



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF ZERUMBONE FROM ZINGIBER ZERUMBET ON
CERVICALCANCER-INDUCED FEMALE BALB/C MICE**

NIRMALA DEVI TAILAN

FPSK(M) 2007 9

06 AUG 2008

**EFFECTS OF ZERUMBONE FROM *ZINGIBER ZERUMBET* ON CERVICAL
CANCER-INDUCED FEMALE BALB/C MICE**

By

NIRMALA DEVI TAILAN

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

MARCH 2007



This thesis is especially dedicated to:

My loving grandmother & Aunty, who are infinitely precious to me,

&

Kaviyarasu and Vasanth, who have filled my life with joy and happiness,

&

My friends, who were there for me!



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

EFFECTS OF ZERUMBONE FROM *ZINGIBER ZERUMBET* ON CERVICAL CANCER INDUCED FEMALE BALB/C MICE

By

NIRMALA DEVI TAILAN

MARCH 2007

Chairman: Ahmad Bustamam Hj Abdul, PhD

Faculty : Medicine and Health Sciences

In the present study, the chemotherapeutic potential of zerumbone towards HeLa cell line and cervical cancer induced in female Balb/c mice were investigated. The chemotherapeutic potential of zerumbone was compared with cisplatin, a commercial drug used to treat cervical cancer. The cytotoxicity of both zerumbone and cisplatin towards HeLa cell line were determined using MTT assay. The findings showed that the IC_{50} value (\pm S.E.M) of zerumbone towards HeLa cell line was $11.3 \pm 0.2 \mu\text{M}$, whilst the IC_{50} value of cisplatin was $7.5 \pm 0.3 \mu\text{M}$. Both IC_{50} values for zerumbone and cisplatin fall within the very significant group based on the National Cancer Institute Standard. All the values are significant ($P < 0.01$). The HeLa cells were treated with IC_{50} concentration of zerumbone and cisplatin respectively for morphological analysis using inverted microscopy. The results showed significant growth retardation in HeLa



cells exposed to zerumbone and cisplatin at 24, 48 and 72 hours, whilst the control cells are well spread and confluent. Pregnant female Balb/c mice were exposed to Diethylstilbestrol (DES) at 13th to 18th day of gestation. The progeny of the DES-exposed mothers developed cervical intra-epithelial neoplasia. These progenies were divided into 4 groups and were either given treatment with normal saline, 8 mg/kg zerumbone, 16 mg/kg zerumbone and 10 mg/kg cisplatin. The mice were sacrificed following the treatments and their cervical tissues subjected to histological examination, TUNEL Assay and immunohistochemistry. The histological examination revealed that both zerumbone and cisplatin treatments were able to inhibit the progression of cervical dysplasia from becoming more severe dysplasia (CIN 3). In the mice treated with normal saline, the dysplasia had progressed to CIN 3 (severe dysplasia). The TUNEL assay micrographs showed that there was no apoptosis in the cervical tissue of the normal saline treated mice compared to the cervical tissue of mice treated with zerumbone and cisplatin, where abundant apoptotic cells were noticed. The levels of serum IL-6 were suppressed in mice treated with zerumbone and cisplatin. In contrast, mice treated with normal saline showed elevated level of serum IL-6. Immunohistochemistry study demonstrated that the production level of membrane bound IL-6 receptor had been suppressed by the treatment of zerumbone and cisplatin compared to mice treated with normal saline which had a higher concentration of membrane bound IL-6 receptors. This showed that both zerumbone and cisplatin act in a similar manner *in vivo* and *in vitro*. In conclusion, it is suggested that zerumbone, a plant derived compound, could be explored as a new anti cancer agent for treating cervical cancer in the future.



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

KESAN ZERUMBONE DARIPADA *ZINGIBER ZERUMBET* KE ATAS TIKUS MENCIT YANG TELAH DIINDUKSI DENGAN KANSER SERVIKS

Oleh

NIRMALA DEVI TAILAN

MAC 2007

Pengerusi: Ahmad Bustamam Hj Abdul, PhD

Fakulti : Perubatan dan Sains Kesihatan

Dalam kajian ini, keupayaan kemoterapeutik zerumbone terhadap sel selanjara kanser serviks (HeLa) dan tikus mencit Balb/c telah dikaji. Keupayaan terapeutik zerumbone dibandingkan dengan cisplatin, dadah komersial bagi merawat kanser serviks. Kesan sitotoksik zerumbone dan cisplatin terhadap sel HeLa dikaji dengan menggunakan asai MTT. Keputusan menunjukkan bahawa nilai IC_{50} (\pm S.E.M) bagi zerumbone ialah $11.3 \pm 0.2 \mu\text{M}$, manakala bagi cisplatin ialah $7.5 \pm 0.3 \mu\text{M}$. Nilai IC_{50} bagi zerumbone dan cisplatin adalah sangat signifikan berdasarkan garis panduan Institut Kanser Nasional. Semua nilai IC_{50} adalah signifikan ($P < 0.01$). Sel HeLa dirawat dengan zerumbone dan cisplatin dengan nilai IC_{50} masing-masing dan kajian morfologi dijalankan dengan bantuan mikroskopi inverted. Keputusan menunjukkan pertumbuhan sel pada jam ke-24, 48 dan 72 rawatan zerumbone dan cisplatin telah terbantut, manakala sel kawalan

tumbuh dengan sihat dan tersebar luas. Dalam kajian *in vivo*, tikus betina yang hamil diberi suntikan DES (Diethylstilbestrol) pada hari gestasi 13 hingga 18. Progeni betina bagi ibu-ibu yang terdedah kepada DES, telah menggalakan pertumbuhan CIN (Cervical Intra-epithelial Neoplasia). Tikus-tikus betina yang telah terdedah kepada DES dalam rahim ibu dibahagikan kepada 4 kumpulan dan diberi rawatan dengan normal saline, 8 mg/kg zerumbone, 16 mg/kg zerumbone dan 10 mg/kg cisplatin. Tikus-tikus tersebut dibunuh dan tisu serviksnya dianalisis melalui kaedah histologi, asai TUNEL dan kaedah immunohistokimia. Kajian histologi menunjukkan zerumbone dan cisplatin dapat menghalang pertumbuhan CIN 1 (displasia awal) kepada CIN 3 (displasia teruk) pada tisu serviks tikus. Manakala bagi tikus yang hanya diberi saline normal, penyakitnya (displasia) berkembang dan mencapai CIN 3. Hasil kajian TUNEL asai menunjukkan tiada sel apoptosis diperhatikan dalam tisu serviks tikus yang dirawat dengan normal saline, manakala tikus yang dirawat dengan zerumbone dan cisplatin menunjukkan sel apoptosis yang banyak. Paras IL-6 darah bagi tikus yang dirawat dengan zerumbone dan cisplatin adalah sangat rendah berbanding tikus yang dirawat dengan normal saline dimana paras IL-6 adalah amat tinggi. Kajian immunohistokimia menunjukkan penghasilan reseptor IL-6 menurun pada tikus yang dirawat dengan zerumbone dan cisplatin, manakala tikus yang dirawat dengan normal saline menunjukkan peningkatan dalam penghasilan reseptor IL-6 secara mendadak. Kedua-dua zerumbone dan cisplatin bertindak pada kadar yang sama dalam kajian *in vitro* dan *in vivo*. Kesimpulannya zerumbone, sebatian semulajadi daripada tumbuhan dicadangkan untuk digunakan bagi menghasilkan ubatan alternatif untuk merawat kanser serviks pada masa hadapan.



ACKNOWLEDGEMENTS

I would like to take this opportunity to thank all those who gave great support to me while doing the project. First of all, I would like to express my sincere gratitude and special appreciation to my supervisor, Dr. Ahmad Bustamam Hj. Abdul and co-supervisor, Associate Professor Dr. Muhd. Nazrul Hakim Abdullah for their endless support, advice, guidance and encouragement throughout the completion of this thesis as partial fulfillment of the requirement for the degree of Master's Science (Pharmacology and Toxicology)

I would also like to express my deepest appreciation and sincere gratitude to Prof. Nordin Lajis for allowing me to use the phytochemical laboratory, Institute of Bioscience, Universiti Putra Malaysia for the extraction and isolation of the compound used in this research. A special thanks is owed to Mr. Shahrin for his endless help and guidance throughout the extraction and isolation of the compound. I would also like to thank Mrs. Zurina for her priceless guidance and support in completing the HPLC and LCMS analysis of the isolated compound.



My heartfelt appreciation goes to Mrs. Siti Muskinah, who have helped and guided me regardless of time, throughout the completion of animal model studies. I would also like to acknowledge Miss Mohanambal, Miss Uma Nanthini and Mr. Zulfahmi for their invaluable support and help while carrying out the animal model studies.

My sincere appreciation are extended to my colleagues, Miss Ajantha Sinniah and Miss Zetty Nadia for their priceless and invaluable guidance, support, advice and help from the beginning until the end of the research project. Not forgetting my other lab mates, Mrs. Normah and Miss Ooi Suek Chin who gave me a hand in completing my lab work.

I am also grateful to Dr. Hairuszah Ithnin for allowing me to use the medical laboratory (Histology Laboratory) for my research work. Special thanks are also extended to Mrs. Juita, Mrs. Normah and Mr. Yip for their kind help and guidance during my histological and immunohistochemistry staining.

I would also like to extend my sincere appreciation to Associate Professor Dr. Fauziah Othman for giving me permission to use the Laser Scanning Confocal Microscopy in Micro electron and Micro Analysis Unit , Institute of Bioscience, Universiti Putra Malaysia. A special appreciation was extended to Mr. Rafiq and Miss Huey Fern who had helped me in TUNEL Assay staining.



My heartfelt gratitude goes to my fiancé Mr. Kaviyarasu Yellappan, for his endless guidance, support, advice and help throughout the completion of this research project.

I would also like to express my deepest and warmest appreciation to my family members especially my grandmother, Madam Vally Palany, my aunty, Miss Visalachee Suppiah and my cousin Vasanth Palanivelu for their patience, concern and kindness in helping me in every part of this thesis.

Above all, I would like to extend my utmost and deepest gratitude to GOD for blessing me with patience and persistence to complete this research.



TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii-iv
ABSTRAK	v-vi
ACKNOWLEDGEMENTS	vii-ix
APPROVAL	x-xi
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xvii-xx
LIST OF ABBREVIATIONS	xxi-xxii
CHAPTER	
1 INTRODUCTION	1.1-1.3
1.1 Research Objectives	1.4
1.2 Hypothesis	1.5-1.6
1.3 Problem statement	1.7
1.4 Significance of study	1.8
2 LITERATURE REVIEW	
2.1 Gynecological Cancer	2.1
2.2 Cervical Cancer	2.1-2.2
2.3 Screening of Cervical Cancer	2.2-2.3
2.4 Prognosis of Cervical Cancer	2.3
2.5 Cervical Intraepithelial Neoplasia	2.4
2.6 Symptoms of Cervical Cancer	2.4
2.7 Risk Factor for Cervical Cancer	2.5
2.7.1 Human Papilloma Virus (HPV)	2.5-2.7
2.7.2 Human Immunodeficiency Virus (HIV)	2.8-2.9
2.7.3 Herpes Simplex Virus (HSV)	2.9
2.7.4 Oral Contraceptives	2.10
2.7.5 Smoking	2.11-2.12
2.7.6 Other co-factors	2.12-2.13
2.8 DES (Diethylstilbestrol)	2.14-2.16
2.8.1 Diethylstilbestrol (DES) and Cancer	2.16-2.17



2.9	Natural Products	2.18
2.10	Zingiber Zerumbet from Zingiberaceae	2.19-2.20
	2.10.1 Zerumbone from Zingiber Zerumbet	2.21-2.23
2.11	Cisplatin Overview	
	2.11.1 Basic Information	2.24-2.25
	2.11.2 Toxicity	2.25-2.26
	2.11.3 Modes of Action of Cisplatin	2.26-2.27
2.12	Apoptosis	2.28-2.30
	2.12.1 Induction of Apoptosis	2.30-2.33
2.13	Overview of Interleukin-6	2.34-2.35
	2.13.1 Interleukin 6 and Cervical Cancer	2.36-2.37
2.14	Overview of Interleukin-6 Receptor	2.38-2.39
	2.14.1 Trans signaling via IL-6/sIL-6R	2.40-2.41

3 METHODOLOGY

3.1	Materials and Equipments	
	3.1.1 Extraction of zerumbone Assay	3.1
	3.1.2 Cell culture and MTT Cytotoxicity	3.1-3.2
	3.1.3 High Performance Liquid Chromatography	3.2
	3.1.4 Liquid Chromatography Mass Spectrometry	3.2-3.3
	3.1.5 Induction of DES in female balb/c mice	3.3
	3.1.6 Hematoxylin and Eosin staining	3.3
	3.1.7 TUNEL Assay	3.4
	3.1.8 ELISA IL-6	3.4
	3.1.9 Immunohistochemistry staining	3.5
3.2	Extraction and Isolation of zerumbone from zingiber zerumbet	
	3.2.1 Crude Extraction	3.6-3.7
	3.2.2 Separation of non-polar compound from polar compound	3.8-3.9
	3.2.3 Fractionation of non-polar compound	3.10
	3.2.4 Recrystallization and purification of zerumbone	3.11-3.12
	3.2.5 High Performance Liquid Chromatography (HPLC) of zerumbone.	3.13
	3.2.6 Liquid Chromatography Mass Spectrometry (LCMS) of zerumbone.	3.13
3.3	Cell culture and maintenance	3.14
	3.3.1 Cryopreservation of cell line	3.15
	3.3.2 Thawing cryopreserved cell line	3.15-3.16
	3.3.3 Cytotoxicity Assay	3.16-3.17
	3.3.4 Morphological studies	3.17



3.4	Induction of cervical dysplasia in female balb/c mice	3.18
3.5	Treatment of offspring exposed to DES in utero	3.19-3.20
3.6	Hematoxylin and Eosin staining	3.21-3.23
3.7	Detection of apoptosis using TUNEL Assay system	3.24-3.27
3.8	Serum IL-6 detection using anti-mouse IL-6 ELISA kit	3.28-3.29
3.9	ImmunoHistoChemistry (IHC) staining to detect IL-6 receptor	3.30-3.31
4	RESULTS	
4.1	High Performance Liquid Chromatography and Liquid Chromatography Mass Spectrometry analysis of zerumbone.	4.1-4.4
4.2	Effects of zerumbone on cell viability using MTT cytotoxicity Assay.	4.5-4.7
4.3	Effects of zerumbone of cell morphology using inverted microscopy.	4.8-4.11
4.4	Effects of zerumbone on progression of dysplasia using histological procedure.	4.12-4.17
4.5	Effects of zerumbone on apoptosis using TUNEL Assay.	4.18-4.25
4.6	Effects of zerumbone on secretion level of IL-6 using IL-6 ELISA Assay.	4.26-4.28
4.7	Effects of zerumbone on membrane and cytoplasmic bound IL-6 receptors using immunohistochemistry.	4.29-4.34
5	DISCUSSION	5.1-5.12
6	CONCLUSION AND RECOMMENDATIONS	
6.1	Conclusion	6.1-6.3
6.2	Future work and Recommendation	6.4
	REFERENCES	R.1-R.25
	APPENDICES	A.1-A.13
	BIODATA OF THE AUTHOR	B.1



LIST OF TABLES

Table	Page
3.1 Treatment groups.	3.20
3.2 Hematoxylin and eosin staining method.	3.22
3.3 Grading of Cervical Intra-epithelial Neoplasia (abnormalities of cervix).	3.23
3.4 Preparation of rTdT Incubation Buffer for experimental reactions.	3.25
4.6.1 The table shows various concentrations of serum IL-6 with Standard Error Mean (\pm S.E.M), according to treatment groups in mice.	4.28
A.1 Treatment concentration of zerumbone and cisplatin towards HeLa cells.	A.4



LIST OF FIGURES

Figure	Page
2.1 <i>Zingiber zerumbet</i> plant (A) Whole plant and (B) Rhizome	2.20
2.2 Molecular structure of zerumbone	2.22
2.3 Molecular structure of cisplatin	2.24
2.4 Mode of action of cisplatin	2.27
2.5 Apoptotic cell death in the nematode worm <i>C. elegans</i>	2.28
2.6 A caspase cascade.	2.32
3.1 Extraction of crude extract from zingiber zerumbet	3.7
3.2 Separation of non-polar compounds from crude extract.	3.9
3.3 Fractionation of non-polar compounds and recrystallization and purification of zerumbone	3.12
4.1.1 HPLC analysis graph of the isolated compound showed only one single peak at retention time of 16 minutes.	4.2
4.1.2 The LCMS analysis graph of zerumbone.	4.3
4.13 The LCMS analysis graph of zerumbone showed only single high fragment.	4.4
4.2.1 The effects of zerumbone to inhibit growth proliferation of human cancer cells, HeLa after 72-hours post-treatment of the compound at concentrations, 5 μ M to 35 μ M.	4.6
4.2.2 The effects of cisplatin to inhibit growth proliferation of human cancer cells, HeLa after 72-hours of post-treatment with the compound at concentrations, 2.5 μ M - 100 μ M.	4.7
4.3.1 Micrographs showing morphological changes towards human cancer cells, HeLa, as viewed under light contrasting inverted microscope after 24-hours post-treatment with compounds, zerumbone and cisplatin.	4.9



4.3.2	Micrographs showing morphological changes towards human cancer cells, HeLa, as viewed under light contrasting inverted microscope after 48-hours post-treatment with compounds, zerumbone and cisplatin.	4.10
4.3.3	Micrographs showing morphological changes towards human cancer cells, HeLa, as viewed under light contrasting inverted microscope after 72-hours post-treatment with compounds, zerumbone and cisplatin.	4.11
4.4.1	Histological micrograph showing normal cervical epithelial cells with low nuclear: cytoplasmic ratio, without noticeable angiogenesis.	4.13
4.4.2	Histological micrograph showing normal cervical epithelial cells with low nuclear: cytoplasmic ratio, with no apparent angiogenesis.	4.13
4.4.3	Histological micrograph showing treatment of induced cervical cancer in female Balb/c mice with 10mg/kg cisplatin.	4.14
4.4.4	Histological micrograph showing treatment of induced cervical cancer in female Balb/b c mice with 10mg/kg cisplatin.	4.14
4.4.5	Histological micrograph showing treatment of induced cervical cancer in female Balb/c mice with 16mg/kg zerumbone.	4.15
4.4.6	Histological micrograph showing treatment of induced cervical cancer in female Balb/c mice with 16mg/kg zerumbone.	4.15
4.4.7	Histological micrograph showing treatment of induced cervical cancer in female Balb/c mice with 8mg/kg zerumbone.	4.16
4.4.8	Histological micrograph showing treatment of induced cervical cancer in female Bbalb/b c mice with 8mg/kg zerumbone.	4.16
4.4.9	Histological micrograph showing treatment of induced cervical cancer in female Balb/c mice with normal saline.	4.17



4.4.10	Histological micrograph showing treatment of induced cervical cancer in female Balb/c mice with normal saline.	4.17
4.5.1	TUNEL micrograph showing cervix tissue of normal female mice.	4.20
4.5.2	TUNEL micrograph of cervical tissue of normal female mice exhibiting less apoptotic cells.	4.20
4.5.3	TUNEL micrograph of cervical tissue of female mice induced with cervix cancer, using normal saline for treatment.	4.21
4.5.4	TUNEL micrograph of cervical tissue of female mice induced cervix cancer, using normal saline for treatment.	4.21
4.5.5	TUNEL micrograph of the cervix tissue of female mice induced cervical cancer with follow-up treatment of zerumbone at 8 mg/kg dosage.	4.22
4.5.6	TUNEL micrograph of the cervical tissue of female mice induced cervical cancer with follow-up treatment of zerumbone at 8 mg/kg dosage.	4.22
4.5.7	TUNEL micrograph of the cervical tissue of female mice induced cervical cancer with follow-up treatment of zerumbone at 16 mg/kg dosage.	4.23
4.5.8	TUNEL micrograph of the cervical tissue of female mice induced cervical cancer with follow-up treatment of zerumbone at 16 mg/kg dosage.	4.23
4.5.9	TUNEL micrograph of cervical tissue of female mice induced cervical cancer with follow-up treatment of cisplatin at 10 mg/kg dosage.	4.24
4.5.10	TUNEL micrograph of cervical tissue of female mice induced cervical cancer with follow-up treatment of cisplatin at 10 mg/kg dosage.	4.24
4.5.11	The mean percentage of apoptotic cells in cervical tissue sectioning of female balb/c mice induced with cervical cancer after treatments with zerumbone, cisplatin and normal saline.	4.25



4.6.1	A standard curve of absorbance versus known concentrations of IL-6. A linear graph shows that the absorbance increases proportionately as concentration of IL-6 increases.	4.27
4.7.1	Immunohistochemistry micrograph of cervical tissue of normal female mice.	4.30
4.7.2	Immunohistochemistry micrograph of cervical cancer tissue of female balb/c mice treated with cisplatin at 10 mg/kg dosage.	4.31
4.7.3	Immunohistochemistry micrograph of cervical cancer tissue of female balb/c mice treated with cisplatin at 10 mg/kg dosage.	4.31
4.7.4	Immunohistochemistry micrograph of cervical cancer tissue of female balb/c mice treated with zerumbone at 10 mg/kg dosage.	4.32
4.7.5	Immunohistochemistry micrograph of cervical cancer tissue of female balb/c mice treated with zerumbone at 16 mg/kg dosage.	4.32
4.7.6	Immunohistochemistry micrograph of cervical cancer tissue of female balb/c mice treated with zerumbone at 8 mg/kg dosage.	4.33
4.7.7	Immunohistochemistry micrograph of cervical cancer tissue of female balb/c mice treated with zerumbone at 8 mg/kg dosage.	4.33
4.7.8	Immunohistochemistry micrograph of cervical cancer tissue of female balb/c mice treated with normal saline.	4.34
4.7.9	Immunohistochemistry micrograph of cervical cancer tissue of female balb/c mice treated with normal saline.	4.34
A.1.	Serial dilutions for standard preparation for IL-6 ELISA.	A.12



LIST OF ABBREVIATIONS

AIDS	Acquired Immuno Deficiency Syndrome
ATCC	American Type Culture Collection
CIN I	Mild dysplasia
CIN II	Moderate dysplasia
CIN III	Severe dysplasia
CIN	Cervical Intra-epithelial Neoplasia
CIS	Carcinoma in situ
CNTF	Ciliary Neurotrophic Factor
COX-2	Cyclooxygenase-2
DDP	Diamminedichloroplatinum (cisplatinum)
DES	Diethylstilbestrol
DESAD	National Cooperative Diethylstilbestrol Adenosis
DESAD	National Cooperative Diethylstilbestrol Adenosis
DMSO	Dimethylsulphoxide
DNA	Deoxyribonecleic Acid
ELISA	Enzyme Link Immuno Sorbent Assay
FCS	Feotal Calf Serum
FDA	Food and Drug Administration
FIGO	Federation of Gyneecology and Obstetrics
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography



HPV	Human Papilloma Virus
HSV-2	Herpes Simplex Virus type-2
HTC	Hepatome Tissue Culture
IL-6	Interleukin-6
IL-6R	Interleukin-6 Receptor
LCB	Liquid Base Cytology
LCMS	Liquid Chromatography Mass Spectrometry
LIF	Leukemia Inhibitory Factor
MT	Metallothionein
MTT	Micro Tetrazolium
NCI	National Cancer Institute
OC	Oral Contraceptives
OSM	Oncostatin M
PBS	Phosphate Buffer Saline
RNA	Ribonucleic Acid
TdT	Terminal Deoxynucleotidyl Transferase
TNF- α	Tumour Necrosis Factor-Alpha
TPA	Tetradecanoylphorbol-13-Acetate
TUNEL	TdT-mediated dUTP Nick-End-Labeling
UV	Ultra Violet
VEGF	Vascular endothelial Growth Factor



CHAPTER 1

INTRODUCTION

Cervical cancer remains the second common cancer among women worldwide. It is, however, the most common cancer in women in developing countries accounting for 80% of all cases. Majority of tumors in these women are diagnosed at advanced stages with a resulting high mortality (Rowlands and de Barros Lopez, 2001). Human papilloma virus (HPV) has been implicated as the primary causative agent for cervical squamous cancer, whereby 99.7% of all cervical cancers had been identified to have HPV DNA (Rowlands and de Barros Lopez, 2001).

In 1943, Papanicolaou and Traut published their experience on the role of cervical cytology in identifying cancer of the cervix. The technique known as Pap smear, was rapidly incorporated as a screening test for invasive and pre-invasive lesions (Rowlands and de Barros Lopez, 2001). Despite the success of the Papanicolou Smear (Pap smear), cervical carcinoma still causes high mortality but is treatable and curable if diagnosed at early stage. Moreover, the malignant transformation of cervical epithelial cells has been reported to have a long latency period of up to 10 years (Ho et al., 1998).

To date, the most effective single chemotherapeutic agent for cervical carcinoma is cisplatin, which normally achieves a response rate of between 23% and 50% (Ozols,



2003). Even though chemotherapy has been successful for treatment of some tumors such as testicular cancer and certain leukemia, its success rate for treatment of common epithelial tumors such as breast, colon, cervical, ovarian and lung has been less impressive (Johnstone et al., 2002). A chemotherapeutic drug used as an anti-cancer drug destroys the cancerous cells by interfering with the ability of the cancer cells to divide and reproduce. A drawback to this chemotherapeutic drug, however, is that these drugs also affect normal cells.

Apoptosis is also known as programmed cell death and is a mechanism for eliminating unwanted cells or damaged cells. The cells initiate a suicide sequence which results in a quick and systematic approach in triggering cell death. It is widely believed that tumor cells treated with anticancer agents, including radiation, will die by apoptosis and the tumors that do not readily undergo apoptosis are resilient towards treatment (Lowe and Lin, 2000). Furthermore, response to tumor treatment that employs plant derived-bioactive substances in cancer patients have been implicated by apoptosis induction in tumor cells (Smets, 1994; Paschka et al., 1998).

Most of the earliest pharmaceuticals were plant-derived compounds whereby plants have been used initially to treat diseases (Peter et al., 1998). The plant-derived drugs that are useful in clinical oncology include those of flavonoids, coumarins, cinnamates or phenolics.

