ANTIMICROBIAL ACTIVITY OF *Psidium guajava* AND *Piper betle* EXTRACTS ON SELECTED FOODBORNE BACTERIA

By

HENIE EDWARD FRANCIS PARILLON

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

March 2006

Dedication

To my deceased parents (Everina and Alixford), for their continued guidance through the years.

Also, to my biological mother (Githa) who continues to extend her love and support, my friends and my "self-attained" family who have supported and encouraged me throughout this journey.

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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Chairman: **Associate Professor Zaiton bt Hassan, PhD**

Faculty: **Food Science and Technology**

Five plants, namely *Psidium guajava* (guava), *Illicium verum* (star anise), *Annona squamosa* (sugar apple) and two cultivars of *Piper betle* Linn. (betel leaf), were screened for antimicrobial activity against sixteen foodborne bacteria using the agar disc diffusion method. The plants were selected on the basis of folklore medicinal reports and as practiced by people in Malaysia and the Caribbean. The methanolic extracts of all plants used in preliminary screening had antibacterial activity against at least on one bacterium with guava and the red vein (rv) and green vein (gv) cultivars of *Piper betle* L., demonstrating greater antimicrobial activity. *I. verum* extract was effective against *Aeromonas hydrophila* and *Citrobacter freundii* while the *Annona squamosa* extract was effective only against *Vibrio parahaemolyticus*. The sequentially extracted crude material from the leaves of *P. betle* L. (rv), *P. betle* L. (gv), and guava were evaluated for effectiveness against the bacteria. The methanolic extract of *P. betle* L. (gv) was found to be effective against both Gram-positive and Gram-negative pathogens while the methanolic extract of guava was

mainly effective against the Gram-positive bacteria. The hexane and ammoniacal chloroform extracts of *P. betle* L. (rv) inhibited the growth of most Gram-negative pathogens.

The minimum inhibitory concentration (MIC) of the methanolic extracts of *P. betle* L. (gv) (PBME) and guava (GME) against *Kocuria rhizophila, Staphylococcus aureus, A. hydrophila, Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* and *V. parahaemolyticus* was investigated. The bacteria most sensitive to the GME (MIC = 1 mg/ml) were *S. aureus*, *K. rhizophila* and *E. coli* O157:H7 while *S. aureus* was the most sensitive to the PBME at the same concentration. *A. hydrophila* and *V. parahaemolyticus* were more resistant to the PBME with an MIC of 10 mg/ml. The extracts were also investigated for their killing kinetics against four bacteria. The GME completely inhibited the growth of *K. rhizophila* in 12 h at a concentration of 1 mg/ml while at 5 mg/ml, complete inhibition was obtained in 4 h. PBME also killed all the bacterial cells of *L. monocytogenes*, at a concentration of 1 mg/ml in 32 h while at 5 mg/ml, complete inhibition was obtained in 4 h. At the concentration of 1 mg/ml the rate of killing of the GME was faster than that of the PBME for all the bacteria studied.

The effect on cell viability and cellular leakage of *K. rhizophila, S.* Typhimurium, *L. monocytogenes* and *E. coli* O157:H7 was measured after exposure to PBME and GME at a concentration of 5000 ppm (5 mg/ml). Assay of filtrates of all treated cell suspensions revealed a significantly higher ($P \leq 0.05$) release of nuclear material, K^+ ions and protein than that of untreated controls, thereby indicating disruptive action on the cytoplasmic membrane of bacterial cells. The PBME caused a higher leakage

of K^+ ions than the GME for all the bacteria tested while total protein released by *K. rhizophila* and *S.* Typhimurium was also higher in cells treated with PBME than with GME. The PBME caused a noticeably higher ($P \leq 0.05$) release of nuclear material in the Gram-positive organisms while the GME caused a higher release in the Gram-negative. While GME caused a higher release of 260nm-absorbing materials than PBME for all the organisms investigated, both extracts appeared to have disrupted the integrity of the lipopolysaccharide (LPS) layer of the Gram-negative microorganisms. Several mechanisms of action may be involved in the growth inhibition of bacteria. The loss of cell viability and leakage of intracellular constituents observed in this study suggests that membrane damage was a major cause of lethal effect for any of the extracts.

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

POTENSI ANTIMIKROBIAL EKSTRAK *Psidium guajava* (JAMBU BATU) DAN EKSTRAK *Piper Betle* (SIREH) KE ATAS BAKTERIA BAWAAN MAKANAN

Oleh

HENIE EDWARD FRANCIS PARILLON

March 2006

Pengerusi: **Profesor Madya Zaiton bt Hassan, PhD**

Fakulti: **Sains Makanan dan Tecknologi**

Lima tumbuhan, *Psidium guajava* (jambu batu), *Illicium verum* (cengkih) dan *Annona squamosa* (nona) dan dua kultivar *Piper betle L.* (urat merah dan urat hijau) diuji untuk aktiviti antimikroorganisma ke atas enam belas bakteria bawaan makanan dengan menggunakan kaedah 'agar disc diffusion'. Tumbuhan ini dipilih berdasarkan laporan perubatan dan amalan warisan yang diamalkan oleh masyarakat di Malaysia dan di kawasan Caribbean. Ekstrak methanol bagi semua jenis tumbuhan yang dikaji pada peringkat saringan menunjukkan aktiviti antibakteria terhadap sekurang-kurangnya satu bakteria dengan ekstrak jambu batu dan kedua-dua daun sireh (urat merah dan hijau) menunjukkan aktiviti antimikrobial tertinggi berbanding ekstrak tumbuhan lain yang dikaji. Ekstrak *I. verum* efektif ke atas *Aeromonas hydrophila* dan *Citrobacter fuendi* manakala ekstrak *A. squamosa* efektif ke atas *Vibrio parahaemolyticus*. Ekstrak sebatian kasar daun *P. betle* L. (urat merah dan urat hijau) dan jambu batu dinilai keberkesanannya terhadap bakteria. Ekstrak methanol daun sireh (urat hijau) didapati berkesan terhadap patogen Gram-positif

dan negatif, manakala ekstrak methonol bagi jambu batu hanya berkesan terhadap mikroorganisma Gram-positif. Ekstrak hexana dan kloroform beramonia daun sireh (urat merah) didapati dapat merencat pertumbuhan kebanyakan patogen Gram-negatif.

Seterusnya ekstrak methonal daun sireh (urat hijau) (PBME) daun jambu batu (GME) dikaji untuk kadar perencatan minimum (MIC) terhadap *Kocuria rhizophila, Staphylococcus aureus, A. hydrophila, Escherichia coli* O157:H7*, Salmonella* Typhimurium*, V. parahaemolyticus, Listeria monocytogenes* dikaji. Bakteria yang paling sensitif kepada GME (MIC=1 mg/ml) adalah *S. aureus, K. rhizophila* dan *E. coli* O157:H7 manakala *S. aureus* juga sangat sensitif kepada PBME pada kepekatan yang sama. *A. hydrophila* dan *V. parahaemolyticus* mempunyai ketahanan yang lebih kepada PBME yang mempunyai MIC berkepekatan 10 mg/ml. Ekstrak-ekstrak tersebut juga dikaji corak kinetik membunuh ke atas empat jenis bakteria. GME merencat sepenuhnya pertumbuhan *K. rhizophila* selepas 12 jam mendedahkan kepada ekstrak pada kepekatan 1 mg/ml manakala pada kepekatan 5 mg/ml, perencatan pertumbuhan sepenuhanya diperolehi selepas 4 jam perlakuan. PBME juga membunuh semua sel *L. monocytogenes* pada kepekatan 1 mg/ml dalam masa 32 jam manakala pada kepekatan 5 mg/ml, perencatan sepenuhnya diperolehi dalam masa 4 jam. Pada kepekatan 1 mg/ml, kadar pembunuhan GME adalah lebih cepat daripada PBME untuk semua bakteria yang dikaji.

Kesan ke atas kemampuan hidup sel dan kebocoran selular untuk *K. rhizophila, S.* Typhimurium, *L. monocytogenes* dan *E. coli* O157:H7 diukur selepas pendedahan kepada PBME dan GME pada kepekatan 5,000 ppm (5 mg/ml). Penentuan hasil turas

bagi semua ampaian sel yang dirawat menunjukkan peningkatan bererti ($P \leq 0.05$) bagi perembesan bahan nukleik, ion K^+ dan protein berbanding kawalan. Ini menunjukkan berlakunya tindakan pemusnahan membran sitoplasmik sel bakteria. PBME menyebabkan kebocoran ion K^+ yang lebih tinggi daripada GME bagi kesemua bakteria yang diuji. Sementara protein keseluruhan yang dirembes oleh *K. rhizophila, S.* Typhimurium adalah lebih tinggi bagi sel yang dirawat dengan PBME berbanding dengan GME. PBME juga menyebabkan perlepasan bahan nukleik yang lebih ketara ($P \leq 0.05$) bagi bakteria Gram-positif sementara GME menyebabkan pelepasan bahan nukleik yang lebih tinggi bagi bakteria Gram-negatif. Walaupun GME menyebabkan perlepasan bahan penyerapan pada 260nm yang lebih tinggi berbanding PBME untuk semua jenis bakteria yang dikaji, namun kedua-dua ekstrak ini mampu merosakkan integriti lapisan lipopolisakarida (LPS) bakteria Gram-negatif. Beberapa mekanisma tindakbalas mungkin terlibat dalam perencatan pertumbuhan bakteria. Kehilangan kebolehan hidup sel dan rembesan bahan selular yang diperhatikan dalam kajian ini mencadangkan kerosakan membran adalah merupakan punca utama kesan letal sel oleh semua ekstrak.

ACKNOWLEGEMENTS

All praises to the Almighty Lord, the Most Gracious and Merciful, who is omnipotent and all giving, for affording me the strength and determination to complete this study. I owe a great deal of gratitude to many people and institutions for their kind assistance and support at various stages of my research work. Words are insufficient to adequately express my sense of indebtedness to these individuals, however, it would be remise of me not to make special mention of those persons.

It is my pleasure to express my sincere gratitude and appreciation to the Chair of my thesis supervisory committee, Associate Professor Zaiton Hassan, who was also my main advisor throughout the duration of my study, for her critical assessment, helpful suggestion, guidance and patience in the writing of this thesis. Special thanks also to the lone other member of my supervisory committee, Professor Suhaila Mohamed for her constant review of my work as it progressed, her advice and sharing of her vast knowledge on various aspects of my work. I thank them both for all their critical remarks and suggestions in undertaking my research work and for their time and assistance in writing up this thesis. Without this invaluable help from these two formidable women, this level of success would have been unattainable. Special thanks to them for helping me understand the scope of my research work. It was their kind encouragement, critical assessment, invaluable and fruitful discussion that made my work more meaningful. Finally and most importantly, I want to thank them for doing something very simple… defying the gods and not giving up on me.

It is also my pleasure to thank particularly my lab mates especially Daniel, Yin Sze, Lesley, Yousr, Levina, Tunung, Chandrika, Jurin and other colleagues such as AbdulKarim, Sagineedu, Tenaw, Abdullah and AbdulKadar who taught me a lot and explained many simple, yet important concepts to me. Their efforts proved very instrumental in helping me remember some of the things I learnt during my undergraduate years. Our brief, occasional chats were invaluable and their assistance will never be forgotten. I am indebted to them in ways words cannot express. Special thanks are also extended to the Lecturers and staff of the Faculty of Food Science and Technology, UPM, for their guidance and support in one way or the other throughout my study. Thanks also to my colleagues, especially Shandil from Fiji for his constant taunts to get my work done and Demis from Italy, who insisted that I focus on my work instead of going out to party. My heart also goes out to members of the Catholic Students' Society (CSS) on campus for welcoming me in their fold and contributing to my having spent a memorable time in Malaysia. Appreciation also goes to Erin, Melchiol "Snoop" Ambunda, Andreas and Ignatius. I would also like to express my sincerest thanks to Lim for his efforts and contribution to this research project.

It would be remise of me not to make special mention of and thank the love of my life Lenita, and my dearest of friends, Gido, Gray, Aunty Vern, Lyndelle, Camo, Riviere, Delia, Shernelle, Fr. Auguiste and Lian among others, who have basically carried me (spiritually and otherwise) through this period of time spent in Malaysia. Finally and very importantly, I would like to say I am exceptionally appreciative of and extremely grateful to the Government and People of Malaysia for having awarded me an MTCP Scholarship, which primarily facilitated my study here.

I certify that an Examination Committee has met on 6th June 2006 to conduct the final examination of Henie Edward Francis Parillon on the Master of Science thesis entitled "Antimicrobial activity of psidium guajava and piper betle extracts on selected foodborne bacteria", in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis 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 are as follows:

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Date:

DECLARATION

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

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HENIE EDWARD FRANCIS PARILLON

Date:

TABLE OF CONTENTS