DETERMINATION OF CHEMICAL COMPOSITION AND POTENTIAL ANTICANCER ACTIVITIES OF ALLIUM ATROVIOLACEUM EXTRACT ON SELECTED CANCER CELL LINES

SOMAYEH KHAZAEI

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By

SOMAYEH KHAZAEI

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

September 2016

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DEDICATION

I dedicate the thesis to the people who without them I wouldn't be able to reach what I have reached,

My beloved husband
My dear father
My caring mother

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Doctor of Philosophy

DETERMINATION OF CHEMICAL COMPOSITION AND POTENTIAL ANTICANCER ACTIVITIES OF *ALLIUM ATROVIOLACEUM* EXTRACT ON SELECTED CANCER CELL LINES

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September 2016

Chairman: Professor Patimah Ismail, PhD
Faculty: Medicine and Health Sciences

The present study was carried out to determine the phytochemicals and the chemotherapeutic potentials of *Allium atroviolaceum* flower (FA) and bulb (BA) crude extracts in selected reproductive cancer cell lines, human hormone dependent breast cancer (MCF7), human hormone independent breast cancer (MDA-MB-2341), human cervical cancer (HeLa), and human liver cancer (HepG2) and non-malignant murine fibroblast (3T3) cell lines.

The extracts were found to induce anti-proliferative effect on all the studied cells. 70% methanol extracted after 9 hours gave the most promising IC_{50} values. Exposure of breast, cervical, and liver cancer cell lines to FA and BA extracts demonstrated growth inhibition of cells in both dose- and time-dependent manners. The IC_{50} value for each cell line at 24, 48, and 72 hours respectively, is given as follows: MCF7 (65, 41, and 24 µg/ml for FA, while 91, 88, and 75 µg/ml for BA), MDA-MB-231 (77, 67, and 51 µg/ml for FA, while 150, 114, and 101 µg/ml for BA), HeLa (68, 51, and 37 µg/ml for FA , while 100, 98, and 74 µg/ml for BA), and HepG2 (57, 44, and 26 µg/ml for FA, while 97, 70, and 58 µg/ml for BA). On the other hand, the IC_{50} value of normal 3T3 cell line was found to be > 100, indicating that FA and BA extracts were not cytotoxic to normal cells.

In addition, the interaction of FA and BA extracts with tamoxifen (against MCF7 and MDA-MB-231) and doxorubicin (against HeLa and HepG2) revealed a significant dose-reduction in IC_{50} value.

Phase contrast and fluorescent microscopy (AO/PI) analyses demonstrated apoptotic features after treatment with FA and BA for 24, 48, and 72 hours.

Analyses of DNA content and cell cycle with PI staining confirmed that the time and dose course treatments of FA and BA crude extracts displayed the ability in promoting S phase arrest, G2/M phase arrest, as well as apoptosis in MCF7, MDA-MB-231, and HepG2. However, the event was both time- and dose-dependent, although G0/G1 phase arrest was observed in HepG2 treated with BA in low concentration. Meanwhile, in HeLa cells, the extract enhanced only the percentage of cells present in sub-G0 in both time- and dose-dependent manners.

The Annexin V assay revealed that FA and BA extracts induced cell death by stimulating early apoptosis in low and middle concentrations (IC_{25} and IC_{50}), as well as shorter incubation time (24 and 48 hours), while inducing late apoptosis/necrosis in high concentration (IC_{75}) and longer time course (72 hours) in all the cell lines.

The effect of FA and BA extracts at 24 hours on activity caspases illustrated that the apoptotic effects of FA and BA were via activation of specific inflammatory and initiator caspase, which led to the activation of final effector, caspase-3 or 6. In some cases, increment of caspase-8 and -9 activities indicated that both extrinsic and intrinsic pathways were activated by those extracts that were depended on the type of cell lines. Moreover, the activity of caspase-3 as the main executioner caspase was evaluated with more time course, 48 and 72 hours. As a result, more time of exposure to both FA and BA led to less caspase-3 activity in MCF7 and HeLa, while in MDA-MB-231 and HepG2, longer time incubation resulted in more activities of caspase-3 executioner caspase.

In addition, gene expression analysis using the qPCR conducted on three target genes (*BCL2*, *CDK1*, and *p53*) showed downregulation of anti-apoptotic *BCL2* in MCF7, MDA-MB-231, and HeLa, while only FA-treated HepG2 exhibited enhanced apoptosis. Additionally, upregulation of *p53* only after treatment with BA in MDA-MB-231 and HepG2 illustrated induced apoptosis through a *p53*-dependent mitochondrial pathway, while *p53*-independent mitochondrial pathway in other treated cells. More to the point, *CDK1* was downregulated in MCF7, MDA-MB-231, HeLa, and slightly in HepG2 after exposure to both extracts that might be the main mechanism used by both extracts to exert G2/M cell cycle arrest and ultimately, the final fate of cells, apoptosis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia Sebagai memenuhi keperluan untuk Doktor Falsafah

PENENTUAN KOMPOSISI KIMIA DAN AKTIVITI ANTI KANSER POTENSI ALLIUM ATROVIOLACEUM EKSTRAK ON TERPILIH KANSER CELL LINES

Oleh

SOMAYEH KHAZAEI

September 2016

Pengerusi: **Profesor Patimah Ismail, PhD**
Fakulti: **Perubatan dan Sains Kesihatan**

Kajian ini dijalankan untuk menentukan sifat fitokimia dan evaluasi potensi agen kemoteraputik ekstrak mentah bunga (FA) dan bebawang (BA) *Allium atroviolaceum* terhadap sel selanjar kanser terpilih iaitu sel selanjar kanser payudara manusia yang bergantung kepada hormon (MCF-7), sel selajar kanser payudaya manusia yang tidak bergantung kepada hormon (MDA-MB-2341), sel selanjar kanser pangkal rahim manusia (HeLa) dan sel selanjar kanser hati manusia (HepG2) dan normal 3T3.

Ekstrak didapati merangsang kesan anti-proliferatif ke atas semua sel-sel selanjar kanser yang dikaji. Ekstrak metanol 70% selama 9 jam memberikan nilai IC_{50} paling menggalakan. Rawatan dan pendedahan ekstrak FA dan BA terhadap sel selanjar kanser payudara, pangkal rahim dan hati menunjukkan perencatan pertumbuhan sel adalah bergantung kepada dos dan tempoh dedahan. Nilai IC_{50} untuk setiap sel selanjar pada 24, 48, dan 72 jam pendedahan kepada ekstrak adalah seperti berikut: MCF7 (65, 41 dan 24 µg/ml untuk FA., manakala 91, 88 dan 75 µg/ml untuk BA.), MDA-MB-231 (77, 67 dan 51 µg/ml untuk FA., manakala 150, 114 dan 101 µg/ml untuk BA.), HeLa (68, 51 dan 37 µg/ml untuk FA, manakala 100, 98 dan 74 µg/ml untuk BA.), dan HepG2 (57, 44 dan 26 µg/ml untuk FA., manakala 97, 70 dan 58 µg/ml untuk BA.). Malahan, nilai IC_{50} sel selanjar fibroblast normal murine (3T3) adalah melebihi 100 µg/ml memberi indikasi bahawa esktrak FA dan BA adalah tidak toksik kepada sel-sel normal.

Kajian juga mendapati interaksi ekstrak FA dan BA dengan ubat tamoxifen (terhadap MCF7 dan MDA-MB-231) dan doxorubicin (terhadap HeLa dan HepG2) menunjukkan pengurangan ketara nilai dos IC_{50} dengan interaksi ubat-ekstrak.

Analisis mikrokop fasa kontras dan mikroskop pendafluor menunjukkan ciri-ciri apoptotik terhadap sel-sel selanjar kanser selepas pendedahan dan rawatan terhadap FA dan BA selama 24, 48 dan 72 jam yang.

Analisis kandungan DNA dan kitaran sel dengan menggunakan pewarnaan PI mengesahkan bahawa masa dan dos rawatan ekstrak mentah FA dan BA menyebabkan pengumpulan sel pada fasa S dan G2/M selain menyebabkan apoptosis pada MCF7, MDA-MB-231 dan HepG2. Walaupun kitaran sel adalah bergantung kepada masa dan dos rawatan, pengumpulan sel pada fasa G0/G1 dapat diperhatikan pada kepekatan dos yang rendah terhadap sel selanjar kanser HepG2 manakala terdapat penambahan peratusan sel pada fasa sub-G0 terhadap sel selajar kanser HeLa.

Analisis Annexin V menunjukkan ekstrak mentah FA dan BA merangsang kematian sel dengan merangsang apoptosis awal pada kepekatan dos rendah dan sederhana (IC_{25} dan IC_{50}) dan juga pada waktu pendedahan yang pendek (24 dan 48 jam). Manakala kepekatan dos yang tiggi (IC_{75}) dan tempoh rawatan serta dedahan yang lebih panjang (72 jam) menyebabkan ransangan apoptosis akhir/nekrosis pada semua sel selanjar kanser.

Ekstrak mentah FA dan BA pada tempoh rawatan dan dedahan 24 jam terhadap aktiviti 'caspase' menggambarkan bahawa kesan apoptotik yang disebabkan oleh ekstrak mentah FA. dan BA. adalah melalui pengaktifan reseptor inflamasi khusus dan pemula 'caspase' yang akhirnya menyebabkan pengaktifan 'caspase'-3 dan -6. Selain itu, dalam beberapa kes, bergantung kepada jenis sel selanjar kanser, peningkatan aktiviti 'caspase'-8 dan -9 menunjukkan bahawa ekstrak mentah *Allium atroviolaceum* menyebabkan pengaktifan kedua-dua jalur laluan intrinsik dan ektrinsik. Tambahan pula, aktiviti 'caspase'-3 sebagai 'caspase' eksekutor utama dapat diperhatikan pada tempoh rawatan dan dedahan yang lebih panjang iaitu pada 48 dan 72 jam. Selain itu, tempoh rawatan dan dedahan yang lebih panjang terhadap ekstrak mentah FA. dan BA. menyebabkan pengurangan aktiviti 'caspase'-3 dalam sel selanjar kanser MCF7 dan HeLa .Namun, dalam sel selanjar kanser MDA-MB-231 dan HepG2 aktiviti 'caspase' eksekutor iaitu 'caspase'-3 menunjukkan peningkatan aktiviti seiring dengan tempoh rawatan dan dedahan.

Analisis gen menggunakan qPCR yang dijalankan ke atas tiga gen sasaran (*BCL2*, *p53*, dan *CDK1*) menunjukkan regulasi menurun pada gen anti-apoptotik *BCL2* pada MCF7, MDA-MB-231 dan HeLa, manakala hanya rawatan dan pendedahan FA. terhadap HepG2 menunjukkan peningkatan apoptosis. Selain itu, regulasi menaik *p53* hanya dapat diperhatikan pada MDA-MB-231 dan HepG2 menunjukan rangsangan apoptosis melalui jalur laluan *p53* yang berkaitan dengan mitokondria manakala bagi sel selanjar kanser lain, apoptosis adalah melalui jalur laluan *p53* yang tidak berkaitan dengan mitokondria. Lebih penting lagi regulasi menurun gen *CDK1* dapat diperhatikan pada sel selanjar kanser MCF7, MDA-MB-231, HeLa dan sedikit pada sel selanjar kanser HepG2 selepas rawatan dan dedahan terhadap kedua-dua ekstrak mentah yang mungkin menjadi mekanisma utama yang membawa kepada pengumpulan sel pada fasa G2/M kitaran sel dan akhirnya menyebabkan kematian sel secara apoptosis.

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In the name of God, The Lord of Majesty and Bounty, The Inspirer of Faith. Praised to Him for enlightening my path and surrounding me with wonderful people.

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I certify that a Thesis Examination Committee has met on 2 September 2016 to conduct the final examination of Somayeh Khazaei on her thesis entitled **"DETERMINATION OF CHEMICAL COMPOSITION AND POTENTIAL ANTICANCER ACTIVITIES OF *ALLIUM ATROVIOLACEUM* EXTRACT ON SELECTED CANCER CELL LINES"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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ADP-ribose	DNA repair enzyme poly
ANOVA	One-way Analysis of Variance
Apaf-1	Apoptotic protease activating factor-1
ASR	Age standardized incidence rate
BA	Bulb of *Allium atroviolaceum*
BAD	*BCL2*-associated death promoter
BAX	*BCL2*-associated X protein
BH3	*BCL2* homology domain
BID	BH3 interacting-domain death agonist
BRCA1	Breast cancer type-1 susceptibility genes
BRCA2	Breast cancer type-1 susceptibility genes
CARD	Caspase recruitment domain
Caspases	Cysteinyl aspartate-specific proteases
CI	Combination index
CDK	Cyclin dependent kinase
cDNA	Complementary DNA
DCIS	Ductal carcinoma *in situ*
DD	Death domain
DED	Death effector domain
DISC	Death inducing signaling complex
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E2F	E2 factor transcriptional factor
ER+	Estrogen receptor positive
FA	Flower of *Allium atroviolaceum*
FADD	Fas-associated death domain
Fas/FasL	Fibroblast-associated cell-surface and Fasligand
FBS	Fetal bovine serum
FDA	Food and drug administration
HBV	Hepatitis B viruses
HCC	Hepatocellular carcinoma
HCV	Hepatitis C viruses
HTS	High-throughput screening
HeLa	Human cervical cancer cell line
HepG2	Human hepatocellular cancer cell line

IC_{50}	Inhibition concentration of 50% of cell death
ICCAM	Interagency Coordinating Committee in the Validation of Alternative Methods
IDC	Invasive ductal carcinoma
ILC	Invasive lobular carcinoma
LACC	Locally advanced cervical cancer
LCIS	Lobular carcinoma *in situ*
LD	Local destruction
LEEP	Loop electrosurgical excision procedure
LOC	Lab-on-a-chip technology
MCF7	Human hormone-dependent breast cancer cell line
MDA-MB-231	Human non-hormone-dependent breast cancer cell line
MDR	Multi-drug resistance
MMP	Matrix metalloproteinases
MOMP	Mitochondrial outer membrane permeabilization
MPF	Maturation/M-phase promoting factor
mRNA	Messenger ribonucleic acid
MSI	Microsatellite instability
NACT	Neoadjuvant chemotherapy
NCR	National Cancer Registry
NIEHS	National Institute of Environmental Health Science
NIST	National Institute Standard and Technology
OLT	Orthotopic liver transplantation
PARP	Poly(ADP-ribose) polymerase
PBS	Phosphate buffer saline
PIAF regimen	Regimen combination of cisplatin, interferon, doxorubicin and 5- fluorouracil
PS	Phospholipid phosphatidylserine
RFA	Radiofrequency ablation
Rb	Retinoblastoma
RIN	RNA Integrity Number
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute
SD	Standard deviation
SERM	Selective estrogen receptor modulator
SMAC/DIABLO	Pro-apoptotic protein
SPSS	Statistical Package for Social Sciences

TACE	Trans-arterial chemo-embolization
TAM	Tamoxifen
tBID	Truncated BID
TGF	Transforming growth factor
TM	Trans-membrane
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
T*p53*	Tumour suppressor protein
TRADD	TNFR-associated death domain
TRAIL	Receptors DR-4 and DR-5
WHO	World Health Organization
3t3	Mouse embryonic fibroblast *cell* line
5-FU	5-Fluorouracil
6-MP	6-Mercaptopurine

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Cancer is a disease of cell growth deregulation due to the reposition of mutations in a single cell that lead to gradual phenotypic changes, from a normal to a neoplastic cell. Loss or mutations of genes can lead to deregulation of abnormal amplification of growth signals and signal transduction pathways that conclusively transform normal cells into invasive cancer cells (Shen *et al*., 2003).

Cancer is a major health problem worldwide (Yaacob *et al*., 2010). Statistics illustrate that cancer strikes almost one third of the population (more than 20% of all deaths) (Zaid *et al*., 2010). Despite considerable advances in treatment and early detection of cancer (Mathers *et al*, 2008), it remains a big problem for families and economies (Motaal and Shaker, 2011).

Conventional, advanced and alternative treatment has indicated an evolution in cancer therapy (Ricki, 2005). Chemoprevention, that is a means of cancer control or reverse by natural or synthetic agents, is gaining attention (Amin *et al*., 2009), however, development of resistance to chemotherapeutic drugs prevents effective elimination of the cancer cells. In addition, cardiac and other toxicities are serious side-effects that cause suffering in patients (Yaacob *et al*., 2010). In addition, the toxicity of anticancer drugs to normal fast-growing cells limits their effectiveness. Resistance of cancerous cells to a specific drug which initially suppressed them is another problem of chemotherapy drugs. Hence, using several drugs in combination may be more effective (Alan, 2008). Furthermore, the physiological and mechanistic deregulations responsible for initiation and promotion of cancer signify hundreds of genes or signaling cascades. Therefore, it appears that multi-target drugs are necessary to overcome cancer. The multiple therapeutic effects of natural compounds in traditional medicine motivate researchers to evaluate the anti-tumor effect of natural compounds and understand their mechanism of action (Teitene *et al*., 2010).

Natural products are considered powerful sources for novel drug discovery and development (Van Slambrouck *et al*., 2007).

Phytochemicals, the heterogeneous class of molecules, include vitamins (carotenoids) and food polyphenols, such as flavonoids, phenolic acids and sulfur rich compounds. Biological targets of phytochemicals in mammalian cells are involved in inflammatory processes and oncogenic transformation, for instance variation of cell cycle control, apoptosis evasion and metastases (Russo *et al*., 2010); therefore, phytochemical agents might have potential as lead compounds for clinical anticancer drugs development.

Allium atroviolaceum (A.atroviolaceum) is a species in the genus *Allium. Allium* is the largest genus in the widely distributed *Alliaceae* family (Dahlgren *et al*., 1985). The health benefits of *Allium* species such as garlic (*A.sativum*) and onion (*A.cepa*) have been known for thousands of years; however, interest in other species has been increasing recently (Štajner *et al*., 2006). The *Allium*s are commonly valued for food, medicine, and garden ornamentals, and range from plants to weeds. Onions, garlic, leek, chives and Welsh onions are some of the most commonly valued *Allium*s (Uhl, 2000).

In this study, crude flower (FA) and bulb (BA) extracts of *A.atroviolaceum* were tested to investigate the anti-proliferation activity of cancer cells such as human hormone-dependent breast cancer (MCF7), human hormone-independent breast cancer (MDA-MB-231), human cervical cancer (HeLa), human liver cancer (HepG2) and also its effect towards normal cells (3T3) were monitored to discover any probable harmful effect on normal cells. A study then was carried out with the aqueous-methanol extract of FA and BA to investigate their potential. The different intracellular mechanisms affected by FA and BA will be detailed hereafter.

Taken together, research in the field of natural products is in high demand to help humans overcome many newly emerging and known diseases, particularly cancers.

1.2 Problem Statement

Malignant cancers are a major cause of death, and they continue to increase. While suitable cures and therapies are able to assist patients to recover at the primary stages, mortality and short survival time of malignant cancers at an advanced stage are inescapable (Bhattacharyya *et al*., 2010). In addition, chemotherapy has some unavoidable side effects resulting in the lack of optional cytotoxicity (Ahmed *et al*., 2003) and many existing anticancer drugs share a common mechanism of action that could lead to drug resistance (Alessandro et al., 2007). For these reasons, new agents with new mechanisms need to be developed and tested. Since the use of natural products in cancer chemotherapy have growth significantly, the study of mechanism and mode of action of plant extracts and metabolites become more important. Some components of *Allium* vegetables are reported to block several stages of carcinogenesis, although the underlying mechanisms of action are generally unclear. Association between the consumption of *Allium* vegetables and the risk of cancer has been assessed which point to lower risks for cancers of the stomach, colon, esophagus, and perhaps breast (Sengupta *et al*., 2004). Moreover, although *A.atroviolaceum* have been used traditionally as medication against various diseases, its medicinal values, particularly against different type of cancer have not been documented yet. This prompted us to investigate the effect of *Allium atroviolaceum* on human breast, cervical and liver cancers.

1.3 Significance of the Study

Natural origin is defined as natural products, derivatives of natural products or synthetic pharmaceuticals based on natural product models (Shoeb, 2006). With only approximately 15% of the world's known plant resources screened for their therapeutic values and over 60% of the currently used anticancer agents derived from natural sources including plants, marine organisms and micro-organisms, it is clear that plants have, and will play, a crucial role in the development of new anti-cancer agents. Due to few medical facilities, low income and cultural and religious beliefs, most of people in developing countries (~80%) use traditional medicines obtained from plants (Boukes and van de Venter, 2011). Natural products medicines are effective in the treatment of specific characteristics while also reducing side effects (Bhadury *et al*., 2006).

A.atroviolaceum is one of the lesser known species of *Allium;* its pharmaceutical value remains undiscovered. Study of the anticancer effect of *A.atroviolaceum* and understanding of its effects at a molecular level may lead an effective cancer treatment and a promising approach to cancer control.

1.4 Hypothesis

The extract of aerial and underground parts of *A.atroviolaceum* exhibit activity against breast, cervical and liver tumor cells, including a selective cytostatic effect that potentiate use as anticancer drugs. Furthermore, the extract may contain multiple bioactive compounds that could work alone or in combination to restrict cell survival. Moreover, the extracts will demonstrate cytotoxicity towards breast, cervical and liver cancer cells by inducing cell death.

1.5 Objectives

1.5.1 General objective

To investigate the phytochemical and cytotoxic activities of flower and bulb extracts of *A.atroviolaceum* on human breast (MCF7, MDA-MB-231), cervical (HeLa) and liver (HepG2) and non-malignant murine fibroblast (3T3) cell lines.

1.5.2 Specific objectives

1. To identify the phytochemical composition of FA and BA based on the effects of multi-factor experiments.

2. To study the *in vitro* cytotoxic effects of the methanol crude extracts of FA and BA on human hormone-dependent breast cancer (MCF7), human non-hormone-dependent breast cancer cell line (MDA-MB-231), human cervical cancer (HeLa) and human liver cancer (HepG2) and non-malignant murine fibroblast (3T3) cell lines.

3. To evaluate the combination effect of the crude extracts and chemotherapeutic drugs, tamoxifen and doxorubicin.

4. To determine the cell cycle profile of human hormone-dependent breast cancer (MCF7), human non-hormone-dependent breast cancer cell line (MDA-MB-231), human cervical cancer (HeLa) and human liver cancer (HepG2) cell lines induced by FA and BA methanol extracts.

5. To evaluate the anti-proliferative effects and apoptosis induction of FA and BA methanol extracts on human hormone-dependent breast cancer (MCF7), human non-hormone-dependent breast cancer cell line (MDA-MB-231), human cervical cancer (HeLa) and human liver cancer (HepG2) cell lines by using fluorescent microscopy (AO/PI) and flow cytometry (AnexinV).

6. To identify the possible mechanism of cell death pathways by assessing the alteration in activity of caspase1,2,3,5,6,8 and 9 induced by FA and BA methanol extracts on human hormone-dependent breast cancer (MCF7), human non-hormone-dependent breast cancer cell line (MDA-MB-231), human cervical cancer (HeLa) and human liver cancer (HepG2) cell lines.

7. To determine the expression of apoptotic related genes (*BCL2*, *CDK1* and *p53*) by FA and BA methanol extracts on human hormone-dependent breast cancer (MCF7), human non-hormone-dependent breast cancer cell line (MDA-MB-231), human cervical cancer (HeLa) and human liver cancer (HepG2) cell lines.

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BIODATA OF STUDENT

Somayeh Khazaei was born in December 18th, 1983 in Nowshahr, Iran. She begin her primary school in Nowshahr from 1990 to 1997 and continue her high school from 1997 to 2000. After graduation from high school she started to study foundation in 2001. Her enthusiasm for learning and the great support and encouragement from her parents led her to peruse further education. She studied Bachelor of Science in the College of Science/Biology Department/ Mazandaran University and graduated for the academic year 2003/2007 Obtained the B.Sc degree in Plant biology branch with a grade good and an average of 75. Then she decided to continue her education and started her further education in Master Degree (M.Sc in Plant Physiology) in College of Medicine/Azad University of Tonekabon and graduated in 2009 with an average of 94.5. After her marriage in 2010 she moved to Malaysia where her husband was living. Her interest to further study and the good quality of Universities in Malaysia led her to start her Phd in Genetic at Faculty of Medicine and Health Sciences in Universiti Putra Malaysia (UPM) from September of 2012.

List of Publications

Khazaei, S., Abdul Hamid, R., Zakaria, Z.A., Mohd Esa, N., Ramachandran, V., Etemad, A., Ismail, P. Comparative Evaluation of Chemometric Profile of 12 Various Flower and Bulb Extracts of *Allium atroviolaceum* with Gas Chromatography Mass Spectrometric Technique.

Somayeh Khazaei., Norhaizan Mohd Esa., Roslida Abdul Hamid., Vasudevan Ramachandran., Ashok Kumar., Patimah Ismail. *In vitro* Antiproliferative and Apoptotic Effect of *Allium atroviolaceum* Extract on Breast, Cervical and Liver Cancer Cells.

Somayeh Khazaei, Norhaizan Mohd Esa, Vasudevan Ramachandran, Roslida Abdul Hamid, Patimah Ismail. Cytotoxicity and apoptosis inducing effects of *Allium atroviolaceum* flower Extract is mediated through cell cycle arrest, caspase-dependent and *p53*-independent pathway in breast cancer cell lines.

Khazaei, S., Abdul Hamid, R., Mohd Esa, N ., Ramachandran, V ., Aalam GHomi Tabatabaee, F ., Ismail, P. Mechanism of the promotion of HepG2 cell apoptosis by flower of *Allium atroviolaceum.*

Khazaei, S., Abdul Hamid, R., Mohd Esa, N., Ramachandran, V., Aalam GHomi Tabatabaee, F., Ismail P [1]*. Flower Extract of *Allium atroviolaceum* Triggered Apoptosis, Activated Caspase-3 and Down-Regulated the antiapoptotic *BCL2* gene in HeLa Cancer Cell Line.