UNIVERSITI PUTRA MALAYSIA

SYNTHESIS AND CONTROLLED RELEASE PROPERTIES OF ANTICANCER DRUG NANODELIVERY SYSTEMS BASED ON PROTOCATECHUIC AND CHLOROGENIC ACIDS USING LAYERED HYDROXIDE INORGANIC HOSTS

FARAHNAZ BARAHUIE

FS 2015 75

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By

FARAHNAZ BARAHUIE

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

February 2015

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DEDICATION

I dedicate my thesis to my patient mother, who endured being far from me and to my brothers, nephew and niece.

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

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February 2015

Chairman: Professor Mohd Zobir Bin Hussein, PhD
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Nanoscience and nanotechnology are the design, characterization, production, and application of structures by controlled manipulation of size and shape at the nano scale (1-100nm) that produces new materials with unique and superior properties compared to their counter regime parts.

Anticancer drugs are used to treat malignancies or cancerous cell growths by preventing the development, maturation and proliferation of neoplasms. These drugs could destroy cancer cells but also have many side effects, because treatment destroys the body's normal cells in addition to cancerous cells.

The use of nanotechnology in medicine or the so called nanomedicine has created new, safe and effective method of delivering anticancer drugs which releases the drug in predetermined time, reduces the undesired fluctuation of the drug levels in blood circulation, decreased duration therapy, improved patient compliance due to less frequent of drug administration, increases the drug solubility and reduces adverse side effects while enhanced therapeutic response.

Layered hydroxides (LHs) are considered as promising new generation materials which can be used as hosts to construct organic-inorganic nanocomposites. These inorganic nanomaterials are composed of nanolayers with two-dimensional infinite layers similar to that of mineral brucite, $Mg(OH)_2$ and offer wide applications in diverse areas.

These inorganic nanolayer materials have extensively been used as unique delivery nanocarrier for anticancer drugs. In this study, protocatechuic acid (PA) and chlorogenic acid (CA) were intercalated into zinc layered hydroxide, magnesium-aluminium and zinc-aluminium layered double hydroxides in order to increase the therapeutic efficiency. Protocatechuic acid was intercalated into

magnesium-aluminium-layered double hydroxide at Mg^{2+}/Al^{3+} ratio of 4 (R=4) to form its nanocomposites and were synthesized by direct co-precipitation and ion exchange methods, labelled as PAND and PANE, respectively. This drug was also intercalated into zinc-aluminium-layered double hydroxide (R=2) to form its nanocomposites by direct method (PZAC) and nanocomposite by ion-exchange method (PZAE). Furthermore, protocatechuic acid was also intercalated into zinc layered hydroxide in order to obtain protocatechuic acid-zinc layered hydroxide nanocomposite (PAN).

Chlorogenic acid was intercalated into magnesium-aluminium-layered double hydroxide (R=4) using direct co-precipitation and ion-exchange method for the formation of a nanocomposite by direct method (CMAC) and a nanocomposite by ion-exchange method (CMAE). This drug was also encapsulated into zinc-aluminium-layered double hydroxide (R=4) to form a nanocomposite using direct method (CZAC) and a nanocomposite using ion-exchange method (CZAE). Moreover, chlorogenic acid was also intercalated into zinc layered hydroxide to form chlorogenic acid-zinc layered hydroxide nanocomposite (CAN). All the nanocomposites exhibit the properties of mesoporous-type material, with greatly enhanced thermal stability of the intercalated drug compared to its free counterpart. X-ray diffraction pattern showed expansion of the basal spacing of the nanocomposites due to the monolayer arrangement of drug anions between the interlayer lamellae of the layered hydroxides. Furthermore, the FTIR result of nanocomposites corroborated the strong interaction between the guest and inorganic host in these nanocomposite materials.

The release of drugs from the nanocomposites occurred slowly and in a sustained manner, so that it was 4000 (83%), 7500 (59%), 6706 (79%), 8612 (86%), 7001 (87%), 7088 (79%), 5141 (78%), 11470 (88%), 6610 (75 %) and 9660 (99%) minutes at pH 7.4 compared to 900 (98%), 1000 (85%), 2391 (91%), 3068 (98%), 1592 (95%), 3855 (93%), 3044 (89%), 1435 (99%), 1350 (97%), 4800 (88%) minutes at pH 4.8 from PAND, PANE, PZAC, PZAE, PAN, CMAC, CMAE, CZAC, CZAE and CAN nanocomposites, respectively, indicating that the nanocomposites are potential drug controlled release formulations.

In vitro cytotoxicity assay studies showed that PAND and PANE nanocomposites had greater suppression effect on human breast cancer (MCF-7) and human cervical cancer (HeLa) cell lines compared to free protocatechuic acid, without toxicity on 3T3 normal fibroblust cell after 72 hours incubation. In addition, the cell viability test of magnesium-aluminium-layered double hydroxide indicated the absence of toxicity toward normal fibroblast (3T3) cells and both cancer cell lines.

PZAE and PZAC nanocomposites exhibit better cytotoxicity effect than the free PA in human cervical (HeLa), colorectal (HT29) and human liver (HepG2) cancer cells. However, they did not show cytotoxicity in 3T3 normal fibroblast cells, after 72 hours treatment. The anticancer activity of PZAE and PZAC was

more extensive particularly in HepG2 and HT29 cancer cell lines. In addition, increasing of zinc-aluminium-layered double hydroxide (R=2) concentration lead to killing the 3T3 normal fibroblast and cancer cells.

The inhibition of cancer cell growth in cervical adenocarcinoma (HeLa), liver hepatocarcinoma (HepG2) and colorectal adenocarcinoma (HT29) cancer cells was higher for the PAN nanocomposite than for free protocatechuic acid. In this work, the tumor growth suppression of PAN was more prominent in HT29 and HepG2 cell lines. Furthermore, the high concentration exposure of zinc oxide suppressed cell growth in 3T3 normal fibroblast and cancer cell lines.

The nanocomposites, CMAE and CMAC, showed better cytotoxicity properties against various human cancer cells namely human breast (MCF-7), human cervical (HeLa) and human lung (A549) cancer cells particularly the liver cancer cells (HepG2) in a dose-dependent manner, compared to free chlorogenic acid. In addition, these nanocomposites did not produce any toxicity behavior in normal fibroblast cells.

CZAE and CZAC nanocomposites possess significant anti-tumor properties in cervical, HeLa and breast, MCF-7 cancer cells particularly liver cancer, HepG2 and lung cancer, A549 cells in a dose-dependent manner compared to chlorogenic acid without showing any toxicity on normal fibroblast cells. In addition, the CZAC exerted better cytotoxic effects than the CZAE compound, particularly in liver cancer cells. However, there was no inhibition in cell proliferation of normal fibroblast and cancerous cells when they were exposed to zinc-aluminium-layered double hydroxide (R=4).

CAN nanocomposite indicates an increased in cytotoxicity compared to the free form of chlorogenic acid in MCF-7 and HepG2 cancer cell lines tested, particularly in HepG2 liver cancer cell lines in a dose-dependent manner without a toxic effect on normal fibroblast, HeLa and A549 cancer cells.

In essence, all nanocomposites showed the sustained release properties and can therefore be used as controlled-release formulations and the introduction of zinc layered hydroxide, magnesium-aluminium and zinc-aluminium layered double hydroxides layers in protocatechuic and chlorogenic acid improved the anticancer efficacy and selectivity feature of the compounds.

All the works presented in the thesis have been published in the journals of the international repute, which reflect the quality of this research work.

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

SINTESIS DAN SIFAT-SIFAT LEPASAN TERKAWAL SISTEM NANO-PENGHANTARAN DRUG ANTI KANSER BERDASARKAN ASID PROTOKATEKUIK DAN ASID KLOROGENIK MENGGUNAKAN PERUMAH HIDROKSIDA BERLAPIS TAKORGANIK

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Nanosains dan nanoteknologi adalah reka bentuk, pencirian, penghasilan, dan penggunaan struktur oleh manipulasi saiz terkawal dan bentuk terkawal pada skala nano (1-100nm) yang menghasilkan bahan-bahan baru dengan ciri-ciri unik dan unggul berbanding dengan rakan mereka.

Ubat anti-kanser yang digunakan untuk merawat tumor atau ketumbuhan sel barah dengan menghalang pertumbuhan, kematangan dan percambahan neoplasma. Ubat-ubatan ini boleh memusnahkan sel-sel kanser tetapi juga mempunyai banyak kesan sampingan, kerana rawatan akan juga memusnahkan sel-sel normal badan selain daripada sel-sel kanser.

Penggunaan teknologi nano dalam perubatan, yang juga dikenali sebagai nanoperubatan telah mencipta kaedah baru yang selamat dan berkesan dalam menyampaikan ubat-ubatan antikanser, yang membebaskan dadah anti kanser ini dalam masa yang telah ditetapkan, mengurangkan turun naik tahap dadah antikanser yang tidak diingini dalam edaran darah, menurunkan jangka masa terapi, pesakit lebih patuh kerana pengambilan dadah kurang kerap, meningkatkan kelarutan dadah dan mengurangkan kesan buruk disamping meningkatkan tindak balas terapeutik.

Hidroksida berlapis (LHs) dianggap sebagai bahan generasi baru yang memberang sangkan untuk digunakan sebagai perumah untuk menjana nanokomposit organik-organik. Nanobahan bukan organik ini terdiri daripada nanolapisan dengan lapisan tak terhingga dua dimensi sama dengan mineral brucite, Mg $(OH)_2$ yang dapat menawarkan aplikasi yang luas dalam pelbagai bidang.

Hidroksida berlapis telah meluas digunakan sebagai pembawa nano yang unik untuk ubat-ubatan anti-kanser. Dalam kajian ini, asid protokatekuik dan asid klorogenik telah diinterkalasi ke dalam lapis zink berlapis hidroksida, magnesium aluminium dan zink aluminium berlapis dua hidroksida untuk meningkatkan kecekapan terapeutik. Asid protokatekhuik telah diinterkalasi ke dalam lapisan magnesium/aluminium hidroksida berlapis dua pada nisbah Mg^{2+} / Al^{3+} 4 (R = 4) untuk membentuk PAND dan nanokomposit PANE disintesis melalui kaedah pemendakan bersama dan pertukaran langsung ion. Ubat ini juga diinterkalasi ke dalam lapisan zink/aluminium hidroksida berlapis dua (R = 2) untuk membentuk nanokomposit PZAC dengan kaedah langsung dan nanokomposit PZAE dengan kaedah pertukaran ion. Tambahan pula, asid protokatekhuik telah diinterkalasi ke dalam lapisan zink hidroksida berlapis untuk mendapatkan nanokomposit PAN.

Asid klorogenik telah diinterkalasi ke dalam lapisan magnesium-aluminium hidroksida-berlapis dua (R = 4) dengan menggunakan kaedah pemendakan langsung dan kaedah pertukaran ion untuk pembentukan masing-masing CMAC dan CMAE. Ubat ini juga terkandung dalam lapisan zink/aluminium hidroksida berlapis dua (R = 4) untuk membentuk nanokomposit CZAC menggunakan kaedah langsung dan nanokomposit CZAE menggunakan kaedah pertukaran ion. Selain itu, asid klorogenik telah diinterkalasi ke dalam lapisan zink hidroksida berlapis untuk membentuk CAN.

Semua nanokomposit mempamerkan sifat-sifat daripada bahan mesoporous, dengan kestabilan terma dadah diinterkelasi menunjukkan peningkatan yang tinggi berbanding dengan rakan bebas mereka. Corak pembelauan Sinar-X menunjukkan jarak perkembangan basal daripada nanokomposit disebabkan oleh susunan lapisan mono anion ubat antara kepingan lapisan dalam di hidroksida berlapis. Tambahan pula, hasil FTIR bagi nanokomposit menyokong interaksi kuat antara tetamu dan tuan rumah bukan organik dalam bahan-bahan nanokomposit tersebut.

Pembebasan ubat dari pada nanokomposit berlaku secara perlahan-lahan dan dengan cara yang mampan, supaya ia adalah 4000 (83%), 7500 (59%), 6706 (79%), 8612 (86%), 7001 (87%), 7088 (79 %), 5141 (78%), 11470 (88%), 6610 (75%) dan 9660 (99%) minit pada pH 7.4 berbanding dengan 900 (98%), 1000 (85%), 2391 (91%), 3068 (98%), 1592 (95%), 3855 (93%), 3044 (89%), 1435 (99%), 1350 (97%), 4800 (88%) minit pada pH 4.8 daripada masing-masing PAND, PANE, PZAC, PZAE, PAN, CMAC, CMAE, CZAC, CZAE dan CAN nanokomposit. Ini menunjukkan bahawa nanokomposit menunjukkan perlepasan ubat terkawal yang berpotensi.

Dalam kajian vitro asei sitotoksisiti, nanokomposit PAND dan PANE telah menunjukkan pengurangan yang lebih ke atas MCF-7 kanser payudara dan HeLa serviks garisan sel kanser manusia berbanding asid protokatekuik bebas, tanpa racun ke 3T3 sel fibroblust normal selepas inkubator 72 jam. Di samping itu, ujian daya maju sel magnesium-aluminium-lapis dua hidroksida menunjukkan

ketiadaan sifat toksik keatas 3T3 fibroblast biasa dan kedua-dua bahagian sel kanser.

PZAE dan PZAC nanokomposit mempamerkan kesan sitotoksiti lebih baik daripada PA bebas bagi HeLa (pangkal rahim manusia), HT29 (kolorektal) dan HepG2 (hati) sel-sel kanser, tetapi, tidak menunjukkan sitotoksisiti bagi sel-sel fibroblast 3T3 biasa, selepas 72 jam rawatan. Aktiviti anti-kanser daripada PZAE dan PZAC lebih luas terutamanya dalam HepG2 dan HT29-bahagian sel kanser. Selain itu, peningkatan kepekatan zink aluminium lapis dua hidroksida (R = 2) dapat membunuh sel kanser, 3T3 fibroblast dan sel-sel normal.

Perencatan pertumbuhan sel kanser bagi HeLa adenokarsinoma serviks, HepG2 hepatocarcinoma (hati) dan HT29 (kolorektal) adalah lebih tinggi untuk nanokomposit PAN daripada asid protokatekuik bebas. Dalam kajian ini, pengurangan pertumbuhan tumor daripada PAN adalah lebih menonjol dalam bahagian sel HT29 dan HepG2. Tambahan pula, pendedahan kepekatan zink oksida yang tinggi mengurangkan pertumbuhan sel dalam 3T3 fibroblast dan sel kanser normal.

Nanokomposit, CMAE dan CMAC, menunjukkan ciri-ciri sitotoksiti yang lebih baik terhadap pelbagai sel-sel kanser manusia iaitu MCF-7 (payudara), HeLa (pangkal rahim) dan A549 (paru-paru) sel-sel kanser terutamanya sel-sel kanser hati, HepG2 dan bergantung kepada dos berbanding dengan asid klorogenik bebas. Di samping itu, nanokomposit ini tidak menunjukkan apa-apa ciri-ciri toksik dalam sel-sel fibroblast.

nanokomposit CZAE dan CZAC memiliki sifat-sifat anti-tumor yang ketara dalam sel-sel kanser HeLa dan MCF-7 terutamanya kanser hati, HepG2 dan kanser paru-paru, A549 yang bergantung kepada dos berbanding asid klorogenik yang tanpa menunjukkan sebarang keracunan ke sel-sel fibroblast biasa. Di samping itu, CZAC yang dikenakan kesan sitotoksik lebih baik daripada sebatian CZAE, terutamanya dalam sel-sel kanser hati. Walau bagaimanapun, tiada perencatan dalam percambahan sel fibroblast normal dan sel-sel kanser apabila mereka didedahkan terhadap zink aluminium lapis dua hidroksida (R = 4). nanokomposit CAN menunjukkan peningkatan dalam sitotoksisiti berbanding dengan bentuk bebas asid klorogenik bagi sel kanser MCF-7 dan HepG2 yang telah diuji, terutamanya dalam HepG2 (hati) ia bergantung kepada dos tanpa kesan toksik pada fibroblast biasa, HeLa dan sel-sel kanser A549.

Pada dasarnya, semua nanokomposit menunjukkan sifat-sifat kelegaan berterusan dan dengan itu boleh digunakan sebagai rumusan lepasan terkawal dan pengenalan berlapis zink hidroksida, magnesium aluminium dan zink aluminium berlapis hidroksida lapisan berganda dalam asid klorogenik protokatekhuik memperbaiki keberkesanan anti-kanser dan ciri pemilihan sebatian.

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I certify that a Thesis Examination Committee has met on 18 February 2015 to conduct the final examination of Farahnaz Barahuie on her thesis entitled "Synthesis and Controlled Release Properties of Anticancer Drug Nanodelivery Systems Based on Protochatechuic and Chlorogenic Acids using Layered Hydroxide Inorganic Hosts" 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 Doctor of Philosophy.

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LIST OF ABBREVIATIONS

LHs	Layered Hydroxides
LDH	Layered Double Hydroxide
LHS	Layered Hydroxide Salt
ZLH	Zinc Layered Hydroxide
Mg/Al-LDH	Magnesium/Aluminum Layered Double Hydroxide
Zn/Al-LDH	Zinc/Aluminum Layered Double Hydroxide
PA	Protocatechuic Acid
CA	Chlorogenic Acid
PAND	Protocatechuic Acid-Magnesium/Aluminum Layered Double Hydroxide Nanocomposite Synthesized by Co-precipitation Method
PANE	Protocatechuic Acid-Magnesium/Aluminum Layered Double Hydroxide Nanocomposite Synthesized by Ion-exchange Method
PZAC	Protocatechuic Acid-Zinc/Aluminum Layered Double Hydroxide Nanocomposite Synthesized by Co-precipitation Method
PZAE	Protocatechuic Acid-Zinc/Aluminum Layered Double Hydroxide Nanocomposite Synthesized by Ion-exchange Method
PAN	Protocatechuic Acid-Zinc Layered Hydroxide Nanocomposite
CMAC	Chlorogenic Acid-Magnesium/Aluminum Layered Double Hydroxide Nanocomposite Synthesized by Co-precipitation Method
CMAE	Chlorogenic Acid-Magnesium/Aluminum Layered Double

	Hydroxide Nanocomposite Synthesized by Ion-exchange Method
CZAC	Chlorogenic Acid-Zinc/Aluminum Layered Double Hydroxide Nanocomposite Synthesized by Co-precipitation Method
CZAE	Chlorogenic Acid-Zinc/Aluminum Layered Double Hydroxide Nanocomposite Synthesized by Ion-exchange Method
CAN	Chlorogenic Acid-Zinc Layered Hydroxide Nanocomposite
PXRD	Powder X-ray Diffraction
FTIR	Fourier Transform Infrared Spectroscopy
CHNS	Carbon, Hydrogen, Nitrogen, and Sulfur
ICP	Inductively Coupled Plasma Atomic Emission Spectrometry
TGA/DTG	Thermogravimetric and Differential Thermogravimetric
FESEM	Field Emission Scanning Electron Microscope
UV-VIS	Ultraviolet-Visible Spectrophotometer
TEM	Transmission Electron Microscopy
BET	Brunauer, Emmet and Teller
BJH	Barret–Joyner–Halenda

CHAPTER 1

INTRODUCTION

1.1 Background of study

Nanoscience and nanotechnology, as the most fruitful scientific advances in the recent century are the waves of future, and investment in this technology has continued to increase (Oh et al., 2006a).

They are a new domain in science and engineering with ability of work in atomic and molecular levels to create products with at least one dimension in nanoscale or one billionth of a meter, and incredible unusal properties and highest performance (Giersig and Khomutov, 2006; Alexis et al., 2008).

The resulting nano-structured materials have a large surface area to volume ratio and their physico-chemical properties, such as friction and interaction with other molecules, are distinct from equivalent materials at a larger scale (Ferrari, 2005).

This advanced technology provides significant scientific and technological advances in various fields including optics (Groblacher et al., 2013), electronics (Beaumont, 1996), medicine (Boisseau and Loubaton, 2011), sensors (Zhang et al., 2014a), water treatment (Elkhattabi et al., 2013), space (Diez et al., 2013), food industry (Chellaram et al., 2014), air pollution remediation (Yunus et al., 2012), fuel, energy (Abdin et al., 2013), flame retardants (Xu et al., 2012), catalysis (Baikousi et al., 2013) and agriculture (Bashi et al., 2013).

The first concept of nanotechnology was presented by an American physicist, Richard Feynman in 1959 (Neumann, 1966), while the application of nanotechnology on realm of medicine gained considerable attention and enthused scientists at the end of 1960, that the enormous efforts and research was started in this area (Boisseau and Loubaton, 2011).

Nanomedicine, i.e. the medical application of nanotechnology enhanced advances in detection, diagnosis, monitoring, prevention and treatment of diseases using nanoscale materials to deliver drugs to specific cells or diseased sites. It has potential to play great impact on improving health and prolonging life due to interesting possibilities for enhancing drug delivery (Boisseau and Loubaton, 2011).

The conventional drug administration in cancer therapy does not provide the efficient therapy in cancer diseases due to the rapid release of drugs, with no control over release rate and fluctuation in drug concentration levels in blood flow, multi-dose drug administration, low drug water solubility, drug

degradability and damage to normal and healthy cells (Shen et al., 2010; Trikeriotis andGhanotakis, 2007).

Cancer nanotechnology is a revolution in cancer therapy by creation of the biocompatible nanocarriers for anticancer drug delivery systems which improve the properties of cancer cell targeting and maintain steady state therapeutic levels of drugs over an extended period of time. Therefore, this technology is the main solution for cancer disease treatment (Misra et al., 2010; Larina et al., 2005).

Layered hydroxides have shown great potential as nanovehicle for delivering anticancer drugs and will be discussed in details in the review paper.

In this work, protocatechuic and chlorogenic acids were intercalated into the lamella of layered hydroxides to obtain new nanocomposites for the formation of the smart anticancer drugs with controlled release and cancer cell targeting properties, which will maintain constant drug concentration at therapeutic level in blood circulation and minimize the adverse effects. These factors would improve the cancer patients compliance to the treatment and would shorten the treatment duration.

1.2 Problem statement

Cancer is a global issue and continues to be the main cause of deaths in the world. According to the World Health Organization report, the total cancer cases in the world will increase to more than double in 2030. It is an incomprehensible and complex disease (Boyle and Levin, 2009; Kawasaki and Player, 2005). Current anticancer drugs; antimetabolites, natural products, alkylating agent have not provided the effective therapy because of degradation, drug resistance, short plasma half-life, low water solubility and inability to discriminate between normal and cancer cells that this indiscriminate action leads to the adverse side effects (Yang et al., 2006; Nie et al., 2007).

Protocatechuic acid or 3, 4-dihydroxybenzoic acid (PA) (Figure 1.1) is a natural phenolic acid isolated from a number of popular medicinal plants (Tseng, 1998; Jürgenliemk and Nahrstedt, 2002; Ellnain-Wojtaszek, 1997). Previous studies have shown that protocatechuic acid has an amazing antioxidant property which terminates the attacks of free radicals through its scavenging and chelating activities (Li et al., 2011). Further, protocatechuic acid demonstrates other pharmacological activities such as anticancer, (Yin et al., 2009) antitumor (Nakamura et al., 2000) antimutagenic (Stagos et al., 2006) antibacterial (Liu, W. H. et al., 2008) anti-inflammatory (Liu et al., 2002) antigenotoxic (Anter et al., 2011) cardioprotective, and chemopreventive (Tanaka et al., 2011). It has been shown to cause apoptotic effects in the treatment of several types of cancer cells, including human leukemia (pa-2000-leukemia), cervix, breast, lung, liver, and prostate. It induces cell death via increasing DNA fragmentation, decreasing mitochondrial membrane potential, lowering Na-K-ATPase activity, and

elevating caspase-3 and caspase-8 activities in cancerous cells. But, protocatechuic acid has low water solubility and very short plasma half-life and is released very fast (Yin et al., 2009).

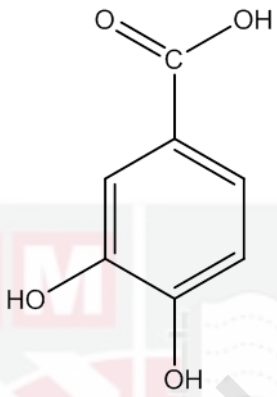

Figure 1.1 Structure of protocatechuic acid

Chlorogenic acid (CA) (Figure 1.2), is a naturally occurring organic compound that is well known for its biological activities, including its antioxidant activity via free radical scavenging and metal ion chelation, which functions to prevent oxidative damage (Yen et al., 2005; Paganga et al., 1999; Kono et al., 1998). It also has anti-HIV, (McDougall et al., 1998) anti-inflammatory (Krakauer, 2002), anti-carcinogenic (Kasai et al., 2000) and antitumor activities (Shimizu et al., 1999; Matsunaga et al., 2002). CA induces apoptotic cell death via a H_2O_2-mediated oxidation mechanism, DNA fragmentation and activation of caspases (Matsunaga et al., 2002).

However, CA has low water solubility and will be decomposed at 60 °C. This anticancer agent has short half-life and its high dose causes some unwanted effects such as, vomiting, asthma, pruritus, shock, diarrhea, liver and kidney injury, and even death (Du et al., 2013).

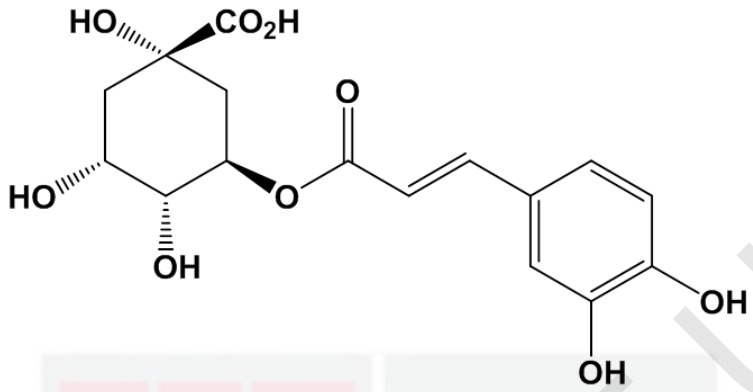

Figure 1.2 Structure of chlorogenic acid

Therefore, development of new anticancer agent is critical and urgent requirement. Cancer nanotechnology is a field of science which provides new avenues that conventional technology is not able to make especially in the prevention, diagnosis and therapy of cancer diseases. It offers the design of nanoscale materials and devices with unique therapeutic properties that increase the solubility, half-life and bioavailability of attached drugs in order to deeply infiltrate tumors with a high level of specificity and administer novel therapies to curb the problems of drug delivery in cancer (Nie et al., 2007; Bharali et al., 2011).

The inorganic nanolamellar solids, layered hydroxides have demonstrated their suitability for different applications in the pharmaceutical industry and attract considerable attention for encapsulation and stabilization of anticancer drugs due to their unique properties for anticancer drug delivery (Oh et al., 2009a; Carja et al., 2007; Rives et al., 2009; Li et al., 2009).

CA and PA with having a carboxylic functional group and negative charge can be easily intercalated into the interlayer gallery of layered hydroxides with positively charged layers.

The intercalation of protocatechuic and chlorogenic acid into the interlayer lamella of layered hydroxides leads to the sustained release of intercalated PA and CA and this controlled release system can enhance anticancer property of the drugs. In addition, intercalated PA and CA will have greater thermal stability and water solubility. Moreover, favourable cell endocytosis and better cancer cell targeting property can be obtained using this nanocomposite system because cell with the negative charge wall prevents from cellular uptake of drug with negative charge because of repulsion between negative charge of cell wall and negative charge of the drug. The intercalation of protocatechuic and chlorogenic acid into

the interlayer lamella of layered hydroxides with positive charge neutralize the negative surface charge of PA and CA anions.

1.3 Objectives

The main objectives of this study are:

a) synthesis of protocatechuic acid-magnesium-aluminum layered double hydroxide, protocatechuic acid-zinc-aluminum layered double hydroxide and protocatechuic acid-zinc layered hydroxide nanocomposites.
b) synthesis of chlorogenic acid-magnesium-aluminum layered double hydroxide, chlorogenic acid-zinc-aluminum layered double hydroxide and chlorogenic acid-zinc layered hydroxide nanocomposites.
c) characterization of all the samples using different analytical techniques such as X-ray diffraction (XRD), fourier transformed infrared (FTIR) spectroscopy, CHNS analysis, ultraviolet-visible (Uv/Vis) spectroscopy, thermogravimetric and differential thermogravimetric analysis, field emission scanning electron microscopy (FESEM), inductively coupled plasma atomic emission spectrometry, transmission electron microscopy (TEM) and surface area and porosity analyzer (ASAP).
d) controlled release studies of the drugs from the nanocomposites and fitting them to various kinetic models.
e) studies of the drug molecules orientation in the layered hydroxide inorganic interlayers using ChemOffice software.
f) study the cytotoxicity of zinc oxide, zinc-aluminum and magnesium-aluminum layered double hydroxides, free protocatechuic acid and chlorogenic acid, and their nanocomposites on the 3T3 normal fibroblast, HeLa human cervical adenocarcinoma, MCF-7 human breast adenocarcinoma, A549 human lung adenocarcinoma epithelial, HepG2 human liver hepatocellular carcinoma and HT29 human colorectal adenocarcinoma cell lines.

1.4 Significance of the study

The presented studies in the thesis were performed to discover new anticancer agent with controlled release property and cancer cell targeting features for active anticancer agents; protocatechuic and chlorogenic acid. The protocatechuic and chlorogenic acid nanocomposite formulations prolong the release of encapsulated drug, maintain constant the drug concentration at therapeutic level in blood stream, minimize potential of adverse effects and enhance anticancer properties by targeting tumor cells than normal cells.

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LIST OF PUBLICATIONS

A. PUBLICATIONS

1. **Farahnaz Barahuie**, Mohd Zobir Hussein**,** Sharida Fakurazi and Zulkarnain Zainal. Synthesis of protocatechuic acid-zinc/aluminium layered double hydroxide nanocomposite as an anticancer nanodelivery system. *Journal of Solid State Chemistry*. 2015; 221: 21-31.

2. **Farahnaz Barahuie**, Mohd Zobir Hussein, Sharida Fakurazi, Zulkarnain Zainal. Development of drug delivery systems based on layered hydroxides for nanomedicine. *International Journal of Molecular Sciences*. 2014; 15: 7750-7786.

3. **Farahnaz Barahuie**, Mohd Zobir Hussein, Palanisamy Arulselvan, Sharida Fakurazi, Zulkarnain Zainal. Drug delivery system for an anticancer agent, chlorogenate-Zn/Al-layered double hydroxide nanohybrid synthesised using direct co-precipitation and ion exchange methods. *Journal of Solid State Chemistry*. 2014; 217: 31-41.

4. **Farahnaz Barahuie**, Mohd Zobir Hussein**,** Shafinaz Abd Gani**,** Sharida Fakurazi and Zulkarnain Zainal. Anticancer nanodelivery system with controlled release property based on protocatechuate-zinc layered hydroxide nanohybrid. *International Journal of Nanomedicine*. 2014; 9: 3137-3149.

5. **Farahnaz Barahuie**, Mohd Zobir Hussein**,** Samer Hasan Hussein-Al-Ali, Palanisamy Arulselvan**,** Sharida Fakurazi and Zulkarnain Zainal. Preparation and controlled release studies of a protocatechuic acid-magnesium/aluminium-layered double hydroxide nanocomposite. *International Journal of Nanomedicine*. 2013; 8: 1975-1987.

6. **Farahnaz Barahuie**, Mohd Zobir Hussein, Palanisamy Arulselvan, Sharida Fakurazi, Zulkarnain Zainal. Development of the anticancer potential of a chlorogenate-zinc layered hydroxide nanohybrid with controlled release property against various cancer cells. *Science of Advanced Materials*. 2013; 5: 1983-1993.

7. **Farahnaz Barahuie**, Mohd Zobir Hussein, Palanisamy Arulselvan, Sharida Fakurazi, Zulkarnain Zainal. Controlled in vitro release of the anticancer drug chlorogenic acid using magnesium/aluminium-layered double hydroxide as a nanomatrix. (Under review in Journal of Physics and Chemistry of Solids)

B. CONFERENCES AND WORKSHOPS

1. Workshop on Advanced Materials and Nanotechnology (WAMN 2014), Organized by Institute of Advanced Technology (ITMA), Faculty of Engineering , University Putra Malaysia

2. **Farahnaz Barahuie**, Mohd Zobir Hussein**,** Shafinaz Abd Gani, Sharida Fakurazi and Zulkarnain Zainal. Synthesis of protocatechuic acid-zinc/aluminium layered double hydroxide nanocomposite as an anticancer nanodelivery system. *Fundamental Science Congress (FSC) 2014*, University Putra Malaysia (UPM).

3. **Farahnaz Barahuie**, Mohd Zobir Hussein**,** Shafinaz Abd Gani, Sharida Fakurazi and Zulkarnain Zainal. Synthesis of protocatechuic acid-zinc/aluminium layered double hydroxide nanocomposite as an anticancer nanodelivery system. *International conference on chemical, biological and environmental science (ICCBES'14).* 2014, Kuala lumpur, Malaysia.

4. *The 6th nanotechnology cancer Asia-Pacific (NCAP) Network Meeting*. 2014.

5. **Farahnaz Barahuie**, Mohd Zobir Hussein**,** Sharida Fakurazi and Zulkarnain Zainal. Preparation anticancer drug-Zn/Al- layered double hydroxide nanocomposites by using protocatechuic acid as the active agent and study the controlled release property. *Fundamental Science Congress (FSC) 2013*, University Putra Malaysia (UPM).

6. Workshop on Advanced Materials and Nanotechnology (WAMN 2013), Institute of Advanced Technology (ITMA), University Putra Malaysia.

7. Workshop on animal cell culture June 2012, "Animal cell culture workshop 2012" Laboratory of Vaccines & Immunotherapeutic, Institute of Bioscience (IBS), University Putra Malaysia (UPM).

8. **Farahnaz Barahuie**, Mohd Zobir Hussein**,** Sharida Fakurazi and Zulkarnain Zainal. Synthesis and controlled release properties of antioxidant and anticancer drug, protocatchuic acid intercalated into Mg-Al layered double hydroxide. *Fundamental Science Congress (FSC) 2012*, University Putra Malaysia (UPM).

9. Workshop on Advanced Materials and Nanotechnology (WAMN 2011), Organized by Institute of Advanced Technology (ITMA), Faculty of Engineering & Faculty of Science, University Putra Malaysia.