PRODUCTION AND CHARACTERIZATION OF PHYCOBILIPROTEINS FROM CYANOBACTERIA AND THEIR EFFECTS ON HepG2 CANCER CELL LINES

HASINA BEGUM

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By

HASINA BEGUM

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

April 2014

DEDICATION

To my husband

&

To my parents who always inspire and encourage me to achieve my goal

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

CHARACTERIZATION AND PRODUCTION OF PHYCOBILIPROTEINS FROM CYANOBACTERIA AND THEIR EFFECTS ON HepG2 CANCER CELL LINES

By

HASINA BEGUM

April 2014

Chairman: Professor Fatimah Md. Yusoff, PhD

Institute Bioscience

Phycobiliproteins have a wide range of promising applications in food, nutraceutical and biomedical industries because of their antibacterial, antifungal, anticancer and antioxidant properties. Due to the fact that synthetic drugs for treating cancer have some adverse effects, there is a need for alternative therapy by using natural compounds to inhibit the growth of cancer cells. Therefore, this study emphasized on the effect of cyanobacterial phycobiliproteins on the growth and proliferation of cancer cell lines.

Marine and freshwater cyanobacteria were screened to compare the concentration of phycobiliproteins and purity of phycocyanin subjected to different drying methods. Results from oven dried showed that total phycobiliproteins production (208.1±3.14mg/g) and purity ratio of phycocyanin (1.2) was significantly ($p<0.05$) higher in marine periphytic *Geitlerinema* sp. compared to other cyanobacterial species. Therefore, *Geitlerinema* sp. was used for succeeding experiments.

Phycobiliproteins production and purity ratio was significantly ($p<0.05$) higher in *Geitlerinema* sp. at 35ºC, 30 ppt and pH 8 compared to other temperature, salinity and pH levels. Under these conditions, protein concentration in phycobiliproteins crude extract from marine *Geitlerinema* sp. was 64.5 ± 0.2µg/ml with molecular weight approximately between 19 KDa to 26 KDa. In addition, crude phycobiliproteins extract of *Geitlerinema* sp. have the similar characteristics as commercial phycocyanin.

In vitro screening of cytotoxic effects of crude phycobiliproteins extract (rich fraction of phycocyanin) found that IC_{50} (half maximal inhibitory concentration) value (3.8±1.26 µg/ml) lower in HepG2 cell line. In addition, concurrent study of the crude phycobiliproteins extract with paclitaxel showed IC_{50} value of crude phycobiliproteins extract similar to the paclitaxel and no negative effect on normal liver cell line (Chang liver cell line).

Morphological changes of HepG2 treated with crude phycobiliproteins extract (rich fraction of phycocyanin) exhibited cell shrinkage, chromatin condensation, membrane blebbing, cytoplasomic extrusions and formation of apoptotic bodies. The Annexin V assay revealed apoptotic induction in HepG2 cells exposed to the crude phycobiliproteins extract in a time-dependent manner, whereas DNA fragmentation of HepG2 cells were detected using 1.0% agarose gel electrophoresis. Cell cycle analysis showed that there was significant ($p<0.05$) G1 phase arrest at each time point from 12 to 72 h. Crude phycobiliproteins extract significantly ($p<0.05$) stimulated both caspase 9 and 3 activities.

Results from the apoptotic gene expression showed significant ($p<0.05$) down-regulated expression level of anti-apoptotic Bcl2 gene, up-regulated expression level of Bax, Bak that increases mitochondrial membrane potential (MMP) and pore formation. Subsequently, cytochrome C and up-regulated expression level of Apaf-1 were released. These effects were associated with the induction of caspase-9, -3 activities, and subsequent DNA fragmentation by blocking PARP followed by intrinsic pathway of apoptosis. Meanwhile, crude phycobiliproteins extract significantly ($p<0.05$) down-regulated or inhibited NF-kB2 and NF-kB1 respectively which may directly or indirectly regulate Bcl2 through the modulation of anti-apoptotic genes to induce intrinsic pathway of apoptosis. In addition, it was found that phycobiliproteins crude extract from the *Geitlerinema* sp. significantly ($p<0.05$) inhibited TNF-α, FADD, TRADD, Interleukin-6, down regulation of IFN-gamma and JNK-1 gene but no activation of caspase 8 which strongly suggested that induction of apoptosis in HepG2 cells is without involvement of extrinsic pathway. Thus, crude phycobiliproteins extract (rich fraction of phycocyanin) from marine cyanobacteria *Geitlerinema* sp. has significant induction of apoptosis on HepG2 cells via multiple signal transduction pathways involving intrinsic pathway and may have potential therapeutic value as anticancer towards human hepatocellular carcinoma.

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

PENGHASILAN DAN PENCIRIAN FIKOBILIPROTEIN DARIPADA SIANOBAKTERIA DAN KESANNYA KE ATAS BARISAN SEL KANSER HepG2

Oleh

HASINA BEGUM

April 2014

Pengerusi: Profesor Fatimah Md. Yusoff, PhD

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Fikobiliprotein mempunyai banyak aplikasi yang meyakinkan dalam industri makanan, nutraseutikal dan bioperubatan disebabkan oleh ciri-ciri anti-bakteria, anti-kulat, anti-kanser dan anti-oksidannya. Memandangkan perubatan sintetik yang digunakan untuk merawat sel kanser mungkin menyebabkan kesan sampingan, keperluan untuk mencari alternatif terapi menggunakan kompaun semulajadi untuk merencatkan pertumbuhan sel kanser. Oleh itu, kajian ini menekankan ke atas kesan fikobiliprotein sianobakteria ke atas pertumbuhan dan perkembangan barisan sel kanser.

Sianobakteria air tawar dan air masin telah disaring untuk membandingkan kepekatan fikobiliprotein dan ketulenan fikosianin yang didedahkan kepada kaedah pengeringan yang berbeza. Keputusan daripada pengeringan oven menunjukkan penghasilan phycobiliprotein kesuluruhan (280.1±3.14) dan nisbah ketulenan fikosianin (1.2) adalah lebih tinggi signifikannya dalam perifitik air masin Geitlerinema sp. dibandingkan dengan spesies sianobakteria yang lain. Oleh itu, Geitlerinema sp. digunakan dalam eksperimen yang seterusnya.

Penghasilan fikobiliprotein dan nisbah ketulenan dalam Geitlerinema sp. lebih tinggi signifikannya pada suhu 35° C, 30 ppt dab pH 8 jika dibandingkan dengan suhu, kemasinan dan paras pH yang lain. Dalam keadaan ini, kepekatan protein dalam ekstrak fikobiliprotein kasar daripada Geitlerinema sp. air masin telah dikenalpasti pada 64.5±0.2 µg/ml dengan berat molecular ekstrak fikobiliprotein kasar daripada Geitlerinema sp. adalah kira-kira 19KDa dan antara 19 dan 26 KDa. Tambahan pula, ekstrak

fikobiliprotein kasar Geitlerinema sp. mempunyai ciri yang sama dengan fitosianin komersial.

Kesan sitotoksik invitro ekstrak fikobiliprotein (pecahan fikocyanin yang tinggi) kasar telah dikesan memiliki nilai IC50 (separuh kepekatan rencatan maksimum) yang rendah pada 3.8±1.26 µg/ml dalam barisan sel HepG2. Tambahan pula, kajian ekstrak fikobiliprotein kasar yang berikutnya dengan paclitaxel menunjukkan nilai IC50 ekstrak fikobiliprotein kasar adalah sama dengan paclitaxel dan tiada kesan negatif pada barisan sel hati normal (Barisan sel hati Chang)..

Perubahan morfologikal ke atas HepG2 yang dirawat dengan ekstrak fikobiliprotein kasar menampakkan pengecutan sel, kendensasi kromatin, pengenduran membran, penyemperitan sitoplasomic dan pembentukan jasad apoptotik. Esei Annexin V mendedahkan induksi apoptotik dalam sel HepG2 yang didedahkan kepada ekstrak fikobiliprotein kasar dalam cara kebergantungan masa, manakala pemecahan DNA sel HepG2 telah dikesan menggunakan elektroforesis gel agaros 1.0%. Analisa kitar sel menunjukkan bahawa terdapat penahanan fasa G1 yang signifikan ($p<0.05$) pada setiap takat masa daripada 12 hingga 72h. Ekstrak fikobiliprotein kasar secara signifikan ($p<0.05$) distimulasikan pada kedua-dua aktiviti caspase 9 dan 3.

Keputusan menunjukkan ekspresi gen apoptotic menunjukkan penurunan secara signifikan ($p<0.05$) tingkat gen anti-apoptotic Bcl2, peningkatan ekspresi Bax, Bak yang meningkatkan potensi membran mitokondria (MMP) dan pembentukan liang. Seterusnya, sitokrom C dan peningkatan tingkat ekspresi Apaf-1 telah dilepaskan. Kesan-kesan ini adalah berkaitan dengan induksi aktiviti caspase-9, caspase-3 dan pemecahan DNA dengan menghalang PARP diikuti laluan intrinsik apoptosis. Sementara itu, ekstrak fikobiliprotein kasar menurunkan secara signifikan ($p<0.05$) atau merencatkan NF-kB2 dan NF-kB1 yang mungkin secara langsung atau tidak langsung mengawal Bcl2 melalui modulasi gen anti-apoptotic untuk menginduksi laluan intrinsik apoptosis. Sebagai tambahan, ekstrak fikobiliprotein kasar daripada Geitlerinema sp. telah dikenalpasti merencatkan TNF-α, FADD, TRADD, Interleukin-6, dan mengawalturun IFN-gamma dan JNK-1 secara signifikan ($p<0.05$) tetapi tiada pengaktifan caspase 8 yang mencadangkan bahawa induksi apoptosis sel HepG2 tanpa penglibatan laluan ektrinsik. Jadi, ekstrak kasar fikobiliprotein daripada sianobakteria air masin Geitlerinema sp. mempunyai induksi apoptosis yang signifikan ke atas sel HepG2 melalui beberapa laluan isyarat transduksi yang melibatkan laluan intrinsic dan mungkin mempunyai potensi nilai terapeutik sebagai antikanser terhadap hepatocellular carcinoma manusia.

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I certify that an examination committee met on 29th April, 2014 to conduct the final examination of Hasina Begum on her Doctor of Philosophy thesis entitled "Production and characterization of phycobiliproteins from cyanobacteria and its effects on HepG2 cancer cell lines" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µl	Microliter
AIF	Apoptosis inducing factor
ANOVA	Analysis of variance
AO	Acridine orange
APAF-1	Apoptotic protease activating factor-1
APC	Allophycocyanin
APS	Acrylamide ammonium persulfate
Arg	Arginine
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
Bak	Bcl2 homologous antagonist/killer
Bax	Bcl2 associated X protein
Bcl2	Anti-apoptotic protein
BSA	Bovine serum albumin
CAD	Caspase activated DNA
caspases	Cystein-aspartic proteases
CEM-ss	Human T4-lymphoblastic leukemia cell line
cm	Centimeter
CO_2	Carbon dioxide
DD	Death domain
DED	Death effector domain
DFF	DNA fragmentation factor
DISC	Death inducing signaling complex
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ERK	Extracellular signal regulated kinase
FADD	FAS-associated death domain
FasL	Fas ligand
FBS	Fetal bovine serum
g	Grams
G_0	Gap 0 at cell cycle
G_1	Gap 1 at cell cycle
G_2	Gap 2 at cell cycle
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
Glu	Glutamic
Gly	Glycine
h	Hour
H_2O_2	hydrogen peroxide
HBV	Hepatitis B virus
HCC	Human hepatocellular carcinoma
HeLa	Cervical cancer cell line
HepG2	Human hepatocellular liver carcinoma cell line
His	Histidine
IAP	Inhibitor of apoptosis

IBS	Institute of Bioscience
IC_{50}	Inhibitory concentration (50%)
ICAD	Inhibitor of caspase activated DNA
IL-6	Interleukin-6
Ile	Isoleucine
K562	Human immortalised myelogenous leukaemia line
Kan	Kanamycin
Kbp	Kilo base pair
kDa	Kilo dalton
KPa	Kilo pascal
Leu	Leucine
M	Molar
MAPK	Mitogen activating protein kinase
MCF7	Estrogen receptor positive breast cancer cell line
MDA	Human breast adenocarcinoma
mg	Milligram
min	Minute
ml	Milliliter
mM	Micro molar
MMP	Mitochondrial membrane potential
M-phase	Mitosis
mRNA	Messenger Ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium
MW	Molecular weight
n	Number
N	Normality
N_2	Nitrogen
NaOH	Sodium hydroxide
NCI	National Cancer Institute
NCR	National Cancer Registry Malaysia
OD	Optical density
p	Probability value of test statistics
P	Phosphorus
p53	protein 53 or tumor protein 53
PARP	Poly ADP ribose polymerase
PBS	Phosphate buffer saline
PBS_s	Phycobilisomes
PC	Phycocyanin
PCR	Polymerase chain reaction
PE	Phycoerythrin
PI	Propidium iodide
PIAF	Cisplatin, interferon, doxorubicin and 5-fluorouracil
ppt	Parts per thousand
PS	Phosphatidylserine
ROS	Reactive oxygen species
rpm	Revolutions per minute
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
Ser	Serine

S-phase	Synthesis
TEMED	N,N,N,N'-tetramethylenediamine
TFA	Trifluoroacetic acid
Thr	Threonine
TNF-α	Tumor necrosis factor alpha
TRADD	TNFR1 associated death domain
TRAIL	Tumor necrosis (TNF)-related apoptosis inducing ligand
UPM	Universiti Putra Malaysia
UV	Ultra-violet
v/v	Volume per volume
WHO	World Health Organization
µg	Microgram
µm	Micrometer

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Microalgae are diverse groups of simple, plant like organisms which are unicellular or filamentous microorganisms and are able to harness solar energy to produce large quantities of biomass through the photosynthesis mechanism (Matsunaga et al., 2005). They are classified according to the colour of pigments; *chlorophyceae* (green colour), *rhodophyceae* (red colour), *cyanophyceae* (blue green) and *phaeophycae* (brown colour) (Graham and Wilcox, 2000). Chlorophyll, carotenoids and phycobiliproteins exhibit colours ranging from green, yellow and brown to red. Natural colourants from different microalgae like phycocyanin (blue pigment from *Spirulina*), β-carotene (yellow pigment from *Dunaliella*) and astaxanthin (yellow to red pigment from *Haematococcus*) are gaining importance over synthetic colourants as they are nontoxic and non-carcinogenic (Dufossé et al., 2005).

As microalgae culture is eco-friendly and renewable, there is increase interest to use microalgae in the aquaculture industry as live feed for larviculture industry (Salvesen et al., 1999), such as premix for feed formulation/supplement and bioremediation for improvement of water quality (Khatoon et al., 2007), as well as production of healthy organisms and enhancement of animal colour (astaxanthin) (Guerin and Hosokawa, 2001). In addition, microalgae have been exploited for centuries for food and health care (Dufossé et al., 2005; Richa et al., 2011). Among the different phyla of microalgae, cyanobacteria are oxygenic photosynthetic prokaryotes showing large diversity in their morphological, physiological, ecological and biochemical characteristics.

The name "Cyanobacteria" comes from the colour of the bacteria (Greek κυανός (kayos) = blue) that include blue-green microalgae/blue-green bacteria. They are prokaryotic and have no organized structures such as plasmids and mitochondria; thus more closely resembling bacteria than eukaryotic microalgae (Skulberg et al., 1996). Cyanobacteria or blue-green microalgae are found in a variety of environments including marine environment, fresh water and arid areas where they are a major component of biological soil crusts. In addition, they are present in the aquatic environment as plankton (free floating) or periphyton (attached to surfaces), and at times they also form a valuable ubiquitous component of marine picophytoplankton that contributes significantly to the total carbon biomass and primary productivity of the ocean (Viskari and Colyer , 2003).

Cyanobacteria and microalgae posses a broad range of coloured components including carotenoids, chlorophyll and phycobiliproteins (Gantt, 1981). Phycocyanin pigment aggregates into particles referred to phycobilisomes, which are attached in regular array to the external surface of the thylakoid membrane and perform as the most important light harvesting pigment in cyanobacteria and red microalgae (Romay et al., 1998). Cyanobacteria with pigments (phycobilins) that reflect both blue and green wavelengths are often referred to as blue-green microalgae. These phycobilisomes are composed of phycobiliproteins categorized in family of hydrophilic, brilliantly coloured, stable fluorescent pigment proteins and covalently linked with linear tetrapyrrole prosthetic group (bilins) (Apt et al., 1995; Santiago-Santos et al., 2004).

Cyanobacterial phycobiliproteins are classified into three main groups namely, phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC) based on inherent colour and absorbance properties. Phycocyanin (blue) and phycoerythrin (red) are the two major natural pigments commercially utilized from microalgae. They are photosynthetic accessory pigments collectively called phycobillins. Cyanobacteria/blue-green microalgae are potential source for the commercial production of phycocyanin. In cyanobacteria, significant proportion of the total protein is contributed by the phycobiliproteins (Myers et al., 1956), which are located in granules attached to the photosynthetic membrane. Although their functions are not completely clear, the current theory is that the phycobiliproteins absorb light quanta and transfer the energy to the active chlorophyll molecules involved in photosystem. Phycobiliproteins are water soluble which cannot exist within the membrane like carotenoids. However, they aggregate to form clusters and adhere to the membrane and they are known as phycobilisomes. Phycoerythrin capture the light energy which is then passed on to the reaction centre chlorophyll pair most of the time via the phycocyanin and allophycocyanin (Gantt, 1980; Glazer and Wedemayer, 1995).

Phycocyanin is an oligomeric biliprotein with the linear tetrapyrrole chromophores identified as bilins covalently attached to the apoprotein by a thioether linkage. The molecular weight, position and intensity of the phycocyanin depend on the state of aggregation which is influenced by parameters such as solution pH, temperature, protein concentration and microalgal origin (Gantt, 1981; Huang and Berns, 1981; Berns and MacColl 1989; Houghton, 1996; MacColl 1998). In addition, Huang and Berns (1981) reported that phycocyanin in solution exist in the forms of monomer, trimer, hexamer and some higher aggregates.

The phycoerythrins are found in cyanobacteria (blue-green microalgae), cryptomonads, glaucophytes and red algae (Rhodophyta). Due to excellent spectroscopic properties, the fluorescent chromophore-containing proteins

are extensively applied in the generation of molecular fluorescent probes (Kronick and Grossman, 1983; Glazer and Stryer, 1984; Haugland, 2002). Phycobiliproteins are extensively used in industry, cosmetics and clinical or research immunological laboratories as labels for antibodies, receptors and other biological molecules (Telford et al., 2001). In addition, phycobiliproteins are used as colouring agents in cosmetics, dairy products, ice creams, jellies, in diagnostics, biomedical research and oxidative stress induced diseases as they are protein in nature and posses unique colour, fluorescence and antioxidant properties (Rimbau et al., 1999; Romay et al., 2003). Phycocyanin from *Spirulina* plays an important role in inducing apoptosis on HeLa cells (Li et al., 2006; 2009), enhancing wound healing (Madhyastha et al., 2008), retardation of platelet aggregation (Hsiao et al., 2005; Chiu et al., 2006) and eradication of cancer cells *in vitro* (Wang et al., 2009; Li et al., 2010). In the last six years, various researches have demonstrated anti-cancerous effect of phycocyanin from *Spirulina* and its ability to decrease 49 % proliferation of leukemia cell lines. In addition, phycocyanin also reduces 59 % proliferation of hepatocellular carcinoma cell line and significantly reduce the proliferation of HeLa cells *in vitro* compared to control cells (Capelli et al., 2010). Moreover, phycocyanin combination with selenium has an effective anti-proliferative agent against human melanoma cells and human breast cancer cells (MCF-7 cells) (Capelli et al., 2010).

1.2 Problem statements

Cancer has become the most deadly disease in the world which is characterized by an uncontrolled, abnormal growth of cells appearing in different parts of the body that can spread to other part of the body (Altman and Sarg, 1992). World Health Organization (WHO) reported that there are 7.6 million new cancer cases, with 52 % taking place in developing countries (Jemal et al., 2005; Shukla and Kalra, 2006). In Malaysia, health problems related to cancer are increasing and have become the fourth foremost causes of death. In 2006, it is estimated that 21,773 cancer cases were diagnosed in Peninsular Malaysia and registered in the National Cancer Registry (NCR). Out of these cases, 9,974 males and 11,799 females were diagnosed and the rate of incidence for all cancers regardless of sex was 131.3 per 100,000 in the year 2006. Breast, colorectal, lung, cervix and nasophyrynx cancer are the five most common cancers in Malaysia which are registered in NCR.

Liver cancer is ranked as 6th in Peninsular Malaysia and a total of 793 cases were diagnosed and registered with NCR involving 568 males and 225 males. According to NCR, the age standardized incidence rate was 4.9 per 100,000 for both sexes and for males and females were 7.2 and 2.7 per 100,000 populations, respectively. Hepatocellular carcinoma (HCC) or liver cancer is characterized by common malignancy with high metastasis rates (Barshack et al., 2010). The prevelance of HCC is rising all around the World (Capocaccia et al., 2007; Yuen et al., 2009). Most of the liver cancer

incidence was detected at the later stages of the disease and the recurrence of the disease is more than 70%, even after surgical resection (Guglielmi et al., 2008). Furthermore, survival rate of the disease is minimal with the systematic chemotherapeutic agents due to its toxic effects (Chen et al., 2011). There are some adverse effects (e.g. side effects, ineffective therapy) when synthetic drugs are used in treating cancer. Hence, there is a need for alternative natural compounds to inhibit the growth of cancer cells.

Phycobiliproteins have a wide range of commercial applications including anticancer, but its mechanism in inducing death of cancer cells is poorly understood. Therefore, the aim of this study is to isolate, characterize and produce phycobiliproteins from marine cyanobacteria and to demonstrate their effects on cancer cell lines. With these objectives, the current study was undertaken based on the hypothesis that crude pycobiliproteins extract may induce apoptosis in human hepatocellular carcinoma cell line (HepG2).

1.3 Objectives

The study was undertaken with the following objectives:

Main objective
To study the crude phycobiliproteins extract from potential cyanobacteria and evaluate their anti-cancerous activities on HepG2 cell line.

Specific objectives

- To isolate cyanobacteria with high phycobiliproteins contents and their characterization.
- To optimize the growth parameters for maximum production of phycobiliproteins from the selected marine periphytic cyanobacteria.
- To study the anticancer effects of phycobiliproteins crude extract of selective marine periphytic cyanobacteria on three different cancer cell lines namely HepG2, MDA, CEM-SS and Chang liver cell line *in vitro.*
- To investigate apoptotic induction and the mechanism for anticancer activity of phycobiliproteins crude extract from selected marine periphytic cyanobacteria on HepG2 cell line *in vitro*.

It was hypothesized that:
1. Marine cyanobacteria have higher content of phycobiliproteins compared to freshwater cyanobacteria.
2. Crude phycobiliproteins extract from cyanobacteria are cytotoxic towards HepG2 cancer cell line but less toxic towards Chang liver cell line.
3. Crude phycobiliproteins extract induces apoptosis and cell cycle arrest in the HepG2 cell line.
4. Crude phycobiliproteins extract from cyanobacteria regulate the induction of apoptotic genes to induce anti-proliferation of HepG2 cell line.

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BIODATA OF THE STUDENT

The author, Hasina Begum, daughter of Md. Matiur Rahman and Halima Khatoon, was born on 1st Janury 1982, at Mymensingh, District in Bangladesh. She obtained Secondary School Certificate (10 level) and Higher Secondary Certificate (12 level) from the Education Board of Dhaka 1996 and 1998, respectively. In 2002, she graduated in Doctor of Veterinary Medicine (DVM) from Bangladesh Agricultural University Mymensingh and got first class. From the same Institute, in 2002 she pursued her Master of Science in Microbiology and stood first class and a research thesis entitled 'Enteropathotypic characterization of *Escherichia coli* isolated from diarrhoeic calves and their antibiogram study." She has published several research papers as an author and co-author in international and local journals.

LIST OF PUBLICATION

1. H. Begum, F. M. Yusoff, S. Banerjee, H. Khatoon & M. Shariff. 2014. Availability and utilization of pigments from microalgae. Critical Reviews in Food Science and Nutrition. (In press).

2. H. Sulaiman Rahman, A. Rasedee, A. Bustamam Abdul, H. Hassan Othman, H. Begum, S. Wei Tan, S. Keong Yeap. 2014. Antileukemic Effect of Zerumbone Loaded Nanostructured Lipid Carrier on Murine Leukemic (WEHI-3B) Model. Journal of Nanomedicine. (Submitted)

3. H. Begum, M. P. Siddique, S. J. Shammi, M. T. Rahman, and M. S. R. Khan. 2009. Enteropathotypic characterization of *Escherichia coli* isolated from diarrhoeic calves and their antibiogram study. International Journal of Bioresearch 10: 01-05.

4. S. J. Shammi, M. P. Siddique, H. Begum, M. Rahman, M. S. R. Khan and K. A. Choudhury. 2009. Effects of various medicinal plant products on the growth of bacterial flora isolated from guinea pigs. International Journal of Bioresearch 10: 27-31.

Seminars/Conferences/Workshop Attended

1. Infrared Imaging System- The Path to Qualitative, Multiplex western Blot Detection Seminar - Organized by Laboratory of Laboratory of Vaccines and Immunotherapeutics Institute of Bioscience. Held on 12-13th April, 2012.

2. Enzyme purification Workshop “From Crude to Pure” Organized by Laboratory of Laboratory of Vaccines and Immunotherapeutics Institute of Bioscience. Held on 12-13th April, 2012.

3. Fundamental Techniques in Animal Studies Workshop- Organized by Institute of Bioscience. Held on 11-13th October 2011.

4. Flow Cytometry: Application in Biological cells workshop- Organized by Laboratory of Molecular Biomedicine, Institute of Bioscience. Held on 27-28th July, 2010.

5. Animal cell culture workshop – Organized by Institute of Bioscience. Held on 21- 23th June, 2010.

6. Workshop on methodological issues in research workshop: approaches in data analysis - Organized by Veterinary Medicine, Universiti Putra Malaysia. Held on 27- 29th July, 2010.

7. Workshop on SPSS – Organized by IDEC, Universiti Putra Malaysia. Held on 7- 8th October, 2010.

8. Scientific Writing Seminar –Organized by Scholl of Graduate studies University Putra Malaysia on 4th August 2010.

9. Effective Writing Workshop organized by School of Graduate Study Universiti Putra Malaysia 5-6th September, 2009.

10. Conference presentation workshop organized by School of Graduate Study Universiti Putra Malaysia 22-23rd August 2009.

11. Technique and Skill Improvement using use 'integrated' SAS and Excel packages in data handling, statistical analysis and the analysis output and presentation organized by School of Graduate Study Universiti Putra Malaysia 15-16th August, 2009.

Working Experience:

1. Worked as a laboratory executive officer in a multinational company C. P. Bangladesh Ltd. Duration: February, 2007 – May 2009.

2. Experienced in laboratory management, sample collection, preservation and analysis and handling laboratory equipment used for environmental analysis.

Training and Field Visit Attended

1. Training experience in internship program 2004 for 6(six) months in different Organization of Bangladesh as research fellow arranged by Faculty of Veterinary science, Bangladesh agricultural university and "Department Of Livestock" of Peoples Republic of Bangladesh.

2. Study tour with a view to observed different dairy and poultry farm in different district of Bangladesh. I have also visited Central Veterinary Hospital in Dhaka and different Livestock Research Institute in Bangladesh as a part of Study Tour.