



UNIVERSITI PUTRA MALAYSIA

CONSTITUENTS OF SEA CUCUMBER *Holothuria (Mertensiothuria) leucopilota Brandt* WITH BROAD ANTIBACTERIAL ACTIVITIES

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**CONSTITUENTS OF SEA CUCUMBER *Holothuria (Mertensiothuria) leucospilota*
Brandt WITH BROAD ANTIBACTERIAL ACTIVITIES**

By

ABDOULIE CEESAY

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

May 2013

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DEDICATION

This thesis is specially dedicated to my dear family and longtime friends: first and foremost, to mum and dad Fatoumatta Minteh and

Late Alhagie Salieu Ceesay and also Naffisatou and Late Ba

Ousman Ceesay who have been very instrumental in my religious,

social and academic upbringing;

My brothers: Ebrahim, Ba Omar, Muhammed Foday, Ousman, Lamin,

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Sey, Kaira, Saho, Faruq and Ismaila.

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

CONSTITUENTS OF SEA CUCUMBER *Holothuria (Mertensiothuria) leucospilota* Brandt WITH BROAD ANTIBACTERIAL ACTIVITIES

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May 2013

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Compounds derived from natural products are unquestionable sources of antibacterial agents which could be used to combat the threats posed by pathogenic bacteria species in the phase of abrupt antibiotic resistance in the world. *Holothuria leucospilota* sea cucumber has been used in the past in traditional medicine as cure and remedy for illnesses. This research basically aimed at securing antibacterial compounds from *H. leucospilota* that can inhibit the growth of pathogenic human multidrug resistant bacteria species. Solvent combinations of methanol/acetone, isopropanol/ acetone and also ethanol/ distilled water, methanol/distilled water and isopropanol/ distilled water were used to improve the extraction of active constituents. Extracts from *H. leucospilota* were screened for antibacterial activity against staphylococcal cassette chromosome *mec* (SCC*mec*) clonally diverse Sequenced Type (ST) methicillin resistant *Staphylococcus aureus* (MRSA) of ST-1, ST-7, ST-22, ST-188, ST- 1283 including ST-239 which was highly circulated in Malaysia and methicillin susceptible *Staphylococcus aureus* (MSSA) of ST-1, ST-5, ST-8, ST-80, ST-88 and ST-121 clones and also against

extraintestinal pathogenic *Escherichia coli* (ExPEC) extended-spectrum beta-lactamase (ESBL) producing bacteria strains on which this extract has not been tested before. The disc diffusion and the broth micro dilution methods were used to conduct the preliminary susceptibility screening test. The conventional reduction in bacteria population to determine the bacteriostatic and bactericidal effects of the crude extracts, the time kill assay was used. The chemical screening of the extracts showed the presence of antibacterial chemical compounds classes such as terpenoids, phenols, steroids, alcohols, aldehydes, ketones, saponins, tannins and carbohydrates. Gas chromatography-mass spectrometry analysis revealed new and active antibacterial metabolites which have not been reported in this sea cucumber such as 2,4-bis (1,1-dimethylethyl)- phenol, 4-hydroxy-4-methyl-2-pentanone., 3,7-dimethyl-2,6-octadienal-, (Z)- (citral), stigmast-5-en-3-ol, (3.beta.,24S)-, (sitosterol), acetic acid and methyl, 5,11,14,17-eicosatetraenoic acid. Some antibacterial compounds identified in the species were reported earlier such as hexadecanoic acid, arachidonic acid, eicosapentaenoic acid and oleic acid. The fatty acid analyses of the extracts showed the presence of (C8:0) to (C22:1) fatty acids methyl esters. The saturated methyl palmitate (44.86%) and the unsaturated cis-9-oleic methyl ester (45.0%) were revealed as main antibacterial components. The extracts of the body wall, Cuvierian tubules and visceral extracts exerted broad spectrum antibacterial activity against both the SCCmec Sequenced Type Gram-positive MRSA and ST MSSA clones and also the ExPEC ESBL producing Gram-negative bacteria. Minimum inhibition concentration (MIC) against these bacteria was ranged from 8 to 128 mg/mL and the minimum bactericidal concentration was ranged from 32 to >128 mg/mL which was comparable to values

obtained in other studies especially in plant antimicrobial assays. Data from the time kill assay showed that the extract was bactericidal against the Gram-positive ATCC 700698 (MRSA) and ATCC 29247 (MSSA) bacteria after 12 hr interaction and after 8 hr interaction for Gram-negative ATCC 35218 (*E. coli*) bacteria and bacteriostatic after 6 hr interactions for both bacteria group. These results are also comparable to results obtained in other studies even though in some cases the reduction rate of bacteria load in this study appears slower. The results obtained from this work suggest that *H. leucospilota* sea cucumber could be a potential source of compounds that could be incorporated in ointments, dietary supplements as well as therapeutic and antibacterial agents.

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

**KOMPONEN GAMAT *Holothuria (Mertensiothuria) leucospilota* Brandt
DENGAN AKTIVITI ANTIBAKTERIA UMUM**

Oleh

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Sebatian yang diperolehi daripada produk semula jadi adalah ejen antibakteria dari sumber yang tidak boleh dipersoalkan yang boleh digunakan untuk memerangi ancaman yang ditimbulkan oleh spesies bakteria patogenik dalam fasa rintangan antibiotik mendadak di dunia. Gamat *Holothuria leucospilota* telah digunakan pada masa lalu dalam perubatan tradisional sebagai penawar dan ubat untuk penyakit. Kajian ini pada asasnya bertujuan untuk mendapatkan sebatian antibakteria dari *H. leucospilota* yang boleh menghalang pertumbuhan spesies bakteria patogenik manusia yang tahan pada pelbagai ubat. Kombinasi pelarut metanol/aseton, isopropanol/aseton dan juga etanol/air suling, metanol/air suling dan isopropanol/air suling telah digunakan untuk meningkatkan pengeluaran komponen-komponen aktif. Ekstrak dari *H. leucospilota* telah disaring untuk aktiviti antibakteria terhadap pita staphylococcal kromosom *mec* (SCC*mec*) kepelbagaian klon Jenis Jujukan (ST) *Staphylococcus aureus* tahan pada methicillin (MRSA) ST-1, ST-7, ST-22 ST-188, ST - 1283 termasuk ST-239 yang

banyak disebarkan di Malaysia dan *Staphylococcus aureus* rentan methicillin (MSSA) ST-1, ST-5, ST-8, ST-80, ST-88 dan klon ST-121 dan juga terhadap patogen extraintestinal *Escherichia coli* (ExPEC) spectrum-dilanjutkan beta-lactamase (ESBL) menghasilkan jenis bakteria yang mana ekstrak ini belum diuji sebelum ini. Penyebaran cakera dan kaedah pencairan mikro telah digunakan untuk menjalankan ujian kecenderungan semasa awal pemeriksaan. Pengurangan konvensional dalam populasi bakteria untuk menentukan kesan bacteriostatic dan kesan bakteria daripada ekstrak mentah, masa cerakin telah digunakan. Pemeriksaan kimia ekstrak menunjukkan kehadiran kelas kimia antibakteria seperti terpenoid, fenol, steroid, alkohol, aldehid, keton, saponin, tanin dan karbohidrat. Analisis gas kromatografi-spektrometri jisim mendedahkan metabolit baru dan antibakteria aktif yang tidak dilaporkan dalam gamat ini seperti, 4-bis (1,1-dimetilet) fenol, 4-hidroksi-4-metil-2-pentanone, 3,7-dimetil-2,6-oktadienal-(Z) - (citral), stigmast-5-en-3-ol, (3.beta, 24s.) -, (sitosterol), asid asetik dan metil, asid 5, 11, 14, 17-eicosatetraenoic. Beberapa sebatian antibakteria yang dikenal pasti dalam spesies yang dilaporkan sebelum ini seperti asid hexadecanoic, asid arakidonik, asid eicosapentaenoic dan asid oleik. Analisis asid lemak ekstrak menunjukkan kehadiran (C8: 0) hingga (C22: 1) asid lemak metil ester. Palmitate metil tepu (44.86%) dan tidak tepu ester metil cis-9-oleik (45.0%) telah ditemui sebagai komponen antibakteria utama. Ekstrak dinding badan, tubul Cuvierian dan ekstrak mendalam yang dikenakan spektrum luas aktiviti antibakteria terhadap kedua-dua SCCmec Jenis Jujukan MRSA Gram-positif dan klon ST MSSA dan juga ESBL ExPEC menghasilkan bakteria Gram-negatif. Kepekatan perencatan minimum (MIC) terhadap bakteria ini adalah antara 8 hingga 128 mg/mL dan kepekatan bakteria minimum antara

32 hingga > 128 mg/mL yang setanding dengan nilai yang diperolehi dalam kajian yang lain terutamanya dalam loji ujian antimikrobial. Data daripada masa cerakin menunjukkan yang ekstrak itu adalah bakterisidal terhadap Gram-positif ATCC 700698 (MRSA) dan bakteria ATCC 29.247 (MSSA) selepas 12 jam berinteraksi dan selepas 8 jam berinteraksi untuk bakteria Gram-negatif ATCC 35218 (*E. coli*) dan bacteriostatic selepas 6 jam berinteraksi bagi kumpulan kedua-dua bakteria. Keputusan ini juga setanding dengan keputusan yang diperolehi dalam kajian yang lain walaupun dalam beberapa kes kadar pengurangan beban bakteria dalam kajian ini didapati perlahan. Keputusan yang diperolehi daripada kerja-kerja ini mencadangkan bahawa gamat *H. leucospilota* boleh menjadi sumber yang berpotensi untuk bahan-bahan yang boleh dimasukkan dalam ubat sapu, makanan tambahan dan juga sebagai agen terapeutik dan anti-bakteria.

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I also want to thank all those who in one way or the other supported me in various ways through this journey but their names have been omitted should know that it was my own limitation as a human being.

I certify that a Thesis Examination Committee has met on 7 May, 2013 to conduct the final examination of Abdoulie Ceesay on his thesis entitled " Constituents of Sea Cucumber *Holothuria (Mertensiothuria) leucospilota* Brandt with Broad Antibacterial Activities " 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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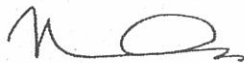
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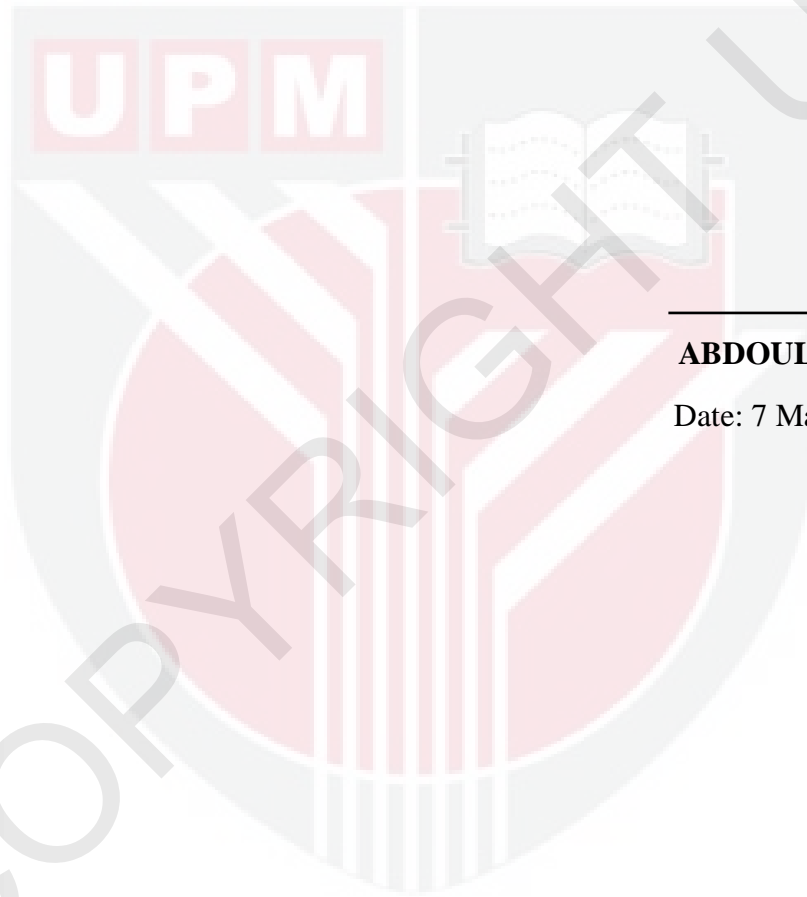
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DECLARATION

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 institutions.



ABDOULIE CEESAY

Date: 7 May 2013

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