UNIVERSITI PUTRA MALAYSIA

ANTIMICROBIAL, IMMUNOMODULATORY AND TUMOR CELLS- SELECTIVE CYTOTOXIC ACTIVITIES OF *BRASSICA OLERACEA* L. AND *VIGNA RADIATA* L.

RAND R. HAFIDH

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By

RAND R. HAFIDH

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

May 2011

DEDICATION

I DEDICATE THE RESULTS OF THIS WORK AND MY DEGREE TO THE PEOPLE WHO WITHOUT THEM I WOULDN'T BE ABLE TO REACH WHAT I HAVE REACHED, MY BELOVED HUSBAND, MY CARING MOTHER, MY FATHER, MY GRANDMOTHER ADILAA AND MY AUNT LAMIAA.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement of the degree of doctor of philosophy

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Chair: Professor Dr. Fatimah Abu Bakar, PhD

Faculty: Food Science and Technology

The world today faces a great challenge in the emergence of drug-resistant microbes and dangerous cancers against most of the commonly known antimicrobial and anticancer drugs, respectively. Screening for effective, new, and cheap remedies from natural resources has become the goal of many scientists all around the world. The plants' methanol extracts used in this study were red cabbage (RC) and mung bean sprout (MBS) which both showed remarkable antiviral activities against herpes simplex virus type 1 (HSV-1) and respiratory syncytial virus (RSV). The mode of action of RC extract was shown to be prophylactic against RSV (the concentration that inhibit 50% of virus cytopathic effect (IC50) = 13.9 mg/ml and selectivity index (SI) = 17.67). Against HSV-1, RC extract showed a remarkable viricidal activity (IC50 = 11.51 mg/ml and SI = 34.52) with less prophylactic activity (IC50 = 32.97 mg/ml and SI = 11.71). MBS extract had viricidal effect (IC50 = 15.62 mg/ml and SI = 14.18), (IC50 = 7.62 mg/ml and SI = 18.23) and to a lesser extent prophylactic (IC50 = 17.23 mg/ml and SI = 12.82), (IC50 = 12.72 mg/ml and SI = 10.9) against

RSV and HSV-1 viruses, respectively. One of the good explaination on the effective prophylactic activities of MBS and RC extracts was their excuisite ability to induce production of high levels of IFNβ, TNFα, IL-1, and IL-6 cytokines (300-900% higher levels when compared to untreated cells; $P<0.0001$) in virally infected cells. These antiviral cytokines proved to play critical and synergistic role in the antiviral resistance of human cells. For the cytotoxic effects of the tested extracts, RC extract showed selective cytotoxic effect against human cervix adenocarcinoma cells (HeLa), (SI = 10.88) while it showed less selectivity against human hepatocellular carcinoma cells (HepG2), (SI = 8.93). On the contrary, MBS extract showed a selective cytotoxic effect against both HeLa and HepG2 cells with SI values of 12.44 and 11.94, respectively. In an attempt to disclose part of the underlying mechansims of cytotoxic effect of both extracts using concentrations lower than IC50, RC induced the production of the anticancer cytokine TNFα (up to 600% increase; $P<0.0001$) whereas MBS induced the production of both anticancer cytokines TNFα (up to 700% increase; $P<0.0001$) and IFNβ (up to 300% increase; $P<0.001$). The increase in the anticancer cytokine levels was dose-dependent. In addition, the IC50 of RC extract induced cell cycle arrest in G0/G1 phase in cancer cells increasing the percentage of cells from 66.87 to 76.96% and from 57.83 to 70.41% in the treated HeLa and HepG2 cells, respectively. On the other hand, the IC50 of MBS extract induced cell cycle arrest in G0/G1 phase only in HeLa cells (62.87 to 80.48%). Both extracts induced remarkably apoptosis in the treated human cancer cells in a dose- and time-dependent manner. The induction of apoptosis in cancerous cells 12-16h after exposure to RC and MBS extracts was significant via caspase-dependent pathways including the extrinsic pathway by upregulating caspase 8 (about 8-32 folds) and the intrinsic pathway by upregulating both caspase 9 (about 32-256 folds)

and Bax genes (about 8-32 folds). Furthermore, both extracts upregulated highly caspase 7 (about 32-512 folds) which could induce apoptosis via caspase-independent pathway. More to the point, Cdk-inhibitor proteins were upregulated (p21: about 8-512 folds, p27: about 4-16 folds, and p53: about 16-32 folds) 12-16h after exposure to both extracts and their upregulation might be the main mechanism used by both extracts to exert G1 cell cycle arrest and ultimately the final fate of cells, apoptosis. For the immunomodulatory effect of tested extracts, RC extract showed potent anti-inflammatory activity by inhibiting the production of the pro-inflammatory cytokine, IFNγ (about 80% decrease; $P<0.01$) in peripheral blood mononuclear cells (PBMC). On the other hand, MBS extract was effective immunomodulator agent as TNFα and IFNβ cytokines are also potent immunomodulators. In addition, MBS extract showed an immunopolarizing effect by inducing IFNγ (about 300% increase; $P<0.01$) and inhibiting IL-4 production (about 75% decrease; $P<0.01$) in PBMC. The immunopolarization effect of MBS extract was dose-dependent. For the antibacterial and antifungal potential of the tested extracts, antibacterial and antifungal activities against multiple drug resistant (MDR), non-MDR bacteria, and fungi were shown to be significant for both extracts. The antibacterial and antifungal activities were dose-dependent. Remarkably, antibacterial activity of RC extract was evident particularly against highly infectious microorganisms such as Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Salmonella enterica* serovar Typhimurium as well as against human fungal pathogens, *Trichophyton rubrum* and *Aspergillus terreus*. MBS extract revealed potential antibacterial and antifungal activities against MRSA, *Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Klebsiella pneumonaie*,

Staphylococcus aureus, and *Salmonella* Typhimurium as well as against human fungal pathogens, *Trichophyton rubrum* and *Trichoderma harzianum*. The findings of the electron microscopy together with phenotype microarray (PM) supported these results for the antibacterial activity. PM screening was a valuable tool in the search for compounds that can inhibit bacterial growth by affecting certain metabolic pathways like peptidoglycan synthesis pathway. This pathway appeared to be targeted by both RC and MBS extracts at metabolic points differ from that of other available drugs. These points could be targets for discovering new therapeutic agents for the currently studied microbes. The considerable results of this study could be explained by the powerful antioxidant activity of RC and MBS which is most likely due to the phenolic and antioxidant compounds present in these plants which act individually or synergistically to bring to light the importance of these plants as candidates for new therapeutic agents.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah dari doktor falsafah

AKTIVITI ANTIMIKROB, IMUNOMODULATOR DAN SEL-SELEKTIF TUMOR SITOTOKSIK DARIPADA *BRASSICA OLERACEA* L. DAN *VIGNA RADIATA* L.

Oleh

RAND R. HAFIDH

Mei 2011

Pengerusi: Profesor Dr. Fatimah Abu Bakar, PhD

Fakulti: Sains dan Technologi Makanan

Dunia saat ini menghadapi cabaran besar dengan munculnya mikrob resistan terhadap ubat dan kanser berbahaya terhadap sebahagian besar antimikrob umumnya dikenali dan ubat antikanser, masing-masing. Penyaringan untuk Penemuan remedi yang berkesan, baru, dan murah daripada sumber alam semulajadi menjadi matlamat ramai saintis di seluruh dunia. Ekstrak metanol yang digunakan dalam penelitian ini adalah kubis merah (RC) dan kacang hijau tunas (MBS) yang keduanya menunjukkan aktiviti antivirus yang luar biasa terhadap jenis virus herpes simpleks 1 (HSV-1) dan virus RSV (RSV). Mod tindakan RC ekstrak terbukti profilaksis terhadap RSV (kepekatan yang menghalang 50% daripada kesan virus sitofatik (IC50) = 13.9 mg / ml dan indeks selektivitas (SI) = 17.67). Terhadap HSV-1, RC ekstrak menunjukkan aktiviti virisidal luar biasa (IC50 = 11.51 mg / ml dan SI = 34.52) dengan aktiviti profilaktik kurang (IC50 = 32.97 mg / ml dan SI = 11.71). Kesan virisidal ekstrak MBS (IC50 = 15.62 mg / ml dan SI = 14.18), (IC50 = 7.62 mg / ml dan SI = 18.23) dan ksan profilaksis tahap yang lebih rendah (IC50 = 17.23 mg / ml dan SI = 12.82), (IC50 = 12.72 mg / ml dan SI = 10.9) terhadap RSV dan

HSV-1 virus, masing-masing. Salah satu alasan yang baik menjelaskan kegiatan profilaksis berkesan ekstrak MBS dan RC adalah kemampuan mereka untuk menginduksi pengeluaran peringkat tinggi IFNβ, TNFa, IL-1, dan IL-6 sitokin (tahap 300-900% lebih tinggi bila dibandingkan dengan yang tidak dirawat sel; P <0.0001) pada sel yang dijangkiti virus. Sitokin ini terbukti memainkan peranan kritikal dan sinergis dalam kesan rintangan sel antivirus manusia. Untuk antikanser ekstrak, RC ekstrak menunjukkan aktiviti anti-kanser selektif terhadap sel adenokarsinoma serviks manusia (Hela), (SI = 10.88), sementara kurang selektiviti terhadap sel karsinoma hepatoseluler manusia (HepG2), (SI = 8.93). MBS ekstrak masing-masing menunjukkan aktiviti anti-kanser selektif terhadap kedua HeLa dan sel-sel HepG2 dengan nilai SI 12.44, dan 11.94. Dalam usaha untuk mendedahkan sebahagian daripada mekanisma yang mendasari aktiviti anti-kanser daripada kedua-dua ekstrak dengan kepekatan yang lebih rendah daripada IC50, RC mengaruh pengeluaran sitokin TNFa antikanser (meningkat hingga 600%, P <0.0001) sedangkan MBS mengaruh pengeluaran antikanser sitokin TNFa (sehingga 700% meningkat; L <0,0001) dan IFNβ (hingga mencapai 300%, P <0,001). Peningkatan kadar sitokin antikanser adalah dos-bergantung. Selain itu, IC50 dari ekstrak RC mengaruh sel penangkapan kitaran sel pada fasa G0/G1 dalam sel kanser dengan meningkatkan peratusan sel 66.87-76.96% dan 57.83-70.41% dalam sel HeLa dan HepG2, masing-masing. Selain itu IC50 dari ekstrak MBS mengaruh penangkapan kitaran sel pada fasa G0/G1 hanya dalam sel Hela (62.87-80.48%). Kedua-dua ekstrak mengaruh apoptosis dalam sel kanser manusia dengan cara-dos dan masa-dependen. Induksi apoptosis dalam sel kanser selepas pendedahan 12-16h untuk ekstrak RC dan MBS yang signifikan melalui laluan caspase-dependent termasuk laluan ekstrinsik menggunakan upregulating caspase 8 (sekitar 8-32 lipatan) dan laluan intrinsik

dengan upregulating kedua-dua caspase 9 (tentang 32-256 lipatan) dan gen Bax (sekitar 8-32 lipatan). Seterusnya, kedua-dua ekstrak terselaras tinggi terhadap caspase 7 (sekitar 32-512 lipatan) yang boleh mengaruh apoptosis melalui laluan bebas-caspase. Lebih penting lagi, protein Cdk-inhibitor terselaras (p21: sekitar 8-512 lipatan, p27: sekitar 4-16 lipatan, dan p53: sekitar 16-32 lipatan) 12-16h setelah terdedak kepada untuk kedua-dua ekstrak dan upregulation mungkin merupakan mekanisme utama yang digunakan oleh kedua-dua ekstrak untuk mengerahkan penangkapan kitaran sel G1 dan akhirnya, apoptosis. Untuk kesan imunomodulator dari ekstrak diuji, RC ekstrak menunjukkan aktiviti anti-inflamasi tinggi dengan menghalang pengeluaran sitokin pro-inflamasi, IFNγ (sekitar 80% menurun, P <0.01) pada sel mononuklear darah periferi (PBMC). MBS ekstrak sebagai agen imunomodulator berkesan sitokin TNFα dan IFNβ. Selain itu, MBS ekstrak menunjukkan kesan immunopolarizing dengan mengaruh IFNγ (sekitar 300% meningkat; P <0.01) menghalas dan pengeluaran IL-4 (penurunan sekitar 75%, P <0.01) pada PBMC. Kesan immunopolarization ekstrak MBS adalah dos-bergantung. Untuk potensi antibakteria dan antikulat dari ekstrak diuji, aktiviti antibakteria dan antikulat terhadap beberapa dadah rintang bakteria (MDR), non-MDR, dan kulat menunjukkan kesan signifikan bagi kedua-dua ekstrak. Aktiviti antibakteria dan antikulat adalah bergantung dos. Hebatnya, aktiviti antibakteria ekstrak RC adalah jelas terutama terhadap mikroorganisme yang sangat berjangkit seperti *Staphylococcus aureus* resistan methicillin-(MRSA), *Escherichia coli* O157: H7, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, dan *Salmonella enterica* serovar Typhimurium serta terhadap kulat patogen manusia, *Trichophyton rubrum* dan *Aspergillus terreus*. Ekstrak MBS menunjukkan aktiviti antibakteria dan antikulat berpotensi terhadap MRSA, *Escherichia coli* O157: H7,

Pseudomonas aeruginosa, *Klebsiella pneumonaie*, *Staphylococcus aureus*, dan *Salmonella* Typhimurium serta kulat patogen manusia, *Trichophyton rubrum* dan *Trichoderma harzianum*. Penemuan menggunakan mikroskop elektron bersama-sama dengan microarray fenotipe (PM) menyokong keputusan untuk aktiviti antibakteria. Penyaringan PM adalah alat yang berharga dalam mencari bahan yang boleh menghalang pertumbuhan bakteria dengan mempengaruhi pusat metabolisme tertentu seperti pusat sintesis peptidoglikan. Laluan ini ampaknya menjadi sasaran oleh kedua-dua ekstrak RC dan MBS pada titik metabolisme yang berbeza dari ubat lain yang sedia ada. Titik-titik ini boleh menjadi sasaran untuk mencari agen terapi baharu bagi mikrob yang dihaji. Keputusan kajian ini menunjukkan aktiviti antioksidan yang kuat dari RC dan MBS berkemungkinan besar disebabkan oleh sebatian antioksidan fenolik yang wujud dalam tanaman yang bertindak secara individu atau secara sinergis tik untuk menjadikan pentingnya tanaman ini sebagai sumber baru agen terapi.

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I certify that an Examination Committee has met on **3rd May 2011** to conduct the final examination of **Rand R. Hafidh** on her **PhD** thesis entitled "**Antimicrobial, Immunomodulatory and Tumor Cells-Selective Cytotoxic Activities of *Brassica oleracea* L. and *Vigna radiata* L."** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded Doctor of Philosophy degree.

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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:

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Date: 14th July 2011

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 institution.

RAND R. HAFIDH

Date: 2nd June 2011

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