



**UNIVERSITI PUTRA MALAYSIA**

**ANTIBACTERIAL ACTIVITY OF SEA CUCUMBER EXTRACT AND  
ELUCIDATION OF MEMBRANE SYNTHESIS GENES IN  
*Staphylococcus aureus***

**NORFARRAH BINTI MOHAMED ALIPIAH**

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By

**NORFARRAH BINTI MOHAMED ALIPIAH**

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

**JULY 2011**

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

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**July 2011**

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Sea cucumber is widely used in South East Asian country folk medicine for the treatment of wound and skin infections. It is a new alternative source of antibiotics that are from valuable biological resource for future commercial development in pharmaceutical use. This study investigated the antibacterial activity of *Stichopus badionotus* methanolic extract to elucidate its use in traditional medicine. Antimicrobial activity was assayed by the disc diffusion and broth macro dilution method. From the results, it appeared that the methanolic extract of *S. badionotus* displayed antibacterial activities against three non resistant and three multiple resistant strains of *Staphylococcus aureus*. The 3.75 mg/mL minimum inhibitory concentration (MIC) value of the extract successfully inhibited the growth of non resistant *S. aureus* (MSSA) and against multi resistant strains of *S. aureus* (MRSA) the effective MIC value is 7.50 mg/mL. The *S. badionotus* extract then

combined with the antibiotics, penicillin and ampicillin gave fractional inhibitory concentration (FIC) as low as 0.375 indicating synergism activity of the mixture against MSSA and MRSA strains. This activity indicates the lower concentration usage of antibacterial agents in inhibiting resistant strains of *S. aureus*. Furthermore, based on healing rates and dosage tests in rats with wound infection model, the combination treatment of extract and antibiotics was better than antibiotic alone treatment. With the low dosage in-combination treatment of the *S. badionotus* extract 0.94 mg/mL combine with penicillin 0.125 mg/mL or ampicillin 0.05 mg/mL, the wound healed by day 6. While single treatment using high dosage of antibiotic ampicillin 0.2 mg/mL heals on day 5. These results indicate the potential of *S. badionotus* extract to become alternative choices of antimicrobial agents for in combination mode of treatment against MRSA infection.

MRSA strains treated with *S. badionotus* extract exhibited inhibitory activity as shown by the plate and tube assays. The extract then proves to interrupt selected genes encoding for cytoplasmic membrane of bacterial structures that are important for bacterial survival. Disruption in membrane integrity will result in leakage of internal contents followed by cell death suggest the membrane a practical drug target. Based on the bacterial needs to have membrane, studies on MRSA membrane synthesis genes, *msrR* and *mprF*, were conducted via reverse transcriptase polymerase chain reaction approach. Alteration of nucleotide sequences at the RNA level after treatment was observed only in the *mprF* gene but not in the *msrR* gene. The nucleotide changes were up to 35% for MSSA isolates and < 10% for MRSA isolates, which both contribute to < 50% changes in

protein base after translation. A confirmatory experiment, fluorescence assay, used in confirming this gene as a drug target predicted by molecular assays revealed positive results. The extract specifically acts on the MRSA membrane, shown through this assay by the uptake of fluorescent dye upon rupturing of the bacterial membrane after treatment with the extract. The *S. badionotus* extract treatment on MRSA at the MIC level disrupts the membrane after 60 min upon application, showed by a steep curve of the fluorescence intensities indicating the membrane leakage. Selective targeting of the *mprF* gene by the *S. badionotus* extract is an invaluable finding requiring further investigation into the feasibility of utilizing this target gene in the development of anti-infective agents against MRSA.

Abstrak tesis ini dikemukakan kepada senat Universiti Putra Malaysia dalam memenuhi keperluan ijazah Master Sains

**AKTIVITI ANTIBAKTERIA OLEH EKSTRAK MENTIMUN LAUT DAN  
PENENTUAN GEN SINTESIS MEMBRAN DALAM *Staphylococcus aureus***

Oleh

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Mentimun laut banyak digunakan dalam perubatan tradisional di Asia Tenggara bagi merawat luka dan jangkitan kulit. Ia merupakan sumber alternatif baru kepada antibiotik yang berharga untuk dibangunkan secara komersil bagi kegunaan farmaseutikal. Kajian ini menyelidik aktiviti antibakteria oleh ekstrak metanol *Stichopus badiionotus* bagi memastikan penggunaannya dalam perubatan tradisional. Aktiviti antimikrob telah diuji melalui kaedah penyerapan disk dan kaedah pencairan pati media. Hasil penemuan menunjukkan keberkesanan ekstrak metanol *S. badiionotus* sebagai antibakteria terhadap *Staphylococcus aureus* iaitu tiga kultur tidak rintang (MSSA) dan tiga kultur rintang pelbagai antibiotik (MRSA). Kepekatan perencat minimum (MIC) ekstrak bernilai 3.75 mg/mL untuk kultur tidak rintang dan 7.50 mg/mL untuk kultur berkerintangan pelbagai. Ekstrak *S. badiionotus* kemudiannya digabungkan dengan antibiotik; penicillin dan

ampicillin memberi index rencat fraksinasi serendah 0.375 yang menggambarkan hubungan kombinasi sinergi menentang kultur MSSA dan MRSA. Seterusnya, berdasarkan kadar penyembuhan luka dan ujian dos terhadap model infeksi luka pada tikus, menunjukkan rawatan kombinasi ekstrak dan antibiotik adalah lebih baik dari rawatan antibiotik sahaja. Rawatan dos rendah kombinasi ekstrak 0.94 mg/mL digabungkan dengan penicillin 0.125 mg/mL atau ampicillin 0.05 mg/mL, infeksi luka sembuh pada hari ke-enam. Manakala rawatan antibiotik sahaja sebanyak 0.2 mg/mL sembuh pada hari ke-lima. Penemuan ini menunjukkan penggunaan gamat secara tradisional dapat merawat jangkitan *S. aureus*, terutama kultur rintang, dan kajian lanjut perlu dijalankan bagi mengeksploitasi potensinya sebagai agen antibakteria.

Kultur *S. aureus* bekerintangan pelbagai (MRSA) menunjukkan potensi ekstrak sebagai penghalang aktiviti melalui ujikaji piring petri dan tiub ujikaji cairan. Ekstrak dibuktikan dapat merosakkan gen - gen terpilih mengkodkan struktur membran sitoplasmik yang penting untuk kemandirian bakteria. Kerosakan integriti membran bakteria akan menyebabkan lelehan kandungan dalaman seterusnya menyebabkan kematian sel menjadikan struktur ini sebagai target yang praktikal. Berdasarkan keperluan bakteria untuk mempunyai membran, kajian ke atas gen yang mensintesis membran bagi MRSA; gen *msrR* dan *mprF* dijalankan melalui pendekatan bioteknologi molekular.

Pengubahsuaian pada jujukan nukleotid selepas rawatan hanya dilihat pada gen *mprF* dan tiada bukti pengubahsuaian jujukan nukleotid dalam gen *msrR*. Perubahan nukleotid sebanyak 35% untuk kultur MSSA dan < 10% bagi kultur MRSA yang menyebabkan < 50% perubahan dalam jujukan protein selepas pengalihan jujukan. Eksperimen

pengesahan iaitu essei pendafluoran menunjukkan keputusan positif seperti yang digambarkan dari kajian molekular bagi penentuan gen sebagai sasaran agen antibakteria. Ekstrak khususnya bertindak hanya ke atas membran MRSA, ditunjukkan dengan penyerapan bahan pendafloran setelah rawatan dengan ekstrak yang merosakkan membran bakteria. Rawatan ekstrak *S. badionotus* terhadap kultur MRSA pada peringkat MIC adalah sangat tinggi yang merosakkan membran setelah 60 min rawatan yang ditunjukkan melalui keputusan eksperimen pendafloran. Pemilihan gen *mprF* oleh tindakan ekstrak *S. badionotus* adalah penemuan yang bernilai tinggi serta memerlukan kajian yang lebih mendalam terhadap kemungkinan gen ini boleh digunakan dalam membangunkan agen anti-infektif untuk menentang MRSA.



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I certify that a Thesis Examination Committee has met on (the date of viva) to conduct the final examination of Norfarrah Binti Mohamed Alipiah on her thesis entitled 'Antibacterial Activity of Sea Cucumber Extract and Elucidation of Membrane Synthesis Genes in *Staphylococcus aureus*' 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 Degree of Master of Science.

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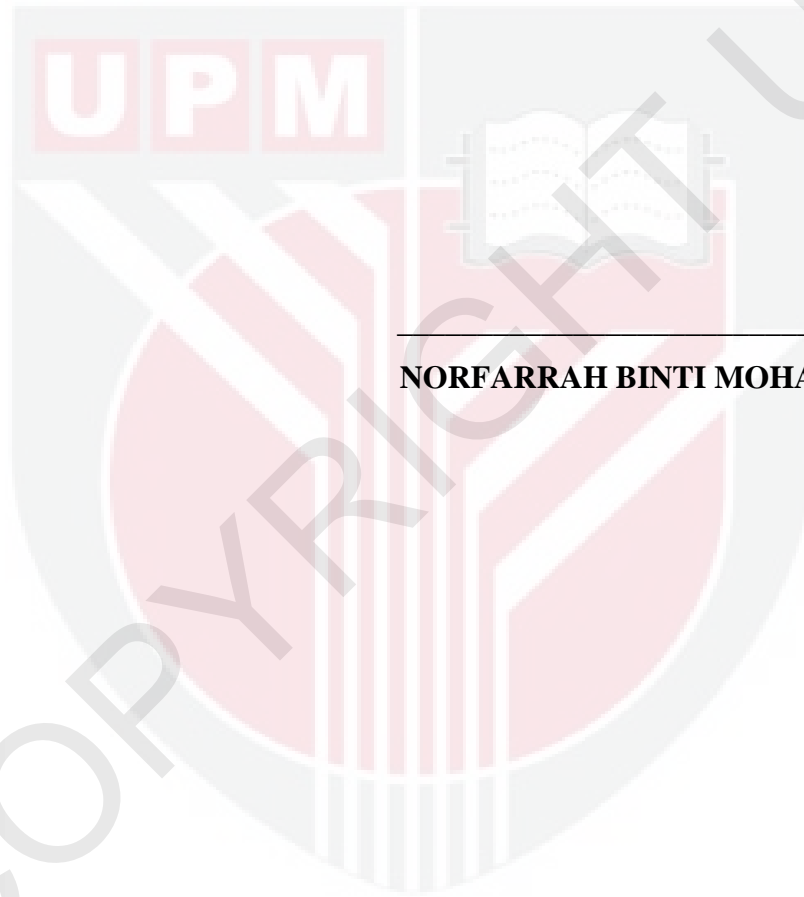
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## DECLARATION

I declare that the thesis is my original work except for the 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 University Putra Malaysia or at any other institution.



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**NORFARRAH BINTI MOHAMED ALIPIAH**

Date: 6 July 2011

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