *Psidium guajava* telah disaring untuk mengenalpasti potensinya sebagai agen kawalan biologi dalam mengawal bakteria patogen tumbuhan. Daun *P. guajava* telah berjaya diekstrak menggunakan n-heksana, etil asetat dan metanol melalui kaedah rendaman. Peratusan hasil ekstrak mentah yang diperolehi bagi setiap ekstrak adalah 0.93% untuk heksana, 1.28% untuk etil asetat dan 1.70% untuk ekstrak metanol daun mentah. Hasil yang lebih tinggi yang diperolehi oleh metanol menunjukkan bahawa metanol mengekstrak lebih banyak sebatian yang mudah larut metanol daripada heksana dan etil asetat. Bagi aktiviti anti-bakteria secara *in vitro*, tujuh spesies bakteria patogen tumbuhan yang berbeza telah digunakan iaitu *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium chrysanthemi*, *Ralstonia*

solanacearum, *Xanthomonas axonopodis* pv. *citri*, *Xanthomonas euvesicatoria*, *Xanthomonas oryzae* pv. *oryzae* dan *Xanthomonas oryzae* pv. *oryzicola*. Bagi kesemua ekstrak mentah, empat kepekatan yang berbeza iaitu 25, 50, 100 dan 200 mg/ml digunakan dalam kaedah peresapan agar cawan-plat. Streptomisin sulfat pada 30 kepekatan µg / ml telah digunakan sebagai kawalan positif, manakala setiap pelarut yang digunakan untuk pengekstrakan daun digunakan sebagai kawalan negatif. Keputusan yang diperolehi dari kajian secara *in vitro* menunjukkan hanya ekstrak metanol memiliki potensi anti-bakteria apabila diuji terhadap bakteria patogen tumbuhan. Heksana dan etil asetat tidak menunjukkan sebarang potensi sebagai anti bakteria terhadap bakteria patogen tumbuhan di mana tiada zon perencatan direkodkan dan diperhatikan untuk ekstrak ini. *X. oryzae* pv. *oryzae* mencatatkan diameter tertinggi zon perencatan bagi semua julat kepekatan yang diperkenalkan diikuti oleh *X. oryzae* pv. *oryzicola*, *X. euvesicatoria*, *X. axonopodis* pv. *citri*, *P. chrysanthemi* dan *P. carotovorum* subsps *carotovorum*. Bagi penentuan kepekatan perencatan minimum (MIC) dan kepekatan minimum bakterisida (MBC), ekstrak metanol digunakan sebagai cerakin kerana hanya ekstrak metanol mempamerkan aktiviti anti bakteria. Kepekatan terendah metanol ekstrak yang digunakan dalam MIC adalah pada 0.39 mg/ml yang menghalang pertumbuhan *X. oryzae* pv. *oryzae*, diikuti oleh *X. euvesicatoria* (0.78 mg/ml), dan *X. oryzae* pv. *oryzicola*, *X. axonopodis* pv. *citri*, *P. chrysanthemi* dan *P. carotovorum* subsp. *carotovorum* pada kepekatan 1.56 mg/ml. Cerakin ini menunjukkan bahawa ekstrak metanol mempunyai kesan bakteriostatik pada kepekatan 0.39, 0.78 dan 1.56 mg/ml untuk spesies yang berbeza bagi bakteria patogen tumbuhan. Kepekatan MBC terendah yang dicatatkan adalah pada 0.78 mg/ml terhadap *X. oryzae* pv. *oryzae*, 1.56 mg/ml terhadap *X. euvesicatoria*, 3.13 mg/ml terhadap *X. oryzae* pv. *oryzicola* dan *X.*

axonopodis pv. *citri*, dan akhirnya pada kepekatan 6.25 mg/ml terhadap *P. chrysanthemi* dan *P. carotovorum* subsp. *carotovorum*. Pemeriksaan fitokimia ekstrak metanol daun *P. guajava* menunjukkan kehadiran alkaloid, flavonoid, tanin, saponin, terpenoid dan fenol. Pemprofilan lapisan kromatografi nipis (TLC) ekstrak metanol menggunakan sistem pelarut heksana dan etil asetat dengan nisbah 7:3 (v/v) memberi 13 lapisan maksimum yang berwarna-warni apabila disembur dengan 10% larutan asid sulfurik etanol, dengan faktor tahanan berbeza, nilai R_f menunjukkan kehadiran pelbagai metabolit sekunder aktif dalam ekstrak metanol. Aktiviti anti bakteria ekstrak metanol juga telah dijalankan melalui teknik bioautografi langsung untuk mengesan lokasi lapisan aktif pada kromatogram yang dibangunkan dengan sistem pelarut yang sama didalam pemprofilan TLC. Nilai-nilai yang R_f paling aktif yang menghalang semua bakteria patogenik yang diuji terdapat pada lokasi nilai R_f yang sama iaitu pada 0.63, 0.85, dan 0.98. Nilai R_f yang diperolehi berbanding dengan kajian yang dilakukan sebelum ini dengan menggunakan sistem pelarut yang sama menunjukkan bahawa nilai R_f yang direkodkan pada >0.90 adalah milik hidrokarbon, dan 0.60-0.80 milik epoksida dan ester yang didapati tergolong dalam kumpulan terpenes teroksigen.

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I certify that a Thesis Examination Committee has met on 7th August 2012 to conduct the final examination of Siti Nur Sarah binti Shafiei on her thesis entitled "*In-vitro* Antibacterial Activity and Phytochemical Screening of Bioactive Compounds from Guava (*Psidium guajava* L.) Crude Leaf Extracts" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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Date: 22 October 2012

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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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

SITI NUR SARAH BINTI SHAFIEI

Date: 7th August 2012

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