UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL COMPOUND DETERMINATION OF Entada rheedei SPRENG AND ITS ANTIFUNGAL ACTIVITY ON SELECTED SOILBORNE FUNGAL PHYTOPATHOGENS

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By

NENI KARTINI BT CHE MOHD RAMLI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

May 2013

DEDICATION

I dedicated this precious effort, the fruit of my thoughts and study to my affectionate mother, brothers and sisters, husband Samsuri and children Nurul Shuhada, Muhammad Shahril Najmi, Muhammad Shahril Nazim, Muhammad Syamil Nabil and Muhammad Syamim Naqib who inspired me to the higher destiny of life.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman : Associate Professor Kamaruzaman Sijam, PhD

Faculty : Agriculture

This study presents the potential use of natural products from *Entada rheedei* Spreng. as biofungicide and their antifungal activity against five common soilborne fungal pathogens. The dried leaves, stems and seeds of *E. rheedei* were extracted by sequential extraction using petroleum ether, chloroform, methanol and water. Among the four solvents used, methanol (MeOH) was proven to be the best extracting solvent by giving the highest quantity of crude extract yielding 7.74g (3.87%) of stems crude extract, 1.861g (0.93%) of leaves crude extract and 26.6g (13.3%) of seed crude extract. The gained crude extracts were then screened via *in vitro* assays for their antifungal activity, using well diffusion method, against five selected soilborne fungal pathogens *Rhizoctonia solani*, *Fusarium commune*, *Fusarium oxysporum f.sp. cubense* (FOC), *Pythium ultimum* and *Phytophthora palmivora*. The petroleum ether and chloroform extracts of stems, seeds and leaves of *E. rheedei* failed to inhibit the growth of all tested soilborne fungal phytopathogens. The best inhibitory effect was elicited by the stems and seeds crude methanol extracts against *Fusarium commune* (51.4% and 50% radial growth inhibition respectively), while

crude water and methanol extracts of seed against FOC showed the least significant effect (15% radial growth inhibition). Nevertheless, seed crude methanol and water extracts gave an impressive inhibitory on radial growth of *F. commune* with 50% and 36.7% inhibition respectively. Moreover, stem crude methanol and water extracts also showed moderately significant inhibitory against FOC (45% and 42.5% inhibitory respectively). The results also showed that all the stem and seed methanol and water crude extracts failed to inhibit the growth of *R. solani, P. ultimum* and *P. palmivora*. The Effective Inhibitory Concentration (EIC) of stem and seed crude methanol and water were determined at 100mg/mL. The phytochemical analysis of the active crude extracts revealed the presence of tannins, saponins, glycosides, triterpenoid, and alkaloid which only present in seed. Light and scanning electron microscopic observation on the effect of active crude extracts on spore germination and hyphal growth of *F. commune* and FOC illustrated that the spore and hyphae were collapsed and lysed; cell wall were degraded and shrank; while the spore (macroconidia) failed to germinate. The active crude extracts were further purified through Vacuum Liquid Chromatography (VLC) fractionation and resulted in the total of 22 fractions with 11 fractions for each stem and seed methanolic extracts. The obtained fractions were then screened for the antifungal activity against the selected soilborne fungal pathogens. Results showed some improvement on antifungal activity of the stem and seed methanolic fractions. The fraction eluted with chloroform: methanol in the ratio of 6:5 (F5) and 5:5 (F6) of stem methanol proved to be the most active fractions against *F. commune* (56.8% and 45.9% growth inhibition) and FOC (48.7% and 43.6% growth inhibition). All fractions of stem and seed methanolic extracts showed some improvement in their antifungal activity against *R. solani* and *P. palmivora,* which were not shown previously by any of their

parent crude extracts. The TLC phytochemical analysis showed the presence of saponin and tannin in the fractions F4, F5, F6 and F7 of stem and seed methanolic extracts. The determination of major active compound in the active fractions via High Performance Liquid Chromatography (HPLC) analysis, to confirm the major constituent contained in the active fractions, showed that the active fractions (F4-F7) of stem and seed methanolic extracts of *E. rheedei* contain saponin as major active compound.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah**.**

PENENTUAN SEBATIAN KIMIA TUMBUHAN *Entada rheedei* SPRENG DAN AKTIVITI ANTIKULATNYA KE ATAS PATOGEN TUMBUHAN KULAT BAWAAN TANAH TERPILIH

Oleh

NENI KARTINI CHE MOHD RAMLI

Mei 2013

Pengerusi : Prof Madya Kamaruzaman Sijam, PhD

Fakulti : Pertanian

Kajian menunjukkan potensi kegunaan hasilan semulajadi daripada pokok *Entada rheedei* Spreng sebagai racun kulat biologi dan aktiviti antikulatnya terhadap lima jenis patogen kulat bawaan tanah. Daun, batang dan biji kering *Entada rheedei* diekstrak melalui pengekstrakan urutan menggunakan empat jenis larutan petroleum ether, kloroform, methanol dan air. Methanol (MeOH) telah terbukti sebagai pelarut pengekstrak terbaik dengan memberikan kuantiti ekstrak mentah terbanyak dengan menghasilkan 7.74g (3.87%) ekstrak mentah batang, 1.861g (0.93%) ekstrak mentah daun dan 26.6g (13.3%) ekstrak mentah biji. Ekstrak-ekstrak mentah yang kedapatan di saringkan untuk aktiviti kulatnya secara *in vitro* dengan kaedah pembauran lubang terhadap lima patogen kulat bawaan tanah terpilih *Rhizoctonia solani, Fusarium commune, Fusarium oxysporum f.sp. cubense* (FOC), *Pythium ultimum* dan *Phytophthora palmivora.* Ekstrak petroleum ether dan kloroform extracts batang, biji dan daun gagal merencat pertumbuhan semua kulat pathogen tumbuhan yang diuji. Perencatan terbaik ditunjukkan oleh ekstrak mentah methanol batang dan buah terhadap *Fusarium commune* (masing-masing dengan 51.4% dan 50% perencatan jejari pertumbuhan), Sementara ekstrak air dan methanol biji memberikan kesan

yang sedikit terhadap FOC (15% perencatan jejari pertumbuhan). Namun, ekstrak mentah methanol dan air biji memberikan perencatan jejari pertumbuhan yang menggalakan terhadap *F. commune* dengan 50% dan 36.7% perencatan masing-masing. Seterusnya, ekstrak mentah methanol dan air batang juga menunjukkan perencatan yang sederhana terhadap FOC (45% dan 42.5% perencatan masing-masing). Keputusan juga menunjukkan semua ekstrak mentah methanol dan air batang dan biji gagal untuk merencat *R. solani*, *P. ultimum* dan *P. palmivora*. Kepekatan Perencatan Berkesan (KPB) ekstrak mentah methanol dan air batang dan biji telah ditentukan pada 100mg/mL. Analisa fitokimia ke atas ekstrak aktif *E. rheedei* mendapati kehadiran tannins, saponin, glycosides, triterpenoid, dan alkaloid yang hanya terdapat dalam biji. Cerapan mikroskopik melalui mikroskop cahaya dan elektron terhadap kesan ekstrak mentah aktif terhadap percambahan spora dan pertumbuhan hifa kulat *F. commune* dan FOC memperlihatkan spora dan hifa kulat meruntuh dan pecah; dinding sel hancur dan mengecut; sementara spora (makrokonidia) gagal untuk bercambah. Ekstrak mentah yang aktif seterusnya di tulinkan melalui fraksinasi Kromatografi Cairan Hampagas (KCH) dan telah menghasilkan sejumlah 22 fraksi dengan 11 fraksi untuk setiap ekstrak mentah methanol batang dan biji. Fraksi yang kedapatan seterusnya disaringkan untuk aktiviti antikulatnya terhadap patogen kulat bawaan tanah terpilih. Keputusan telah menunjukkan peningkatan terhadap aktiviti antikulat fraksi methanol batang dan biji. Fraksi batang methanol yang di alirkan dengan pelarut kloroform: methanol dalam nisbah 6:5 (F5) dan 5:5 (F6) terbukti menjadi fraksi yang teraktif terhadap *F. commune* (56.8% dan 45.9% perencatan pertumbuhan) dan FOC (48.7% and 43.6% perencatan pertumbuhan). Semua fraksi ekstrak methanol batang dan biji menunjukkan peningkatan dalam aktiviti antikulatnya terhadap *R. solani* dan *P.*

palmivora, yang mana sebelumnya tidak ditunjukkan oleh ekstrak mentah induk mereka. Analisa fitokimia TLC menunjukkan kehadiran saponin dan tannin di dalam fraksi-fraksi F4, F5, F6 dan F7 ekstrak methanol batang dan biji. Penentuan sebatian utama yang aktif didalam fraksi yang aktif melalui analisa Kromatografi Cairan Prestasi Tinggi, bagi mempastikan kandungan utama dalam fraksi aktif telah menunjukkan fraksi-fraksi aktif (F4-F7) ekstrak methanol batang dan biji mengandungi saponin sebagai sebatian aktif utama.

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APPROVAL

I certify that a Thesis Examination Committee has met on 31st May 2013 to conduct the final examination of Neni Kartini Che Mohd Ramli on her thesis entitled "Phytochemical compounds determination of *Entada rheedei* Spreng and its antifungal activity against selected soilborne fungal phytopathogens" in accordance with the Universities and University College 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Lau Wei Hong, PhD
Department of Crop Protection,
Faculty of Agriculture,
Universiti Putra Malaysia
(Chairman)

Zainal Abidin Mior Ahmad, PhD
Associate Professor,
Department of Crop Protection,
Faculty of Agriculture,
Universiti Putra Malaysia
(Internal Examiner)

Sariah Meon, PhD
Professor,
Department of Crop Protection,
Faculty of Agriculture,
Universiti Putra Malaysia
(Internal Examiner)

Jamal Ragheb Said Qasem, PhD
Professor,
Department of Plant Protection,
Faculty of agriculture,
University of Jordan
(External Examiner)

_____
SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows

Kamaruzaman Sijam, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Dzolkhifli Omar, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Rita Muhamad, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Siti Zaiton Mad So'ad, Phd
Assistant Professor
Kuliyyah of Pharmacy
International Islamic University Kuantan, Pahang
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

Declaration by graduate student

I hereby confirm that:

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Name and Matric No.: Neni Kartini Che Mohd Ramli (GS19109)

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