



UNIVERSITI PUTRA MALAYSIA

***ANTIFUNGAL ACTIVITY OF *Cosmos caudatus* KUNTH EXTRACT
ON PLANT PATHOGENS AND IDENTIFICATION OF ACTIVE
CONSTITUENTS***

NAZIHAH BINTI MOHD SALEHAN

ITA 2013 2



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CONSTITUENTS**

By

NAZIHAH BINTI MOHD SALEHAN

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

July 2013

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Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirement from the Degree of Master of Science

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July 2013

Chairman : Professor Sariah Meon, PhD

Institute : Institute of Tropical Agriculture, UPM

Increasing concern over adverse environmental effects and decreasing efficacy of synthetic fungicides has brought about the urge for the development of new and natural control alternatives for plant diseases. Thus, extensive exploration on exploitation of plants as natural commercial biopesticides is highly desired. *Cosmos caudatus* Kunth or locally known as 'Ulam Raja' in Malaysia, is a traditional herb to cure and improve human ailments. However, antifungal activity of *C. caudatus* on plant pathogens has not yet been studied. Crude leaf ethanol extract of *C. caudatus* was separated into three fractions; hexane, ethyl acetate (EtOAc) and aqueous fractions and screened for their antifungal activity against selected plant pathogens using agar cup method. The EtOAc fraction was shown to be the most active in inhibiting mycelia growth of *Phytophthora palmivora*, the causal pathogen of black pod of cocoa with percentage inhibition in radial

growth (PIRG) of 52.0%. Sporangia germination was recorded the lowest in EtOAc fraction with values of 15.62%. This was further observed in the scanning electron micrographs of *P. palmivora* treated with EtOAc fraction showing stunted growth and abnormalities in the mycelium with reduced sporangia production. The bio-efficacy of the fractions was further tested on detached cocoa pods. The EtOAc fraction gave the highest inhibition of 57.46% on diameter of lesions. These findings indicated that the EtOAc fraction of *C. caudatus* leaves possesses fungistatic properties against *P. palmivora*. Identification of the active antifungal compound(s) was carried out using bioassay guided fractionation by column chromatography technique. The EtOAc fraction was first fractionated into thirteen fractions, in which the first fraction (F1) showed highest inhibition against *P. palmivora* (PIRG of 54.73%). Fraction F1 was further fractionated into eight sub-fractions (F1-1 to F1-8) and it was found that F1-4, F1-5, and F1-6 as the most active with PIRG of 52.56, 46.92 and 48.05%, respectively. Sub-fraction F1-4 was subjected to Solid Phase Extraction (SPE) and the active constituents were identified using GC-MS analysis. Based on NIST/Wiley data, the compounds suggested to have antifungal activity against *P. palmivora* were identified as oleic acid [9(*Z*)-octadecenoic acid] (**1**), palmitic acid [hexadecanoic acid], 2-hydroxy-1- (hydroxymethyl) ethyl ester (**2**), nonanal (**3**), octanal (**4**), and (*Z*)-2-decenal (**5**). These findings are hoped to benefit in the development of biopesticide for the control of black pod disease, which ultimately will have profound effects on crop production and economic values.

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

**AKTIVITI ANTIKULAT OLEH EKSTRAK *Cosmos caudatus* KUNTH
TERHADAP PATOGEN TUMBUHAN DAN PENGENALPASTIAN
KOMPONEN AKTIF**

Oleh

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Kebimbangan yang semakin meningkat ke atas kesan buruk persekitaran dan penyusutan keberkesanan racun kulat sintetik telah membawa kepada desakan untuk pembangunan alternatif kawalan yang baru dan semulajadi untuk penyakit tumbuhan. Oleh itu, penerokaan yang meluas di dalam mengeksploitasi tumbuhan sebagai racun perosak biologi semulajadi komersil adalah amat diperlukan. *Cosmos caudatus* Kunth atau dikenali sebagai Ulam Raja di Malaysia, merupakan herba tradisional untuk mengubati dan merawat penyakit manusia. Walau bagaimanapun, aktiviti antikulat oleh *C. caudatus* ke atas patogen tumbuhan masih belum dikaji. Ekstrak mentah etanol daun *C. caudatus* telah dipisahkan kepada tiga pecahan; heksana, etil asetat, dan akueous dan disaring untuk aktiviti antikulat terhadap patogen tumbuhan terpilih menggunakan kaedah cawan

agar. Pecahan EtOAc didapati menjadi paling aktif dalam menghalang pertumbuhan mycelia *Phytophthora palmivora*, iaitu patogen penyebab penyakit bintik hitam pada koko, dengan peratusan 52.0% dalam peratusan penyekatan pertumbuhan radius (PIRG). Percambahan sporangia dicatatkan terendah dalam pecahan EtOAc dengan nilai sebanyak 15.62%. Keputusan mikrograf elektron pengimbasan bagi *P. palmivora* yang dirawat dengan pecahan EtOAc dan ia telah menunjukkan pertumbuhan terbantut dan ketidaknormalan dalam miselium dengan penurunan pengeluaran sporangia. Keberkesanan bioefikasi antara pecahan telah diuji pada buah koko terpisah dan pecahan EtOAc telah menunjukkan perencatan tertinggi dalam saiz luka ke atas koko dengan nilai sebanyak 57.46%. Penemuan ini menunjukkan bahawa pecahan EtOAc daun *C. caudatus* mempunyai sifat fungistatik terhadap *P. palmivora*. Pengenalpastian sebatian aktif antikulat telah dijalankan menggunakan bioasai pemeringkatan berpandu dengan teknik kromatografi turus. Pecahan EtOAc telah dipecahkan kepada tiga belas pecahan, di mana pecahan pertama (F1) menunjukkan aktiviti perencatan tertinggi terhadap *P. palmivora* (PIRG 54.73%). Pecahan F1 seterusnya dipecahkan lagi kepada lapan sub-pecahan (F1-1 to F1-8) dan keputusan menunjukkan F1-4, F1-5, dan F1-6 sebagai paling aktif dengan PIRG masing-masing sebanyak 52.56, 46.92 dan 48.05%. Sub-pecahan F1-4 telah melalui Pengekstrakan Fasa Pepejal (SPE) dan komponen aktif telah dikenal pasti menggunakan analisis GC-MS. Berdasarkan keputusan NIST/Wiley, sebatian yang dicadangkan mempunyai aktiviti antikulat terhadap *P. palmivora* telah dikenalpasti sebagai asid oleik [9(Z)-asid oktadesenoik] (**1**), asid palmitik [asid heksadekanoik], 2-hidroksi-1-(hidroksimetil) etil ester (**2**),

nonanal (**3**), oktanal (**4**), dan (*Z*)-2-desenal (**5**). Penemuan ini diharapkan dapat memberi manfaat dalam pembangunan racun perosak biologi untuk kawalan penyakit pod hitam, yang akhirnya akan mempunyai kesan yang mendalam kepada pengeluaran tanaman dan nilai-nilai ekonomi.



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I certify that a Thesis Examination Committee has met on 4 July 2013 to conduct the final examination of Nazihah binti Mohd Salehan on her thesis entitled "Antifungal Activity of *Cosmos caudatus* Kunth Extract on Plant Pathogens and Identification of Active Constituents" 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.

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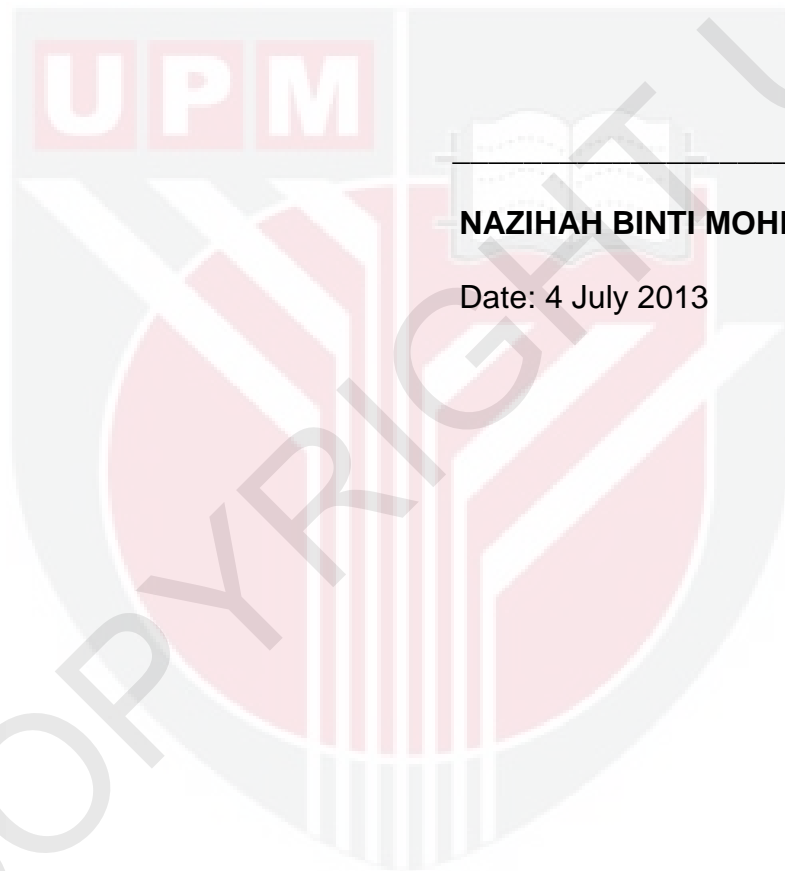
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DECLARATION

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



NAZIHAH BINTI MOHD SALEHAN

Date: 4 July 2013



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