UNIVERSITI PUTRA MALAYSIA

ELUCIDATION OF BASIC MECHANISMS OF FLAVOKAWIN B INHIBITORY EFFECTS ON THE GROWTH OF SELECTED CANCER AND TRANSFORMED NORMAL CELL LINES

NASIR TSAFE UMAR

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BY

NASIR TSAFE UMAR

Thesis submitted in fulfilment of the requirements for the Degree of Master of Science in the Faculty of Medicine and Health Sciences Universiti Putra Malaysia

May 2008

DEDICATION

To all the de-humanised, oppressed,

starving, sub-dued and suppressed

peasants of the world.

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

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Chair: Associate Professor Dr Rozita Rosli, PhD

Faculty: Medicine and Health Sciences

Flavokawin B (FNB) is a hydroxychalcone isolated from a local plant species, Alpinia zerumbet of the Zingiberaceae family. It has been shown to have anti-oxidant and pro-immune properties. The aim of the present study was to assess the basic growth inhibitory mechanisms of FNB, on 21 cell lines, comprising tumour and transformed cell lines. The cells were treated with FNB at 0.1, 1, 3, 10, 30 and 100 µM. Over 3 to 4 days exposure, FNB selectively inhibited the growth of all the cell lines. Tumour cells were more sensitive to FNB than normal cells. MTT cytotoxicity assay was then conducted in order to assess the cytotoxic-indices of FNB. Caco-2, CEM-SS, MCF-7, T-47D and U87MG were found to be the five most sensitive cell lines, with IC50 values of 6.53 ± 0.81, 2.00 ± 0.41, 7.67 ± 0.71, 8.67 ± 1.40 and 14.00 ± 3.00 µM respectively. Tamoxifen (TMX) was used as a positive control for the 5 cell lines, with all the IC50 values found to be comparable to that of FNB. Cell survival analysis confirmed very significant patterns of FNB efficacy

(P<0.001 - P<0.05), in comparison to the untreated/negative controls in all the 5 cell lines. Apoptosis induction was then assessed by life-culture morphology in all the 21 cell lines, out of which 9 indicated apoptosis induction. Nucleoprotein fluorescence analysis was carried out in order to quantify and establish apoptotic frequencies. Combined image captures were used to analyse the apoptotic effects. FNB was found to induce apoptosis at the IC50 concentrations in 9 of the 21 cell lines. The most significant FNB-induced apoptotic frequencies compared to the untreated controls, were found at 70.67 ± 7.51% (P<0.01), 68.17± 6.81% (P<0.01), 49.33 ± 7.32% (P<0.01), 57.5 ± 4.82% (P<0.01) and 52.83 ± 3.62% (P<0.001), for the Caov-3, CEM-SS, CHO, HL-60 and MDA-MB-468 cell lines, respectively. The maximal apoptotic frequency effect induced by FNB was on the Caov-3 cell line, which was more significant than etoposide (ETS) positive control (P<0.05). Apoptosis induction was confirmed only in the 5 most significant (P≤0.01) cell lines, using agarose gel electrophoresis for DNA-laddering. The effect of FNB on oestrogen metabolism at IC50 concentrations, was tested using radioisotope enzymatic assays for oestrone sulphatase (E1STS) and oestradiol-17β hydroxysteroid dehydrogenase (oestrone → oestradiol, E2HD), on four selected human breast cancer cell lines (MCF-7, T-47D, MDA-MB-231, MDA-MB-468). FNB had significant inhibitory effects on the E1STS enzyme in the MCF-7 and T-47D cell lines, with 26.41 ± 0.69% (P < 0.01) and 18.53 ± 1.21% (P < 0.05), respectively at IC50 levels. FNB also significantly inhibited the E2HD enzyme in the T-47D cell line (36.40 ± 1.70%, P < 0.01). Confirmatory assays using the E1STS and E2HD ELISA kits were conducted on the MCF-7 and T-47D cell lines. Similar inhibitory effects of FNB on both enzymes were found in the MCF-7 and T-47D cell lines. On the other hand, FNB stimulated both enzymes in the non-oestrogen dependent cell lines, MDA-MB-

231 and MDA-MB-468. Finally, genotoxicity study was conducted in order to establish a safety profile of FNB in the CHO normal cell line, using ethylmethanesulphonate (EMS) as a positive control. Antigenotoxicity was assayed using a combination of FNB and EMS. The results showed an insignificant FNB clastogenicity ($P>0.05$, at 45 μM) and a significant FNB anti-clastogenicity ($P<0.05$, at 45 μM), in the CHO cells. In conclusion, the overall cytotoxicity, selectivity, cell survival, apoptotic, anti-oestrogenic, non-genotoxic and anti-genotoxic properties of flavokawin B (which are comparably better than TMX, ETS and EMS), forms the basic inhibitory mechanisms, which make this compound potentially an interesting anti-neoplastic agent.

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

PENJELASAN MEKANISMA-MEKANISMA ASAS KESAN-KESAN PERENCETAN FLAVOKAWIN B KE ATAS PERTUMBUHAN TITISAN-TITISAN SE KANSER DAN TERTRANSFORMASI YANG TERPILIH

Oleh

NASIR TSAFE UMAR

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Pengerusi: Associate Professor Dr Rozita Rosli, PhD

Fakuti: Perubatan dan Sains Kesihatan

Flavokawin B (FNB) adalah kumpulan hydroxychalcone yang dipencilkan daripada spesis pokok tempatan, Aplinia zerumbet dalam keluarga Zingiberaceace. FNB telah dikenalpasti mempunyai ciri-ciri anti-oksida dan pro-imun. Kajian ini bertujuan untuk menilai mekanisma perencatan pertumbuhan asas oleh FNB, ke atas 21 titisan sel yang terdiri daripada tumor manusia, dan manusia tertransformasi. Sel-sel diperlakukan dengan FNB (0.1, 1, 3, 10, 30 and 100 μM). Berikutan pendedahan pada ketoksikan piawai selama 3-4 hari, FNB merencat pertumbuhan titisan-titisan sel tersebut secara selektif. Sel-sel tumor lebih sensitif terhadap FNB berbanding sel-sel normal. Asai sitotoksik MTT kemudian dijalankan untuk menentukan indeks sitotoksik bagi FNB. Caco-2, CEM-SS, MCF-7, T-47D dan U87MG merupakan 5 titisan sel yang paling sensitif dengan nilai IC50 6.53 ± 0.81, 2.00 ± 0.41, 7.67 ± 0.71, 8.67 ± 1.40 dan 14.00 ± 3.00 uM masing-masing. Tamoksifen (TMX) telah digunakan sebagai kawalan positif.

Analisis survival sel menentukan corak keberkesanan FNB yang sangat signifikan ($P < 0.001$ – $P<0.05$), dalam kelima-lima titisan sel. Aruhan apoptosis kemudian dinilai menggunakan morfologi kultur-hidup. Analisis floresen nukleoprotin telah dijalankan untuk mengira kuantiti dan menentukan frekuensi apoptotik. Gabungan imej-imej telah digunakan untuk menganalisa kesan apoptosis. FNB didapati telah mengaruh apoptosis pada kepekatan IC50 dalam 9 daripada 21 titisan sel. Frekuensi apoptosis aruhan FNB yang paling signifikan berbanding kawalan tanpa perlakuan adalah 70.67 ± 7.51% ($P<0.01$), 68.17± 6.81% ($P<0.01$), 49.33 ± 7.32% ($P<0.01$), 57.5 ± 4.82% ($P<0.01$), 52.83 ± 3.62% ($P<0.001$), masing-masing bagi Caov-3, CEM-SS, CHO, HL-60 dan MDA-MB-468. Kesan frekuensi apoptosis yang maksima adalah pada titisan sel Caov-3, yang mana lebih signifikan berbanding etoposid (ETS) sebagai kawalan positif ($P<0.05$). Aruhan apoptosis hanya ditentukan pada 5 titisan sel paling signifikan ($P\leq0.01$), menggunakan elektroforesis agaros untuk tetangga-DNA. Kesan FNB ke atas metabolisma estrogen pada kepekatan IC50 telah diuji menggunakan asai radioisotop enzimatik bagi estrone sulphatase (E1STS) dan oestradiol-17ß hydroxysteroid dehydrogenase (oestrone → oestradiol, E2HD), dalam 4 titisan sel terpilih (MCF-7, T-47D, MDA-MB-231 and MDA-MB-468). FNB mempunyai kesan perencatan signifikan ke atas enzim E1STS dalam titisan sel MCF-7 dan T47-D, 26.41 ± 0.69% ($P<0.01$) dan 18.53 ± 1.21% ($P<0.05$) masing-masing. FNB juga merencat secara signifikan enzim E2HD dalam titisan sel T-47D (36.40 ± 1.70%, $P<0.01$). Asai penentuan menggunakan kit ELISA E1STS dan E2HD telah dijalankan ke atas titisan sel MCF-7 dan T-47D. Kesan perencatan yang serupa ditemui ke atas kedua-dua enzim dalam titisan sel MCF-7 dan T-47D. Sebaliknya, FNB telah merangsang kedua-dua enzim dalam titisan sel

bersandar bukan-estrogen, MDA-MB-321 dan MDA-MB-468. Akhirnya, kajian genotoksik telah dijalankan bagi profil keselamatan dalam titisan sel normal CHO, dengan menggunakan ethylmethanesulphonate (EMS) sebagai kawalan positif. Asai antigenotoksik adalah secara gabungan FNB dan EMS. Ujian menunjukkan kesan klastogenik yang tidak signifikan ($P>0.05$, dengan 45 μM), dan anti-genotoksik yang signifikan ($P<0.05$, dengan 45 μM). Kesimpulannya, secara keseluruhan ciri-ciri sitotoksik, selektif, survival, apoptotik, anti-estrogenik, non-genotoksik dan anti-genotoksik oleh flavokawin B (secara bandingannya lebih baik daripada TMX, ETS dan EMS) menjadikan sebatian ini berpotensi sebagai agen anti-neoplastik yang menarik.

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LIST OF ABBREVIATIONS

Abbreviation	:	Full Term
%CV	:	Percentage coefficient of variation
%Ec	:	Percentage counting efficiency
%Rv	:	Percentage recovery value
α	:	Alpha
β		Beta
δ		Delta
µ	:	Micro
µg/L	:	Microgram per litre
µg/ml	:	Microgram per millilitre
µl	:	Microlitre
µM	:	Micromolar
^{14}C	:	Carbon-14
$^{14}CE_2$	:	Carbon-14-oestradiol
^{3}H	:	Tritium
^{3}H-^{14}C	:	Tritiated-carbon-14
$^{3}H\_^{14}C\_Xv$	:	Specific activity value of tritiated-carbon-14
$^{3}HE_1$	:	Tritiated-oestrone
$^{3}H\_Xv$	:	Tritiated specific activity value
ACS	:	American Cancer Society
AGE	:	Agarose gel electrophoresis
AO	:	Acridine orange
ATCC	:	American type culture collection

Avrg.	:	Average
Bax	:	An oncogene involved in apoptosis/cell growth regulation
Bcl-2	:	An oncogene involved in apoptosis/cell growth regulation
Bcl-W	:	A factor involved in apoptosis/cell growth regulation
Bcl-XL	:	A factor involved in apoptosis/cell growth regulation
Bcl-XS	:	A factor involved in apoptosis/cell growth regulation
BHC	:	Biohazard cabinet
bp	:	Base pair
BQN	:	Benzoquinone
CA	:	Chromosome aberration
CCO	:	Corrected carry over
CGM	:	Complete growth medium
CI	:	Confidence interval
CLC	:	Chalcone
CnDF	:	Cell number dilution factor
CO_2	:	Carbon dioxide
contd		Continued
cpm	:	counts per minute
CRC	:	Corrected reference count
DAPI	:	4',6-diamidino-2-phenylindole
DF	:	Dilution factor
DMEM	:	Dulbecocco's modified Eagle's medium
DPH	:	Diphenylheptone
dpm	:	Disintegrations per minute
DTP	:	Diterpene

E_1DF	:	Oestrone-counts dilution factor
E_1S	:	Oestrone sulphate
E_1STS	:	Oestrone sulphatase
E_2	:	Oestradiol
E_2DF	:	Oestradiol-counts dilution factor
E_2HD	:	Oestradiol-17β hydroxysteroid dehydrogenase
E_2HSD		Oestradiol-17β hydroxysteroid dehydrogenase
E_3	:	Oestriol
ECACC	:	European collection of (Applied) cell cultures
EDTA	:	Ethylene diamine tetra acetic acid
ELISA	:	Enzyme-linked immunosorbent assay
EMS	:	Ethylmethanesulphonate
ER	:	Oestrogen receptor
ETS	:	Etoposide
FAS	:	A surface protein for apoptosis (programmed cell death)
Fas-L	:	A ligand surface protein for apoptosis (programmed cell death)
FBS	:	Foetal bovine serum
FCS	:	Foetal calf serum
FITC	:	Fluorescein isothiocyanate
FLV	:	Flavone/flavonol
fmol	:	Femtomole(s)
FNB	:	Flavokawin B
G	:	Gram
H	:	Hour(s)
HEPES	:	4-2-hydroxyethyl-1-piperazineethanesulphonic acid

HF-12	:	Ham's F-12
HPH	:	Hydroxyphenylheptone
i.e.	:	That is
IARC	:	International agency for research on cancer
IC	:	Interphase cells
IC_{50}	:	50 % inhibitory concentration of cell growth
IMDM	:	Iscove's modified Dulbecocco's medium
Kb	:	Kilobase
KPN	:	Kavapyrone
LL-15	:	Leivobitz's L-15
M	:	Molar
MC	:	Mitotic cells
Mc5A	:	McCoy's 5A
Mcl-1	:	A factor involved in apoptosis/cell growth regulation
MEGM	:	Mammary epithelial growth medium
MEME	:	Minimum essential medium Eagle's
mg/ml	:	Milligram per millilitre
MgCl2	:	Magnesium chloride
MI	:	Mitotic index
MIC_{100}	:	100 % of control mitotic index
MIC_{25}	:	25 % of control mitotic index
MIC_{50}	:	50 % of control mitotic index
Ml	:	Millilitre
mRNA	:	Messenger ribonucleic acid
MTD	:	Maximum tolerative dose

MTP	:	Moneterpene
MTT	:	Methylthiazoletetrazolium
MW	:	Molecular weight
Na	:	Sodium
NCI	:	National cancer institute
ng/ml	:	Nanogram per millilitre
Nm	:	nanometre
OECD	:	Organisation for economic corporation and development
P	:	Probability (statistical)
p53	:	A tumour suppressor gene involved in apoptosis/cell growth signal
PBS	:	Phosphate buffered saline
PCN	:	Proanthocynidine
pg/ml	:	Picogram per millilitre
PI	:	Propidium iodide
pMB	:	Promyeloblast
pMC	:	Promyelocytic
PNL	:	Phenolic
ppm		Parts per million
PR	:	Progesterone receptor
pS2		An Oestrogen inducible gene
rpm	:	Revolutions per minute
RPMI	:	Rosewell park memorial institute
SD	:	Standard deviation
SEM	:	Standard error of mean
SQP	:	Sesquiterpene

STDEV	:	Standard deviation
TC	:	Tissue culture
TtC	:	Total cells
TGF	:	Transforming growth factor
TLC	:	Thin layer chromatography
TMX	:	Tamoxifen
TNF	:	Tumour necrosis factor
TNFR	:	Tumour necrosis factor receptor
TNFα	:	Tumour necrosis factor-Alpha
TNFβ	:	Tumour necrosis factor-Beta
TRITC	:	Tetramethyl rhodamine isothiocyanate
UK	:	United kingdom
US	:	United States
USA	:	United States of America
UV	:	Ultraviolet
V	:	Voltage (volt)
v/v	:	Volume/volume
w/v	:	Weight/volume

1 INTRODUCTION

Plants provide an unlimited source of novel and complex chemical structures isolated as bioactive compounds, which could be developed continuously as therapeutic agents, with the goal of direct use as drugs (Fabricant and Farnsworth, 2001). Flavokawin B (FNB, section 2.2.4) is one such compound, a hydroxychalcone purified from a local herb, *Alpinia zerumbet (formerly known as A. nutans)*. The compound also exists in other species of the Zingiberaceae family, such as *A. japonica*, *A. mutica* and *A. rafflesiana* (Habsah, 2002) among others.

A. zerumbet is a perennial, erect herb, with numerous leafy stems usually up to 2 metres tall (Habsah, 2002), occurs widely and mainly in secondary vegetation of bamboo and teak forest under shady conditions of lowlands and hill slopes, throughout Southeast Asia. Locally named *lengkuas hutan*, this plant like other Zingiberaceae species is used as a component of spices, medicines, flavouring agents and as dye-sources (Burkill, 1966). In Malaysia, the *Alpinia* genus is one of the major ingredients of a traditionally prepared 'tonic' called *Jamu*. In northern Brazil however, the aqueous extract of *A. zerumbet* is traditionally used as a sedative (Dias and Takahashi, 1994). Its sister species (*A. speciosa*) is used as a diuretic to control hypertension (Mendonca *et al* 1991). As substantial 'germplasm' of *A. zerumbet* are not yet reported to exist (Oyen and Dung, 1999), this species is now being cultivated by the Laboratory of Phytomedicines and the Plant Genetic Centre, Institute of Bioscience, Universiti Putra Malaysia. Previous studies on *A. zerumbet* have so far only been reviewed and reported for its crude extract effects of anti-microbial, anti-oxidant (Habsah *et al* 2000) and anti-tumour promoting activity (Mackeen *et al* 2000; Habsah, 2002). Further investigations on *A. zerumbet* using bioactivity-guided

fractionation have yielded four compounds that were isolated and found to have antioxidant activities, with FNB as one of the most active (Habsah, 2002). It has been recently reported that FNB possess pro-immune properties of inhibiting nitrogen oxide production in macrophages *in vitro* (Syahida *et al* 2006), being a non-toxic kava-herbal component in rats (Disilvestro *et al* 2007) and as a strong antioxidant (Elzaaweley *et al* 2007).

Cytotoxicity is the degree of harmfulness that causes damage, acute or eventual lethality at general or specific cellular levels. Cytotoxicity is established on the basis of the ability of cells to survive a toxic insult *in vitro* or *in vivo* (Dean and Danford, 1984; Butler and Dawson, 1992; Roper, 1994). It is a destruction capacity that produces structural or functional change in the target cell, which is potentially harmful to the cells or its descendants (Lewis and Besterman, 1998). The general principle of cytotoxicity assays is based on the assessment of a special characteristic of most cytotoxic agents (such as drugs, hormones, nutrients and irradiation). These agents exert cell killing in a manner which may be non-specific, but selective towards cellular proliferative activities, often with acute death, cell cycle blockages and other specificities (Darzynkiewicz *et al* 1995; Skehan, 1995). In other words, these assays are based on the principle that cytotoxic agents inhibit mammalian cell division in culture, so that at effective concentrations of the agent in the culture medium, cells plated in sparse culture, will not grow to confluency in the wells of a microtitre tray (Shier, 1991).

The phrase 'cytotoxicity assay' has traditionally been used to describe methods of measuring the intensity of death resulting from treatment with compounds that cause

cell death (Freshney, 1987; Wilson, 1992). Measurement of toxicity *in vitro* is a purely cellular event that can only mimic the complex pharmaco-kinetics of drug exposure through clearance and excretion (Freshney, 1994). Although it is possible to stimulate most of the *in vivo* parameters (including complex tissue and systemic reactions), most *in vitro* studies concentrate solely on a direct cellular response, thereby gaining the simplicity to be measured by changes in cell survival, metabolism and other effects. In essence therefore, depending on the parameter in question, numerous cytotoxicity assays are widely available for applications to both normal and cancer cells *in vitro* and *in vivo*.

Cancer is a dreaded disease that is due to what is generally understood to be constituent 'mutational' activations, that leads to the 'loss of control' of the network of activities, regulating normal 'life and death' pattern of an individual's own cells (characterised by net tissue expansion), in a particular part of the body (Lowe, 1996; Vogelstein and Kinzler, 1998). Walt Kelly, cited in Pratt *et al* (1994), described cancer as 'an enemy within'.

'Apoptosis', a Greek word originally describing the dropping of leaves from trees (Sluyser, 1996), which was first defined by Kerr and co-authors (Kerr *et al* 1972), is described as a normal, programmed and actively specialised process or mode of cell killing, that occurs in a similar way as normal growth, through proliferation and differentiation, featured by original tissue-localisation (*in vivo*), to becoming eventually contagious, *in vitro* (Wyllie *et al* 1980). In apoptosis, single cells are deleted in the midst of normal living tissue, within an adherent or suspension cell populations, *in vitro* (Kerr *et al* 1994).

Apoptosis, alias ‘suicide’, ‘rescue’, or ‘honour’ death, has been understood to result in all normal cellular developments, for the survival of the neighbourhood populace (Fesus, 1991). Apoptosis is implicated in the steady-state kinetics of healthy adult tissues and accounts for focal deletion of cells, where it serves to clear expired cells during normal embryonic development and metamorphosis (Wyllie *et al* 1980). It is responsible for the fashioning of many tissue structures such as digits-shaping (spaces between our fingers and toes at the very early stage of foetal development) and the hollow cavity development and completion of fusion processes.

The spaces between our fingers and toes, at the very early stage of foetal development, the metamorphosis of tadpole tail to frog, the surplus cell eliminations in the neuron-brain connections or synapses, and the sloughing off of the inner lining of the uterus (endometrium), at the beginning of every regular menstrual process, are all understood to occur by apoptosis. It is an agent of physiological death in normal tissue turnover, and also provides the accomplishing criteria for ‘natural selection’ of the immune system (Wyllie, 1985; 1992; Gregory, 1996).

While cancer is said to result out of abnormal cell regulation, apoptosis being a natural cell homeostatic tool or end-point is a genetically controlled system, which operates through a `network’ or ‘cascade’ of factors. In this process, unrepaired or mis-repaired cell damages are the major, committing and critical determinants that trigger the normal but induced cell death process, sparing the neighbourhood cell populace. The apoptotic homeostasis feature in particular, is what makes apoptosis a very important process that plays a vital role in the kinetics of tumour survival and death (Wyllie,

1992; Lavin and Watters, 1993; Sluyser, 1996; Gregory, 1996), to such an extent that its effective presence basically means cancer suppression and its absence translates in to cancer cell survival. A major problem that appears to be the most serious of all is the prevalence of cancer in terms of 'resistance to treatment' and or 'metastasis'. Considering both genetic and non-genetic factors on the aetiology of cancer, the biggest problem, however, dramatically remains 'nobody is above cancer' and that the exact cause of many cancers remain largely unknown.

It has now been fully reviewed that the general understanding of the regulation of the molecular machinery of apoptosis *in vitro,* has witnessed major advances, in which molecules linking proliferation and apoptosis in healthy cells are being identified, as the apoptotic cell death provides the 'fail-safe' mechanism to counteract excess proliferation. Furthermore, since tumour specificity as ever, is the main issue to be resolved, inhibition of apoptosis in particular, remains a major mechanism of chemotherapeutic resistance (Makin and Dive, 2003; Duffy, 2007; Leen *et al* 2007).

Agents such as FNB that are found to induce cell death through apoptosis in multiple cell lines, are thus expected to provide contributions of paramount importance, towards the clarification of the mechanisms of drug-induced apoptosis, of tumour response. Such contributions are increasingly believed to be capable of providing renewed confidence, where therapeutic approaches based on drug targets within the apoptotic pathways will improve the treatment of cancer patients (Duffy, 2007; Leen *et al* 2007).

Oestrogen are generally understood to play an essential role in regulating the growth and differentiation of mammary gland (Lai, 2002; Reed *et al* 2005). The higher

concentrations of the biologically active oestrogen, oestradiol (E_2), have been shown to be closely related to breast cancer development in breast tumour tissue than in blood. In pre-menopausal women, nearly all the oestrogens are of ovarian origin. Yue *et al* (1998) have observed that after menopause, direct ovarian oestrogen production ceases as a result of which most oestrogens are derived exclusively from the peripheral conversion of androstenedione to oestrone (E_1) by the aromatase enzyme complex. This kind of aromatase activity was markedly observed in stromal cells around the carcinomatous glands in up to 78% of invasive breast carcinoma, where much of the E_1 formed is converted to oestrone sulphate (E_1S) by oestrone sulphotransferase (Sasano *et al* 1996). Several recent studies, have enumerated how the regulation of metabolism, in addition to the mitogenic properties of E_1STS and E_2HSD enzymes plays a chemotherapeutically vital role in the growth and death of oestrogen dependent cancer cells, like the MCF-7 and T-47D cell lines (Abraham *et al* 2006; Olofsson *et al* 2007; Stute *et al* 2007).

The description of the cell lines used in this study are as, indicated in the respective material data sheets provided by the suppliers, the American Type Culture Collections (ATCC) and the European Collections A Cell Culture (ECACC). These are: A-172 (human, brain cancer), Caco-2 (human, colon cancer), Caov-3 (human, ovarian carcinoma), CEM-SS (human, T-cell lymphoblastic leukaemia, Chang (human, transformed liver epithelium), CHO (Chinese hamster, transformed normal ovary), DU-145 (human, prostate carcinoma), HCN-2 (human, transformed glial epithelium), HeLa (human, cervical carcinoma), HepG2 (human, liver carcinoma), HL-60 (human, myelogenous leukaemia), MCF-7 (human, mammary carcinoma, positive for estrogen receptor), MCF-10A (human, transformed mammary

epithelium), MCF-12A (human, transformed mammary epithelium), MDA-MB-231 (human, mammary carcinoma, negative for estrogen receptor), MDA-MB-468 (human, mammary carcinoma, negative for estrogen receptor), PNT2 (human, transformed prostate epithelium), SK-BR-3 (human, mammary carcinoma, negative for estrogen receptor), T-47D (human, mammary carcinoma, positive for estrogen receptor), U87MG (human, Glioblastoma) and VERO (African Green Monkey, transformed normal kidney).

Since cancer is a multifactorial, dreadful disease which is common worldwide, with wide ranging implications of multistage progressive processes, treatment and metastasis, its prevention and eradication efforts therefore requires coherently systematic approaches that are as wholly as the continuous developmental strides for new or novel anti cancer agents. This is a similar view to those shared by Raina and Aggarwal (2007) and Xu, *et al* (2007), on the chemotherapeutic role of apoptosis. With such a problem statement, this study was designed with a hypothesis-driven approach in order to elucidate some of the efficacious mechanisms involved in the proliferative inhibitory potential of FNB. Up to four hypothetical pathways were identified in this study. These are the FNB induction of cytotoxicity and its selectivity patterns, the apoptosis cell death induction pathway, the anti-oestrogenicity pathway and the direct genotoxicity and anti-genotoxicity pathway. The last pathway mainly serves as the pathway for safety potential. Each of these four hypothetical pathways were elucidated using three separate experimental approaches. These are [1] the basic assays: MTT cytotoxicity (Mosmann, 1983; Monks *et al* 1991), live cell apoptosis morphology (D'Herde *et al* 2003), the radioisotopic E_1S to E_1 activity (Ng *et al* 2000; Elsadig *et al* 2001) and mitotic index

(Dean and Danford, 1984), [2] the reproducibility assays: the cytotoxic selectivity (Freshney, 1994; Zachary, 2003) , the AO/PI apoptosis morphology (Singh *et al* 1994; Singh *et al* 1996a; 1996b; Al-Rubeai and Singh, 1998; Ali *et al* 2001), the reductive $E_1 \rightarrow E_2$, coupled with the oxidative $E_2 \rightarrow E_1$ activities (Wong *et al* 2001) and the clastogenic indices modulation (Dean and Danford, 1984), [3] the confirmatory assays: cell survival (Wilson, 1992; Zachary, 2003), apoptosis DNA laddering (Wyllie *et al* 1980; Chomcyznski *et al* 1997; Ali *et al* 2001), the E_1 and E_2 ELISA assays (El-sadig *et al* 2001; Wong, 2002) and genotoxicity and anti-genotoxicty assays (Dean and Danford, 1984; Umar-Tsafe *et al* 2004).

In this research project, it has been hypothesised that due to the medicinal, the antioxidative and the pro-immune response properties of FNB, as highlighted above and reviewed in the literature review section, the compound possesses a strong anticancer cytotoxicity potential. Investigation of the basic mechanisms of its cell growth inhibitory properties, thus require hypothesis-driven objectives as follows:

(i) To evaluate the basic cytotoxic potential of FNB, through the establishment of dose-responses and IC_{50} values for each of the 21 cell lines, as well as to assess the reproducible *in vitro* cytotoxic-selectivity of FNB among the panel of 21 cancer and normal cell lines, using the NCI selective-efficacy guidelines for chemotherapeutic potential.

(ii) To analyse the confirmatory cell survival effects of FNB in the most sensitive and most significant cell lines, using standard guidelines.

(iii) To enumerate the mechanism of apoptosis (as the mode of cell death) in the cell lines that are sensitive to FNB, using basic classical morphology, quantitatively reproducible and confirmatory analysis.

(iv) To elucidate the mechanistic effect of FNB on oestrogen synthesis in four breast cancer cell lines [2 hormone-dependent (MCF-7 and T-47D) and 2 hormone-independent (MDA-MB-231 and MDA-MB-468)], along with two normal-transformed breast epithelial cell line (MCF-10A and MCF-12A).

(v) To establish the safety and mechanistic-efficacy profile of FNB in the CHO normal cell line, using the basic and reproducible MI analysis, and the confirmatory genotoxicity and anti-genotoxicity CA analysis.

REFERENCES/BIBLIOGRAPHY

Abraham, J., Earl, H.M., Pharoah, P.D. and Caldas, C. (2006). Pharmacogenetics of cancer chemotherapy. *Reviews on Cancer*, 766(2):168-183.

Ackerknecht, E.H. (1973). Therapeutics: From the primitives to the twentieth century. Hafner Press, New York.

ACS (2005). Estimated New Cancer Cases and Deaths by Sex for All Sites, US, 2005. Surveillance Research, American Cancer Society, Inc., Washington DC.

ACS (2007). Estimated New Cancer Cases and Deaths by Sex for All Sites, US, 2007. Surveillance Research, American Cancer Society, Inc., Washington DC.

Aden, D.P., Fogel, A., Plotkin, S., Damjanov, I., Knowles, B.B. (1979). Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line. *Nature*, 282: 615-616.

Adler, I. D. (1984). Cytogenetic Tests in Mammals. *In*: S. Venitt and J. M. Parry (eds). Mutagenicity Testing: A Practical Approach. IRL Press Ltd., Oxford.

Adler, I.A. (1980). A review of the coordinated research effort on the comparison of test systems for the detection of mutagenic effects, sponsored by the EEC. *Mutation Res.,* 74: 77-93.

Aggarwal, B.B., Sundaram, C., Malani, N. and Ichikawa, H. (2007).Curcumin: the indian solid gold. *Advances in Experimental Medicine and Biology*, 10.1007/978-0-387-46401-5.

Agrestri, A. (1992). A Survey of Exact Interference for Contingency Tables. *Statistical Science*, 7: 131-153.

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J. D. (1989). Molecular Biology of the Cell, 2nd edition. Garland Publishing Inc., New York.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. (2002). Molecular Biology of the Cell, 4th edition. Garland Science New York.

Ali, A.M., Umar-Tsafe, N., Mohamed, S.M., Inayat-Hussein, S.H., Oo, K.T., Yusoff, K., Osman, A.B. and Din, L.B. (2001). Apoptosis induction in CEM-SS T-lymphoblastic leukemic cell line by goniothalamin. *Journal of Biochemistry, Molecular Biology and Biophysics, 5*(3), 227-235.

Ali, A.M., McKeen, M.M., Intan-Safinar, I., Hamid, M., Lajis, N.H., El-Sharkawy, S.H. and Murakoshi, M. (1996). Antitumor promoting activities of the crude extract from the leaves of *Juniperus chinensis. Journal of Ethnopharmacolology*, 53:165-169.

Ali, M.S., Banskota, A.H., Tezuka, Y., Saiki, I., Kadota, S. (2001b). Antiproliferative activity of diarylheptanoids from the seeds of *Alpinia blepharocalyx*. *Biol Pharm Bull.,* 24(5):525-8.

Ali, M.S., Tezuka Y, Banskota AH, Kadota S. (2001a). Blepharocalyxins C--E, three new dimeric diarylheptanoids, and related compounds from the seeds of Alpinia blepharocalyx. *J Nat Prod.,* 64(4):491-6.

Alink, G.M., Quik, J.T.K., Penders, E.J.M., Spenkelink, A., Rotteveel, S.G.P., Maasc, J.L., Hoogenboezem, W. (2007). Genotoxic effects in the Eastern mudminnow (*Umbra pygmaea* L.) after exposure to Rhine water, as assessed by use of the SCE and Comet assays: A comparison between 1978 and 2005. *Mutation Research*, 63: 93–100.

Alley, M.C., Scudiero, D.A., Monks, A., Czerwinski, M.J., Shoemaker, R.H. and Boyd, M.R. (1986). Validation of an Automated Microculture Tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumour cell lines. *Proc. Am. Assoc. Cancer Res*., 27: 389.

Alley, M.C., Scudiero, D.A., Monks, A., Hursey, M.L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Mayo, J.G., Shoemaker, R.H. and Boyd. M.R. (1988). Feasibility of Drug Screening with Panels of Human Tumour Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Research*, 48: 589-601.

Al-Rubeai, M. and Singh, R. P. (1998). Apoptosis in cell culture. *Curr Opin Biotechnol,* 9: 152-156.

Altman, R. and Sarg, M. (1992). The Cancer Dictionary. Facts on File Inc. New York.

Altmann, K.-H., Gertsch, J. (2007). Anticancer Drugs from Nature-Natural Products as a Unique Source of New Microtubule-Stabilizing Agents *Chem Inform*, 2007, 38(30) DOI: 10.1002/chin.200730264.

Ames, B.N., Gold, L.S. (1997). Environmental pollution, pesticides and the prevention of cancer: misconceptions. *FASEB J.,* 11: 1041-1052.

Ames, B.N., M. Profet, L.S. Gold (1990). Nature's chemicals and synthetic chemicals: comparative toxicology. *Proc. Natl. Acad. Sci.*, 87: 7782-7786.

Ames, B.N. (1983). Dietary carcinogens and anti-carcinogens. *Science*, 221: 1256-1264.

Ames, B.N., J. McCann, E. Yamasaki (1975). Methods for detecting carcinogens and mutagens with Salmonella/mammalian-microsome mutagenicity test. *Mutation Res.,* 31: 347-364.

Anderson, D. (1984). The Dominant Lethal Test in Rodents. *In*: S. Venitt and J. M. Parry (eds). Mutagenicity Testing: A Practical Approach. IRL Press Ltd., Oxford.

Atta-ur-Rahman, M.I. (ed). (1991). Studies in Natural Product Chemistry, vol. 9, Elsevier, Science Publishers, Amsterdam.

Aun, Q.B. (1995). Cytotoxic Activity of Goniothalamin on different types of cancer cell lines. B. Sc. Thesis, UPM-Serdang-Malaysia.

Baker, F.J. and Silverton, R.E. (1985). Introduction to Medical Laboratory Technology. 6th ed. Butterworth Co. Publisher London.

Barber, R.C., Hickenbotham, P., Hatch, T., Kelly, D., Topchiy, N., Almeida, G.M., Jones, G.D., Johnson, G.E., Parry, J.M., Rothkamm, K., Dubrova, Y.E. Radiation-induced transgenerational alterations in genome stability and DNA damage.*Oncogene*, 2006 Nov 30;25(56):7336-7342.

Barch, M.J., ed. (1991). The ACT Cytogenetics Laboratory Manual. Second Edition. The Asssociation of Cytogenetic Technologists / Raven Press, Ltd., New York.

Barranco, S.C. (1979). Drug Effects on the Cell Cycle. In: Effects of Drugs on the Cell Nucleus. Academic Press Inc., London.

Baserga, R. (1995). Measuring Parameters of Growth. *In*: Studzinski, G. P. (ed) Cell Growth and Apoptosis: A Practical Approach. IRL Press / Oxford University Press Inc., New York.

Baserga, R. (1989). Measuring Parameters of Growth. *In*: Baserga, R. (ed.) Cell Growth and Division: A Practical Approach. IRL Press at Oxford University Press, Oxford.

Bellamy, C.O., Malcomson, R.D., Harrison, D.J., (1995). Cell Death in Health and Disease: The Biology and Regulation of Apoptosis. *Semin Cancer Biol.,* 6:3-16.

Benn, J., Su, M Doria, R Schneider (1996). Hepatitis B virus HBx protein induces transcription factor AP-1 by activation of extracellular signal-regulated and c-Jun N-terminal mitogen-activated protein kinases. *J. Virol.,* 70: 4978-4985.

Bensky, D. and Gamble, A. (1993). Chinese herbal medicine: Materia Medica (revised edition). Eastland Press, Inc. Seatle.

Bezerra, D.P., de Castro. F.O., Alves, A.P.N.N., Pessoa, C., de Moraes, M.O., Silveira, E.R., Lima, M.A.S., Elmiro, F.J.M., de Alencar, N.M.N., Mesquita, R.O., Lima, M.W. and Costa-Lotufo, L.V. (20007). *In vitro* and *in vivo* antitumor effect of 5-FU combined with piplartine and piperine. *J. Appl. Toxicol.*, 1261. pp 1-8.

Bhat, S.V., Nagasampagi, B.A., Sivakumar, M. (2005). Chemistry of Natural Products. Springer, Heidelberg, Germany.

Blatt, N.B. and Glick, G.D. (2001). Signaling pathways and effector mechanisms pre-programmed cell death. *Bioorg Med Chem.* 9(6):1371-1384.

Bonney, R.C., Reed, M.J., Davidson, K., Beranek, P.A., and James, V.H.T. (1983). The relationship between 17b-hydroxysteroid dehydrogenase activity and

oestrogen concentrations in human breast tumours and in normal breast tissue. *Clin Endocrinol.,* 19: 727-739.

Boonstra, J. and Post, J.A. (2004). Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells *Gene,* 337:1-13.

Boyd, M.R. 1997. The NCI in vitro anticancer drug discovery screen. In: Teicher, B. (Ed.) Anticancer drug development guide; preclinical screening, clinical trials and approval. Humana Press: Ottowa.

Boyer, M.J. and Tannock, I.F. (1998). Cellular and molecular basis of chemotherapy. In: Tannock, I.F. and Hill, R.P. The basic science of oncology, 3rd edition. The Mc Graw-Hill Inc. Singapore.

Bray, F., McCarron P, Parkin, D.M. (2004). The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Res.*, 6(6):229-39.

Burkill, I.H. (1966). A Dictionary of Economic Products of the Malay Peninsular. Crown Agent Publications, London.

Butler, M. and Dawson, M. (1992). Cell Culture LabFax. BIOS Scientific Publishers Limited, Oxford.

Caelles, C., Helmberg, A., Karin, M. (1994). P53-Dependent Apoptosis in the Absence of Transcriptional Activation of P53-Target Genes. *Nature.* 370:220-3.

Cailleau, R. *et al* (1978). Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. *In Vitro,* 14: 911-915.

Cailleau, R. *et al* (1974). Breast tumor cell lines from pleural effusions. *J. Natl. Cancer Inst.* 53: 661-674.

Cameron, G.R. (1951). Pathology of the Cell. Charles C. Thomas, Springfield.

Canman, C.E. and Kastan, M.B. (1997). Role of p53 in Apoptosis. *In*: Kaufmann, S.H. (ed.). Apoptosis: Pharmacological Implications and Therapeutic Opportunities. Academic Press Ltd., London.

Canman, C.E. and Kastan, M.B. (1995). Induction of Apoptosis by Tumor Suppressor Genes and Oncogenes. *Cancer Biology,* 6:17-25.

Carson, C.F., Hammer, K.A., Riley, T.V. (2006). *Melaleuca alternifolia* (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties. *Clinical Microbiology Reviews,* 19(1): 50-62.

Cemeli, E., Schmid, T. and Anderson, D. (2004) Modulation by flavonoids of DNA damage induced by oestrogen-like compounds. *Environmental and Molecular Mutagenesis* 44 (5) 420-426.

Chakraborty, S., Ghosh, U., Bhattacharyya, N.P., Bhattacharya, R.K., Roy, M. (2006). Inhibition of telomerase activity and induction of apoptosis by curcumin in K-562 cells. *Mutation Research,* 596 (2006) 81–90.

Chang, R.S. (1954). Continuous subcultivation of epithelial-like cells from normal human tissues. *Proc. Soc. Exp. Biol. Med*., 87: 440-443.

Charafe-Jauffret, E., Ginestier C, Monville F, Fientti P, Adelaide J, Cervera N, Fekairi, S., Xerri, L., Jacquemier, J., Birnbaum, D. and Bertucci, F. (2006). Gene expression profiling of breast cancer cell lines identifies potential new basal markers. *Oncogene* 25(15):2273–2284.

Chetrite, G.S., Cortes-Prieto, J., Philippe, J.C., Wright, F. and Pasqualini, J.R. (2000). Comparison of oestrogen concentrations, oestrone sulphates and aromatase activities in normal, and in cancerous, human breast tissues. *Journal of Steroid Biochemistry and Molecular Biology,* 721(1-2):23-27.

Choi, M.A., Kim, SH, Chung, W.Y, Hwang, J.K., Park, K.K. (2005). Xanthorrhizol, a natural sesquiterpenoid from Curcuma xanthorrhiza, has an anti-metastatic potential in experimental mouse lung metastasis model. *Biochem Biophys Res Commun*., 7;326(1):210-7.

Chomcyznski, P., MacKey, K, Drews, R. and Wilfinger, W. (1997). DNAzol®: A Reagent for the Rapid Isolation of Genomic DNA. *Biotechniques*, 22(3): 550-553.

Chun, K.S., Sohn, Y, Kim, H.S., Kim, O.H., Park, K.K., Lee, J.M., Moon, A., Lee, S.S., Surh, Y.J. (1999). Anti-tumor promoting potential of naturally occurring diarylheptanoids structurally related to curcumin. *Mutat Res*., 428(1-2):49-57.

Chun, K.-S., Park, K.-K., Lee, J., Kang, M.., Surh, Y.-J. (2002,).Inhibition of Mouse Skin Tumor Promotion by Anti-Inflammatory Diarylheptanoids Derived From Alpinia oxyphylla Miquel (Zingiberaceae). Oncology Research Incorporating *Anti-Cancer Drug Design*, 13(1): 37-45(9).

Clark, R.A. *et al* (1997). Tenascin supports lymphocyte rolling. *J. Cell Biol.,* 137: 755-765.

Clarke, R., Leonessa, F., Welch, J.N. and Skaar, T.C. (2001). Cellular and Molecular Pharmacology of Antiestrogen Action and Resistance. *Pharmacological Reviews,* 53(1): 25-72.

Clerk, C. (1999). Temozolomide – A Tale of Pharmaceutical Endeavour. *The Pharmaceutical Journal*, 262: 467-69.

Cohen, J. J. (1993). Apoptosis. *Immunol Today,* 14:126-30.

Cohnheim, J. (1889). Lectures on General Pathology: The Pathology of Nutrition, 2nd edition. Translated by McKee, A. B. The New Sydenham Society, London.

Collins, J.A., Schandl, C.A., Young, K.K., Vesely, J. and Willingham, M.C. (1997). Major DNA Fragmentation is a Late Event in Apoptosis. *The J. Histochem and Cytochem.* 45(7):923-934.

Collins, K., Jacks, T. and Paviletich, N. (1997a). The Cell Cycle and Cancer. *Proc. Natl. Acad. Sci. USA,* 94:2776-2778.

Collins, S.J. *et al* (1977). Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. *Nature,* 270: 347-349.

Colognato, R., Coppedè, F., Ponti, J., Sabbioni, E., Migliore, L. (2007). Genotoxicity induced by arsenic compounds in peripheral human lymphocytes analysed by cytokinesis-block micronucleus assay. *Mutagenesis,* 22(4): 255-261.

Cragg, G.M. and Newman, D.J. (2005). Plants as a source of anti-cancer agents. *Journal of Ethnopharmacology,* 100(1-2): 72-79.

Crawford, A.M., Kerr, J.F.R., Currie, A.R. (1972). The relationship of acute mesodermal cell death to the teratogenic effects of 7-OHM-12-MBA in the foetal rat. *Br. J. Cancer,* 26, 498-503.

Cross, S.M., CA Sanchez, CA Morgan, MK Schimke, S Ramel, RL Idzerda, WH Raskind, and BJ Reid. (1995). A p53-dependent mouse spindle checkpoint. *Science,* 267(5202): 1353-1356.

CR-UK (2004). Cancer Research UK News and Resources web site CancerStats, http//:info.cancerresearchuk.org/cancerstats (Page last updated: January 2005, Accessed 11 September, 2007).

Cussenot, O., Berthon, P., Berger, R., Mowszowicz, I., Faille, A., Hojman, F. *et al* (1991). Immortalization of human adult normal prostatic epithelial cells by liposomes containing large T-SV40 gene. *J Urol,* 146, 881–889.

D'Herde, Mussche, S. and Roberg, K. (2003). Morphological Changes in Dying Cells, in Hughes and Mehmet, eds. Cell Proliferation and Apoptosis. BIOS Scientific Publishers, Oxford. Pages 201-231.

Darzynkiewicz, Z., Li, X., Gong, J., Hara, S. and Traganos, F. (1995). Analysis of Cell Death by Flow Cytometry. *In*: Studzinski, G. P. (ed) Cell Growth and Apoptosis: A Practical Approach. IRL Press, Oxford University Press Inc., New York.

David, B., Sevenet, T., Morgat, M., Guenard, D., Moisand, A., Tollon, O. and Wright, M. (1994). Rhazinilam mimics the Cellular Effects of Taxol by Different Mechanisms of Action. *Cell Motility and Cytoskeleton,* 28: 317-326.

de Guzman, C.C. and Siemonsma, J.S. (1999). Plant resources of Southeast Asia, No. 13: Spices. Prosea Foundation, Bogor, Indonesia.

De Vincenzo R. *et al* (1998). Antiproliferative activity of colchicine analogues on MDR-positive and MDR-negative human cancer cell lines. *Anticancer Drug Des*., 13: 19-33.

Dean, B.J. and Danford, N. (1984). Assays for the detection of chemically-induced chromosome damage in cultured mammalian cells, in: S. Venitt and J.M. Parry (Eds.), Mutagenicity Testing: A Practical Approach, IRL Press, Ltd., Oxford.

Dearfield, K.L., M.C. Cimino, N.E. McCarroll, I. Mauer, L.R. Valcovic. Genotoxicity risk assessment: a proposed classification strategy. *Mutation Res.,* 521 (2002) 121-135.

Debinski W. *et al* (1996). Receptor for interleukin (IL) 13 does not interact with IL4 but receptor for IL4 interacts with IL13 on human glioma cells (32550). *J. Biol. Chem.,* 271: 22428-22433.

Deigner, H.P. and Kinscherf, R. (1999). Modulating Apoptosis: Current applications and prospects for future drug development. *Current Medicinal Chemistry*, 6: 399-414.

Deleersnyder, V. *et al* (1997). Formation of native hepatitis C virus glycoprotein complexes. J. Virol. 71: 697-704.

Desnoyers, S. and Hengartner, M.O. (1997). Genetics of Apoptosis. *In*: Kaufmann, S. H. (ed.). Apoptosis: Pharmacological Implications and Therapeutic Opportunities. Academic Press Ltd., London.

Dhar, S., Nygren, P., Csoka, K., Botling, J., Nilsson, K. and Larsson, R. (1996). Anticancer Drug Characterisation Using a Human Cell Line Panel Representing Defined Types of Drug Resistance. *British Journal of Cancer*, 74: 888-896.

Di Marco, (1979). Perspective on the research of new anticancer agents. *In:* Effects of drugs on the cell nucleus. Academic Press inc. London. pp 491-505.

Dias, F.D.L. and Takahashi, C.S. (1994). Cytogenetic evaluation of aqueous extracts of the medicinal plants *Alpinia nutans* Rose (Zingerberaceae) and *Pogostemum hyneanus* benth (Labitae) on Wistar rats and *Allium cepa* Linn (Liliaceae) root tip cells. *Rev. Brasil Genet*., 17: 175-80.

Dihal, A.A., V.C.J. de Boer, H. van der Woude, C. Tilburgs, J.P. Bruijntjes, G.M. Alink, I. M.C.M. Rietjens, R.A. Woutersen, and R.H. (2006). Stierum Quercetin, but Not Its Glycosidated Conjugate Rutin, Inhibits Azoxymethane-Induced Colorectal Carcinogenesis in F344 Rats *J. Nutr.,* 136(11): 2862 - 2867.

Dirks, P.B. and Rutka, J.T. (1997).Current Concepts in Neuro-Oncology: The Cell Cycle-A Review. *Neurosurgery*. 40(5):1000-1015.

DiSilvestro, R.A., Zhang, W., DiSilvestro, D.J. (2007). Kava feeding in rats does not cause liver injury nor enhance galactosamine-induced hepatitis. *Food and Chemical Toxicology,* 45: 1293–1300.

Du, Y, Yin F, Liu C, Hu S, Wang J, Xie H, Hong L, Fan D. (2006). Depression of MAD2 inhibits apoptosis of gastric cancer cells by upregulating Bcl-2 and interfering mitochondrion pathway. *Biochem Biophys Res Commun.,* Jul 7;345(3): 1092-8.

Duffy, M. (2007). Role of Tumour Markers in Patients with Solid Cancers: A Critical Review. *European Journal of Internal Medicine,* 18(3): 175-184.

Duncan, L.J., Goldham, N.G. & Reed, M.J. (1994) The interaction of cytokines in regulating oestradiol 17b-hydroxysteroid dehydrogenase activity in MCF-7 cells. *J Steroid Biochem Mol Biol.,* 49: 63-68.

Duvoix, A., Morceau F, Delhalle S, Schmitz M, Schnekenburger M, Galteau MM, Dicato M, Diederich M. (2003). Induction of apoptosis by curcumin: mediation by glutathione S-transferase P1-1 inhibition. *Biochem Pharmacol. Oct* 15;66(8):1475-83.

Elledge, S. J. (1996). Cell cycle Checkpoints: Preventing an Identity Crisis, *Science,* 274(5293): 1664-1672.

El-sadig, R.E., Reimann, K., Yip, C.H., Lai, L.C. (2001). Inhibition of oestrone sulphatase activity in the MDA-MB-231 breast cancer cell line by breast cyst fluid from Malaysian women. *Anticancer Res*., 21(4A): 2693-6.

El-Sadig, R.E., Lai, L.C. (2000). Oestrogens and Breast Cancer. *The Biochemist Review,* 21(iii): 93.

El-Shemy, H.A., Aboul-Enein, A.M., Aboul-Enein, K.M., Fujita, K. (2007). Willow Leaves' Extracts Contain Anti-Tumor Agents Effective against Three Cell Types. *PLoS ONE*, 2(1): e178. doi:10.1371/journal.pone.0000178.

El-zaawely, A.A., Tran D. Xuan, Haruo Koyamaand Shinkichi Tawata. (2007). Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. *Food Chemistry,* 104(4): 1648-1653.

Evans, E. P., Breckon, G. and ord, C. E. (1964). An Air-Drying Method for Meiotic Preparations for Mammalian Testes. *Cytogenesis,* 3: 289-294.

Evans, R., Cidlowski, J.A. (1995). Apoptosis: Cellular Signaling and Molecular Mechanisms. *In:* Oliver, C. (ed). Cell Biology of Trauma. CRC Press, Boston, MA.

Fabricant, D.S. and Farnsworth, N.R. (2001). The Value of Plants Used in Traditional Medicine for Drug Discovery. *Environmental Health Perspectives*, 109(1): 69 – 75.

Fadeel, B., Orrenius, S. and Zhivotovsky, B. (1999). Apoptosis in human disease: a new skin for the old ceremony? *Biochem Biophys Res Commun,* 266(3):699-717.

Fang, X-P., Anderson, J. E., Chang, C.-J., Fanwick, P. E. and McLaughlin, J. L. (1990). Novel Bioactive Styrylactones: Goniofurfurone, Goniopyrone and 8-acetylgonioltriol from *Goniothlamus giganteus* (Annonaceae*). J. Chem. Soc. Perkin Trans.* I. : 1655.

Fantes, P. and Brooks, R. (1993). The Cell Cycles: A Practical Approach. Oxford University Press, Oxford.

Farnsworth, N. R. and Bingel, A. S. (1977). Problems and Prospects of Discovering New Drugs From Higher Plants By Pharmacological Screening. *In:* Wagner, H. and Wolff, P. (Eds). New Natural Products and Plant Drugs With Pharmacological, Biological or Therapeutical Activity. Springer Verlag, Berlin.

Farnsworth, N. R. and Moris, R. N. (1976). Higher Plants: The Sleeping Giant of Drug Industry. *American Journal of Pharmacy*, 147(2): 46.

Fesus, L., Davies, P. J. A. and Piacentini, M. (1991). Apoptosis: Molecular mechanisms in programmed cell death. *Env. J. Cell Biol*., 56:170-177.

Finn, R.S., Dering, J., Ginther, C., Wilson, C.A., Glaspy, P., Tchekmedyian, N., Slamon, D.J. (2007). Dasatinib, an orally active small molecule inhibitor of both the *src* and *abl* kinases, selectively inhibits growth of basal-type/"triple-negative" breast cancer cell lines growing in vitro. *Breast Cancer Research and Treatment,* 105(3): 319-326.Dowsett, M., Detre, S., Rowlands, M. and Grimshaw, R. (1996). Oestrogen formation in breast: clinical and biological importance. *Journal of Endocrinology*, 150:S59-S63.

Floridata (2007). *Alpinia zerumbet*: Variegated shell ginger. Floridata.com LC, Tallahassee, Florida, USA. (http://www.floridata.com/A/alpi_zer_var.cfm). 18 October, 2007.

Fogh, J., Fogh JM, and Orfeo T. (1977). One hundred and twentyseven cultured human tumor cell lines producing tumors in nude mice. *J Natl Cancer Inst.,* 59: 221–226.

Foley, G.E., Lazarus H, Farber S, Uzman BG, Boone BA, McCarthy RE. (1965). Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer,* 18:522-529.

Freshney, R. I. (1994). Culture of Animal Cells: A Manual of Basic Technique. Third Edition. Wiley-Liss. Inc., New York.

Freshney, R. I. (ed) (1992). Animal Cell Culture: A Practical Approach. 2nd edition. Oxford University Press, New York.

Freshney, R. I. (1987). Culture of Animal Cells: A Manual of Basic Technique. 2nd edition. Alan R Liss. Inc., New York.

Gatehouse, D.G., I.R. Rowland, P. Wilcox, R.D. Callander, R. Forster. (1990). Bacterial mutation assays, in: D.J. Kirkland (Ed.) Basic Mutagenicity Tests:

UKEMS Recommended Procedures, UKEMS/University of Cambridge Press, Cambridge.

Geiger, T. *et al* (1998). Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice. *Anticancer Drug Des.,* 13: 35-45.

GenWay (2004a). ESTRONE-ELISA: Catalogue # 40-056-205046. GenWay Biotech Inc., USA. P.I. Number : 1701116/en Revision Nr : 060727/1: pp 1-7. www.genwaybio.com. 23 June, 2004.

GenWay (2004b). Enzyme immnoassay for the quantitative measurement of oestradiol in serum and plasma (96 determinations). Catalogue # 40-056-205004. Published August 7, 1995. pp 1-2. www.genwaybio.com. 23 June, 2004.

Geran, R. I., Greenberg, N. H., McDonald, M. M., Schumacher, A. M. and Abott, B. J. (1972). Protocols for Screening Chemical Agents and Natural Products Against Animal Tumour and Other Biological Systems. *Cancer Chemotherapy Reports*, 3: 1-103.

Gerlier, D. and Thomasset, N. (1986). Use of MTT Colometric Assay to Measure Cell Activation. *J. Immunol. Methods*, 94: 57-63.

Gewali MB, Tezuka Y, Banskota AH, Ali MS, Saiki I, Dong H, Kadota S. (1999). Epicalyxin F and calyxin I: two novel antiproliferative diarylheptanoids from the seeds of Alpinia blepharocalyx. Org Lett. Dec 2;1(11):1733-6.

Gey, G.O. *et al* (1952). Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. *Cancer Res.,* 12: 264-265.

Ghanei, M., Harandi, A.A. (2007). Long Term Consequences from Exposure to Sulfur Mustard: A Review. *Inhalation Toxicology*, 19:451–456.

Giard, D.J. *et al* (1973). In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J. Natl. Cancer Inst.,* 51: 1417-1423.

Goebel, W., Obermeyer, N., Bleicher, N., Kratzmeier, N., Eibl, H.-J., Doenecke, D., Albig, W. (2007). Apoptotic DNA fragmentation is not related to the phosphorylation state of histone H1. *Biological Chemistry*, 388(2): 197-206.

Gomez-Manzano, C., Fueyo, J. Kyristsis, A. P., McDonnell, T. J., Steck, P. A., Levine, V. A. and Alfred Yung, W. K. (1997). Characterisation of p53 and p21 Functional Interactions in Glioma Cells en Route o Apoptosis. *J Natl cancer Inst,* 89:1036-1044.

Goodenough, U. (1984). Genetics, 3rd edition. Holt-Sandlers College Publishing, Phidalphia. Pp 25-61.

Granada, J.F., David Wallace-Bradley, Htut K. Win, Carlos L. Alviar, Angela Builes, Eli I. Lev, Roberto Barrios, Daryl G. Schulz, Albert E. Raizner, Greg L. Kaluza.

(2007). In Vivo Plaque Characterization Using Intravascular Ultrasound–Virtual Histology in a Porcine Model of Complex Coronary Lesions. *Arterioscler Thromb Vasc Biol*., 27: 387-393.

Grant, W. F. (1999). Higher plant assays for the detection of chromosomal aberrations and gene mutations. *Mutation Res*. 426: 107-112.

Gregory, C. D. (1996). Apoptosis in the Immune System. *In*: Sluyser, M. (ed). Apoptosis in Normal Development and Cancer. Taylor & Francis Ltd., London.

Habsah, M., Lajis, N. H., Ali, A. M., Sukari, M. A., Hin, Y. Y., & Kikuzaki, H. et al (2003). The antioxidative components from *Alpinia nutans*. *Pharmaceutical Biology, 41*(1), 7-9.

Habsah, M. (2002). Search for new antioxidants and other related bioactive compounds from Zingiberaceous species. PhD Thesis. Universiti Putra Malaysia, Serdang.

Habsah, M, Amran, M., Mackeen, M.M., Lajis, N.H., Kikuzaki, H., Nakatani, N., Rahman, A.A., Ghafar and Ali, A.M. (2000). Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. *Journal of Ethnopharmacology* 72: 403-410.

Habsah, M, Amran, M., Mackeen, M.M., Lajis, N.H., Kikuzaki, H., Nakatani, N., Rahman, A.A., Ghafar and Ali, A.M. (2000). Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. *Journal of Ethnopharmacology* 72: 403 – 410.

Hahm, E.R., Park S, Yang CH. (2003). 7, 8-dihydroxyflavanone as an inhibitor for Jun-Fos-DNA complex formation and its cytotoxic effect on cultured human cancer cells. *Nat Prod Res.*, 17(6):431-6.

Hale, A. J., Smith, C. A., Sutherland, L. C., et al (1996). Apoptosis: Molecular Regulation of Cell Death. *Eur J Biochem.* 236:1-26.

Hampton MB, Vanags DM, Porn-Ares MI, et al (1996). Involvement of extracellular Calcium in Phosphatidylserine Exposure During Apoptosis. *FEBS Lett.* 399:277-82.

Haraguchi H, Kuwata Y, Inada K, Shingu K, Miyahara K, Nagao M, Yagi A. (1996). Antifungal activity from Alpinia galanga and the competition for incorporation of unsaturated fatty acids in cell growth. Planta Med. 1996 Aug;62(4):308-13.

Harmon, B. V. and Allan, D. J. (1996). Apoptosis: a 20th century scientific revolution. *In:* Sluyser, M. (ed). Apoptosis in normal development and cancer. Taylor and Francis Ltd, London.

Hawariah, A. L. P. and Stanslas, J. (1998). Antagonistic Effects of Strylpyrone Derivative (SPD) on 7,12-dimethylbenzanthracene-induced Rat Mammary Tumors. *in vivo*, 12(4): 403-410.

He, W., Li, Y., Xue, C., Hu, Z., Chen, X., Sheng, F. (2005). Effect of Chinese medicine alpinetin on the structure of human serum albumin. *Bioorg Med Chem.,* 1;13(5):1837-45.

Healy, G.M., Teleki, S., Serfried, A.V., Walton, M.J. and Macmorine, H.G. (1971). Improved chemically defined basal medium (CMRL-1969) for primary monkey kidney and human diploid cells. *Applied Microbiol.,* : 1-5.

Heinrich, A. (2007). Classification of Urban and Industrial Soils in the World Reference Base for Soil Resources. *Journal of Soils and Sediments*, 7(2): 96-100.

Hideshima, T., Catley, L., Yasui, H., Ishitsuka, K., Raje, N., Mitsiades, C., Podar, K., Munshi, N.C., Chauhan, D., Richardson, P.G., Anderson, K.C. (2006). Perifosine, an oral bioactive novel alkylphospholipid, inhibits Akt and induces in vitro and in vivo cytotoxicity in human multiple myeloma cells. *Blood*, 107(10): 4053-4062.

Hood, R. D. (ed) (1990). Developmental Toxicology: Risk Assessment and the Future. Van Nostran Reinhold, New York.

Hoppe, H.C. *et al* (1997). Identification of phosphatidylinositol mannoside as a mycobacterial adhesin mediating both direct and opsonic binding to nonphagocytic mammalian cells. *Infect. Immun*., 65: 3896-3905.

Horie-Inoue, K. and Inoue, S. (2006). Epigenetic and proteolytic inactivation of 14-3-3σ in breast and prostate cancers. *Seminars in Cancer Biology,* 16(3): 235-239.

Hornby, (1985). Stich, H.F., Hornby, A.P. and Dunn, B.P. A pilot beta-carotene intervention trial with Inuits using smokeless tobacco. *Int. J. Cancer* 36:321-327 (1985).

Huang, M.-T.; Wang Z.Y.; Georgiadis N.; Laskin S.; Conney A.H. (1992). Inhibitory effects of curcumin on tumor initiation by benzoa!pyrene and 7,12-dimethylbenza!anthracene. *CARCINOGENESIS,* 13(11): 2183-2186.

Huang, N. F., Zac-Varghese, S. and Luke, S. (2003). Apoptosis in skin wound healing. *Wounds,* 15(6): 182-194.

Hudziak, R.M., HM Shepard, A Ullrich, BM Fendly (1997). Monoclonal antibodies directed to the Her2 receptor. US Patent 5,677,171 dated Oct 14 1997. Human tumor cells in vitro. New York: Plenum Press.

Hughes, D. and Mehmet, H. (2003). Introduction to Cell Proliferation and Cell Death, in: Hughes, D. and Mehmet, H. eds. Cell Proliferation and Apoptosis. BIOS Scientific Publishers, Oxford. Pages 1-12.

IARC (1997). Biological evaluation of medical devices - Part 3: tests for genotoxicity, carcinogenicity and reproductive toxicity. Second Working Draft for ISO/WD No. 10993-3. ISO & International Organization for Standardization. Munchen, Germany.

IARC (2006). 3.2M new cancer cases in Europe in 2006 released by The International Agency for Research on Cancer. In: J. Ferlay, P. Autier, M. Boniol, M. Heanue, M. Colombet & P. Boyle. Estimates of the cancer incidence and mortality in Europe in 2006. *Annals of Oncology*, doi:10.1093/annonc/mdl498: 1-12.

ICH (1995). Genotoxicity: Guidance on specific aspects of regulatory genotoxicity tests for pharmaceuticals. International Committee on Harmonization. ICH-3BS6a.

Inayat-Hussain, S. H., Osman, A. B., Din, L. B., Ali, A. M., Snowden, R. T., MacFarlane, M. and Cain, K. (1999). Caspases-3 and -7 are activated in goniothalamin-induced apoptosis in human Jurkat T-cells. *FEBS Letters*, 456: 379-383.

IRIS (2003). Mustard gas. Integrated Risk Information System (IRIS). U.S. Environmental Protection. Agency. http://www.epa.gov/iris/subst/index.htm. 28 January, 2003.

Irungu, B.N., G.M. Rukunga, GM Mungai, CN Muthaura (2007). *In vitro* antiplasmodial and cytotoxicity activities of 14 medicinal plants from Kenya. *South African Journal of Botany,* 73(2): 204-207.

Ishidate, M. Jr. (1988). A proposed battery of tests for the evaluation of the mutagenic potential of medicinal and industrial chemicals. *Mutation Res.,* 205: 397-407.

Itokawa, H., Morita, M. and Mikashi, S. (1981). Phenolic compounds from the rhizomes of *Alpinia speciosa*. *Phytochemistry,* 20: 2503-2506.

Jain, S., Shrivastava, S., Nayak and Sumbhate, S., (2007). PHCOG MAG.: Plant Review. Recent trends in Curcuma Longa Linn. *Pharmacognosy Reviews*, 1(1):119-128.

Jantan, I., Pisar, M., Sirat, H.M., Basar, N., Jamil, S., Ali, R.M., Jalil, J. (2004). Inhibitory effects of compounds from Zingiberaceae species on platelet activating factor receptor binding. *Phytother Res.,* 18(12):1005-7.

Jendrossek, V, White, R. (2004). EACR-18: the best of European cancer research. *European Journal of Cancer,* 40(18): 2645-2658.

JIFSAN (2006). Annual Report 2005-2006. The Joint Institute for Food Safety and Applied Nutrition. JIFSAN, Maryland.

Johnson, E M, Jr. (1994). Possible Role of Neuronal Apoptosis in Alzheimer's Disease, *Neurobiology Of Aging,* 15(2):S187-S18.

Johnson, M.L., Grazul-Bilska, A.T., Redmer, D.A., Reynolds L.P. (2007). Effects of Estradiol-17β on Expression of mRNA for Seven Angiogenic Factors and Their Receptors in the Endometrium of Ovariectomized (OVX) Ewes. *Endocrine,* 30(3): 333-342.

Jones, P.A. and Baylin, S.B. (2007). The Epigenomics of Cancer. *Cell,* 128: 683-692.

Joosten, H.F.P., F. A. A. van Acker, D. J. van den Dobbelsteen, G. J. M. J. Horbach and E. I. Krajnc (2004). Genotoxicity of hormonal steroids. *Toxicology Letters,* 151(1): 113-134.

Jost, C. A., Marin, M. C. and Kaelin Jr, W. G. (1997). p73 is a Human p53 Related Protein that can Induce Apoptosis. *Nature*, 389:191-194.

Karlan, B.Y., Boyd J, Strong L, *et al* (1994). Glucocorticoids stabilize HER-2/neu messenger RNA in human epithelial ovarian carcinoma cells. *Gyn. Onc.,* 53: 70-77.

Kaufman, S. (1989). Induction of endonucleotic DNA cleavage in human acute myelogenous leukaemia by etoposide, camptothecin and other anticancer drugs. *Cancer Research,* 49: 5870-5878.

Keren, D. F. (1994). History and Evolution of Surface Marker Assays. *In*: Keren, D. F., Hanson, C. A. and Hurtubise, P. E. (eds.) Flow Cytometry and Clinical Diagnosis. American Society of Clinical Pathologists, Chicago.

Kerr, J.F.R. (1965). A histochemical study of hypertrophy and ischaemic injury of rat liver with special reference to changes in lysosomes. *J. Pathol. Bacteriol.,* 90, 419-435.

Kerr, J.F.R. (1967). Lysosome changes in acute liver injury due to heliotrine. *J. Pathol. Bacteriol.,* 93, 167-174.

Kerr, J.F.R. (1969). An electron-microscope study of liver cell necrosis due to heliotrine. *J. Pathol.,* 97, 557-562.

Kerr, J.F.R. (1971). Shrinkage necrosis: a distinct mode of cellular death. *J. Pathol.,* 105, 13-20.

Kerr, J.F.R., Wyllie, A.H., Currie, A.R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer,* 26, 23-257.

Kerr, J. F. R., Winterford, C. M. and Harmon, B. (1994). Apoptosis - Its Significance in Cancer and Cancer Therapy. *Cancer,* 73: 2013-2026.

Kerr, J. F. R. (1993). Definition of Apoptosis and Overview of its Incidence. *In*: M. Lavin and D. Watters (eds). Programmed Cell Death: The Cellular and Molecular Biology of Apoptosis. Harwood Academic Publishers, Chur, Switzerland.

Kerr, J. F. R., Wyllie, A. H. and Curie, A. R. (1972). Apoptosis: A Basic Biological Phenomenon with Wide-Ranging Implications in Tissue-Kinetics. *British Journal of Cancer*, 26: 239-257.

Kerr, J. F. R. (2002). History of the events leading to the formulation of the apoptosis concept. *Toxicology,* 181-182: 471-474.

Keydar, I., Chen, I., Karby, S., Weiss, F.R., Delarea, J., Radu, M., Chaitcik, S., Brenner, H.J. (1979). Establishment and characterization of a cell line of human breast carcinoma origin. *Eur. J. Cancer,* 15: 659-670.

Kim YU, Son HK, Song HK, Ahn MJ, Lee SS, Lee SK. (2003). Planta Med. Jan;69(1):72-4. Inhibition of 5alpha-reductase activity by diarylheptanoids from Alpinia officinarum.

Kim, H. A., Blanco, F. J. (2007). Cell Death and Apoptosis in Ostearthritic Cartilage. *Current Drug Targets*, 8(2): 333-345.

Kim, S.O., Kundu JK, Shin YK, Park JH, Cho MH, Kim TY, Surh YJ. (2005). [6]-Gingerol inhibits COX-2 expression by blocking the activation of p38 MAP kinase and NF-kappaB in phorbol ester-stimulated mouse skin. Oncogene. [Epub ahead of print Feb 14].

Kimmel, C.B., Sessions, S.K., and Kimmel, R.J. (1981) Morphogenesis and Synaptogenesis of the Zebrafish Mauthner Neuron. *J. Comp. Neurol.* 198:101-120.

Kinghorn, A.D., , N. R., Becher, C. W.W., Soejarto, D.D., Cordell, G.A., Pezzuto, J.M., Wall, M.E., Wani, M.C., Brown, D.M., O'neill, M.J., Lewis, J.A. and Besterman, J.M. (1998). Novel Strategies for the Discovery of Plant-derived Anti-cancer Agents. *In:* Atta-ur-Rahman and Choudhary, eds. (1998). New Trends in Natural Product Chemistry. Harwood Academic Publishers, The Netherlands.

Kirkland, D.J. (1994). Report of the *in vitro* sub-group: International workshop on standardisation of genotoxicity test procedures. *Mutation Res.* 312: 211-215.

Kirkland, D. J. (ed) (1990). Basic Mutagenicity Tests: UKEMS Recommended Procedures. UKEMS/Cambridge University Press, Cambridge.

Kirkland, D. J., ed. (1989). Statistical Evaluation of Mutagenicity Test Data. Cambridge University Press, Cambridge.

Klob, S., Bochennek, K., Huenecke, S., Zimmermann, S.-Y., Kuçi, S., Müller, T., Wels, W.S., Klingebiel, T., Esser, R., Koehl, U. (2007). A novel five-colour

flow cytometric assay to determine NK cell cytotoxicity against neuroblastoma and other adherent tumour cells. *Journal of Immunological Methods*, 325, Issues 1-2, 31 August 2007, Pages 140-147.

Kong, L.Y., Qin, M.J. and Niwa, M. (2000). Diterpenoids from the rhizomes of *Alpinia calcarata*. *J Nat Prod.,* 63: 939-942.

Kreitman, R.J. (1999). Immunotoxins in Cancer Therapy. Curr Opin Immunol., 11(5): 570-578.

Kroemer, G., Zamzami, N. and Susin, S.A.. (1997). Mitochondrial Control of Apotosis. *Immunol Today.* 18:44-51.

Kupchan, S.M. (1976). Plant-derived Tumor Inhibitors. *Cancer Treat Rep.,* 60(8): 111.

Kuroyanagi, M., Noro, T., Fukushima, S., Aiyama, R., Ikuta, A., Itokawa, H., Morita, M. (1983). Studies on the constituents of the seeds of *Alpinia katsumadai*. *Hayata Chem Pharm Bull.,* 31(5): 1544-1550.

Labrie, F., Luu-The, V., Labrie, C., Berube, D., Couet, J., Zhao, H.F., Cagne, R. and Simard, J. (1989). Characterization of two mRNA species encoding human oestradiol 17β-dehydrogenase and assignment of the gene to chromosome 17. *Journal of Steroid Biochemistry*, 34(1-6): 189-197.

Labrie, F., Luu-The, V., Lin, S.X., Simard, J. and Labrie, C. (2000). Role of 17β-hydroxysteroid dehydrogenases in sex steroid formation in peripheral intracine tissues. *Trends in Endocrinology and Metabolism*, 11(10):421-427.

Lahlou, S., Galindo, C.A., Leal-Cardoso, J.H., Fonteles, M.C., Duarte, G.P. (2002). Cardiovascular effects of the essential oil of Alpinia zerumbet leaves and its main constituent, Terpinen-4-ol, in rats: role of the autonomic nervous system. *Planta Med.,* 68(12):1097-102.

Lahlou, S., Interaminense, L.F., Leal-Cardoso, J.H., Duarte, G.P. (2003). Antihypertensive effects of the essential oil of *Alpinia zerumbet* and its main constituent, terpinen-4-ol, in DOCA-salt hypertensive conscious rats. *Fund Clin Pharmacol*., 17(3): 323–330.

Lai, L.C. (2002). Role of steroid hormones and growth factors in breast cancer. *Clin Chem Lab Med.*, 40(10): 969-74.

Larsen, K., Ibrahim, H., Khaw, S,H. and Saw, S.G. (1999). Gingers of Peninsular Malaysia. Natural History Publications (Borneo), Kota Kinabalu.

Lavin, M. and Watters, D. (eds) (1993). Programmed Cell Death: The Cellular and Molecular Biology of Apoptosis. Harwood Academic Publishers, Chur, Switzerland.

Lee, E.T. (1992). Nonparametric Methods for Comparing Survival Distributions. In: Statistical Methods for Survival Data Analysis, 2nd ed. John Wiley & Sons Inc., New York.

Leen, A.M. Rooney, C.M. and Foster, A.E. (2007). Improving T Cell Therapy for Cancer. *Annual Review of Immunology*, 25:243-265.

Lewis, J.A. and Besterman, J.M. (1998). Novel strategies for the discovery of plant-derived anticancer agents. *In*: Atta-ur-Rahman, M. I. and Choudary, M. I. (eds.). New trends in natural products chemistry. Harwood academic publishers, Amsterdam.

Lim, S.T.S., Dragull, K., Tang, C.-S., Bittenbender, H.C., Efird, J.T. and Nerurkar, P.V. (2007). Effects of Kava Alkaloid, Pipermethystine, and Kavalactones on Oxidative Stress and Cytochrome P450 in F-344 Rats. *Toxicological Sciences,* 97(1): 214-221.

Lin, C.Y., Ström A, Li Kong S, Kietz S, Thomsen JS, Tee JB, Vega VB, Miller LD, Smeds J, Bergh J, Gustafsson JA, Liu E.T. (2007). Inhibitory effects of estrogen receptor beta on specific hormone-responsive gene expression and association with disease outcome in primary breast cancer. *Breast Cancer Res.,* 2007;9(2):R25.

Littlewood-Evans, A.J. , Bilbe, G., Bowler, W.B. Farley, D., Wlodarski, B., Kokubo, T., Inaoka, T., Sloane, J., Evans, D.B. and Gallagher, J.A. (1997). The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. *Cancer Res.,* 57: 5386-5390.

Lowe, S.W., Bodis, S., McClatchey, A., Remington, L., Ruley, H.E., Fisher, D.E., Houseman, D.E. and Jacks, T. (1994). p53 Status and Efficacy of Cancer Therapy *In Vivo*. *Science*, 266: 807-810.

Lowe, S.W. (1996). The Role of p53 in Apoptosis. *In*: Sluyser, M. (ed). Apoptosis in Normal Development and Cancer. Taylor & Francis Ltd., London.

Mackeen, M.M., Ali, A.M, Lajis, N.H., Kawazu, K., Hassan, Z., Amran M.; Habsah M.; Mooi L.Y., Mohamed S.M. (2000). Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of Garcinia atroviridis Griff. ex T. Anders. *Journal of Ethnopharmacology*, 72(3): 395-402(8).

Macville, M. Schröck, E., Padilla-Nash, H., Keck, C., Ghadimi, B.M., Zimonjic, D., Popescu, N. and Ried, T. (1999). Comprehensive and definitive molecular cytogenetic characterization of HeLa cells by spectral karyotyping. *Cancer Res.,* 59: 141-150.

Majno, G. and Joris, I. (1995). Apoptosis, Oncosis and Necrosis: An Overview of Cell Death. *American Journal of Pathology*, 146: 3-15.

Majno, G., La Gattuta, M. and Thompson, T.E. (1960). Cellular Death and Necrosis: Chemical, Physical and Morphologic Changes in Rat Liver. *Virchows Arch Pathol Anat*., 333: 421-465.

Makin G. and Dive C. (2003). Recent Advances in Understanding Apoptosis: New Therapeutic Opportunities in Cancer Therapy. *Trends Mol Med*., 9(6): 251-55.

Malcomson, R.D.G., Oren, M., Wyllie, A.H. and Harrison, D.J. (1995). p53-Independent Death and p53-Induced Protection Against Apoptosis in Fibroblasts Treated with Chemotherapeutic Drugs. *British Journal of Cancer*, 72: 952-957.

Marsoni, S., Hoth, D., Simon, R., Leyland-Jones, B., De Rosa, M., Wittes, R.E. (1987). Clinical Drug Development: An Analysis of Phase II Trials, 1970-1985. *Cancer Treat Rep.*, 71(1):71-80.

Matsuda, H, Morikawa, T, Managi, H, Yoshikawa, M. (2003b). Antiallergic principles from Alpinia galanga: structural requirements of phenylpropanoids for inhibition of degranulation and release of TNF-alpha and IL-4 in RBL-2H3 cells. Bioorg Med Chem Lett. 2003 Oct 6;13(19):3197-202.

Matsuda H, Pongpiriyadacha Y, Morikawa T, Ochi M, Yoshikawa M. (2003a). Gastroprotective effects of phenylpropanoids from the rhizomes of Alpinia galanga in rats: structural requirements and mode of action. Eur J Pharmacol. 2003 Jun 13;471(1):59-67.

McLaughlin, J. L., Chang, C. J., David, L. and Smith, D. L. (1991).”Bench-Top” bioassays for the discovery of bioactive natural products: an up-date. *In:* Atta-ur-Rahman, M. I. (ed). Studies in Natural Products Chemistry. vol. 99, Elsevier Press, Armsterdam. Pp. 383-409.

Mendonca, V.L., Oliveira, C.L., Craveiro, A.A., Rao, V.S. and Fonteles, M.C. (1991). Pharmacological and toxicological evaluation of *Alpinia speciosa*. *Mem. Inst. Oswaldo* Cruz, 86(2): 93-97.

Meyn, R. E., Stephens, L. C., Ang, K. K., Milas, L., Hunter, N. R. and Peters, L. J. (1993). *In:* Lavin, M. and Watters, D. (eds) (1993). Programmed Cell Death: The Cellular and Molecular Biology of Apoptosis. Harwood Academic Publishers, Chur, Switzerland.

Miettinen, M. (1999). 17b-hydroxysteroid dehydrogenase types 1 and 2. Expression and activities in various tissues and cell lines and effect of the type 1 enzyme on estrogen-dependent growth of breast cancer cells. University of Oulu, Oulu, Finland.

Miller T, Beausang LA, Meneghini M, Lidgard G. (1993). Death-induced changes to the nuclear matrix: the use of anti-nuclear matrix antibodies to study agents of apoptosis. *Biotechniques,* 15(6):1042-7.

Minn, A J, L H Boise, and C B Thompson (1996). Expression of Bcl-xL and loss of p53 can cooperate to overcome a cell cycle checkpoint induced by mitotic spindle damage. *Genes Dev.,* 10: 2621-2631.

Miyashita, T., Reed JC. (1995). Tumor Suppressor p53 is a Direct Transcriptional Activator of the Human Bax Gene. *Cell.* 80:293-9.

Miyazawa, M., Nakamura, Y., Ishikawa, Y. Insecticidal diarylheptanoid from Alpinia oxyphylla against larvae of Drosophila melanogaster. Nat Prod Lett. 2001;15(1):759.

Moghrabi, N. and Anderson, S. (1998). 17β-hydroxysteroid dehydrogenases: physiological roles in health and disease. *Trends in Endocrinology and Metabolism*, 9(7):265-270.

Mohamad, H., Abas, F., Permana, D., Lajis, N.H., Ali, A.M., Sukari, M.A., Hin, T.Y., Kikuzaki, H., Nakatani, N. (2004). DPPH free radical scavenger components from the fruits of Alpinia rafflesiana Wall. ex. Bak. (Zingiberaceae). *Z Naturforsch*, 59(11-12):811-5.

Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D., Hose, C., Langky, J., Cronise, P., Vaigro-Wolf, A., Gray-Goodrich, M., Campbell, H. J. and Boyd, M. (1991). Feasibility of High Flux Anticancer Drug Screen using a Diverse Panel of Cultured Human Tumor Cell Lines. *J. Natl. Cancer Inst*., 83: 757-766.

Moreno, C.S., Matyunina, L., Dickerson, E.B., Schubert, N., Bowen, N.J., Logani, S., Benign, B.B., McDonald, J.F. (2007). Evidence that p53-mediated cell-cycle-arrest inhibits chemotherapeutic treatment of ovarian carcinomas. *PLoS ONE,* 5(e441): 1-13.

Morgan, D.O. (1995). Principles of CDK Regulation. *Nature.* 374(6518):131-134.

Mosmann, T. (1983). Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *Journal of Immunological Methods,* 65 :55-63.

Muraoka, O., Fujimoto, M., Tanabe, G., Kubo, M., Minematsu, T., Matsuda, H., Morikawa, T., Toguchida, I., Yoshikawa, M. (2001). Absolute stereostructures of novel norcadinane- and trinoreudesmane-type sesquiterpenes with nitric oxide production inhibitory activity from Alpinia oxyphylla. *Bioorg Med Chem Lett*., 11(16):2217-20.

Naitoh, K., Honjo, H., Yamamoto, T., Urabe, M., Ogino, Y., Yasumura, T. and Nambara, T. (1989). Oestrone sulphate and sulphatase activity in human breast cancer and endometrial cancer. *Journal of Steroid Biochemistry,* 33(6):1049-1054.

NCR (2004). The Malaysian cancer incidences. In: Lim, C.C.G., Yahaya, H. (Eds). Second report of the national cancer registry cancer incidence in Malaysia 2003/2004. National Cancer Registry, Kuala Lumpur.

Nesaretnam, K., Jin, Lim, E., Reimann, K., Lai, L.C. (2000). Effect of a carotene concentrate on the growth of human breast cancer cells and pS2 gene expression. *Toxicology,* 151(1-3): 117-26.

Newman, S.P., Purohit, A., Ghilcik, M.W., Potter, B.V.L. and Reed, M.J. (2000). Regulation of steroid sulphatase expression and activity in breast cancer. *Journal of Steroid Boichamistry and Molecular Biology*, 75:259-264.

Ng, J.H., Nesaretnam, K., Reimann, K., Lai, L.C. (2000). Effect of retinoic acid and palm oil carotenoids on oestrone sulphatase and oestradiol-17beta hydroxysteroid dehydrogenase activities in MCF-7 and MDA-MB-231 breast cancer cell lines. .*Int J Cancer*., 88(1):135-8.

Nupponen, N.N. *et al* (1998). Genetic alterations in prostate cancer cell lines detected by comparative genomic hybridization. *Cancer Genet. Cytogenet.,* 101: 53-57.

O`Brien, P.J., B.F. Hales, P.D. Josephy, A. Castonguay, Y. Yamazoe, F.P. (1996). Guengerich. Chemical carcinogenesis, mutagenesis and teratogenesis. Can. J. Physiol. Pharmacol. 74: 565-571.

Oberhammer, F., Wilson, J.W., Dive, C., Morris, I.D., Hickman, J.A., Wakeling, A. E., Walker, P.R. and Sikorska, M. (1993). Apoptotic Death in Epithelial Cells: Cleavage of DNA to 300 and/ or 50kb Fragments Prior to or in the Absence of Internucleosomal Fragmentation. *The EMBO Journal*, 12(9): 3679-3684.

O'Connor, R., Cesano, A., Lange, B., Finan, J., Nowell, P.C., Clark, S.C., Raimondi, S.C., Rovera, G., Santoli, D. (1991). Growth factor requirements of childhood acute T-lymphoblastic leukemia: correlation between presence of chromosomal abnormalities and ability to grow permanently in vitro. *Blood,* 77(7):1534-45.

OECD (1997). OECD guideline for the testing of chemicals: *in vitro* mammalian chromosome aberration test. The organisation for economic coorperation and development (OECD). *OECD Guideline*, 473:1-10.

Olofsson, M.H., Ueno, T., Pan, Y., Xu, R. Cai, F., van der Kuip, H., Muerdter, TE., Sonnenberg, M. Aulitzky, W.E., Schwarz, S., Andersson, E., Shoshan, M.C., Havelka, A.M., Toi, M., Linder, S. (2007). Cytokeratin-18 is a useful serum biomarker for early determination of response of breast carcinomas to chemotherapy. *Clin Cancer Res.,* 13(11):3198-206.

Opferman, J.T. (2007). Apoptosis in the development of the immune system. *Cell Death and Differentiation,* 1350-9047/07: 1–9.

Ormerod, MG. (1996). Further Applications to Cell Biology. *In*: Ormerod, M.G. (ed.). Flow Cytometry: A Pratical Approach, 2nd edition. Oxford University Press, Inc. New York.

Ottenbrite, R.M. and G.B. Butler, eds. (1984). Anticancer and Interferon Agents, Synthesis and Properties, Marcel Dekker, New York.

Owen-Schaub, L.B., Zhang, W., Cusack, J.C. (1995). Wild-Type Human P53 and a Temperature-Sensitive Mutant Induce Fas/APO-1 Expression. *Mol Cell Biol.,* 15:3032-3040.

Oyen, L.P.A. and Dung, N.X. (1999). Plant resources from Southeast Asia, No. 19: Essential oil plants. Prosea Foundation, Bogor, Indonesia. 60-65.

Pai, L.H. and Pastan, I. (1994). Immunotoxins and Recombinant Toxins for Cancer Treatment. *In*: De Vita, V. T., Hellman, S. and Rosenberg, S.A.(eds.) Important Advances in Oncology. Philadelphia: Lippincott.

Paine, T.M. *et al* (1992). Characterization of epithelial phenotypes in mortal and immortal human breast cells. *Int. J. Cancer,* 50: 463-473.

Parry, J.M. ed. (1996). Molecular Cytogenetics. *Mutation Research (Special Issue),* 372(2): 151- 294. Parry JM (ed) (1996).

Pasqualini, J.R. and Chetrite, G. (1999). Oestrone sulphatase versus oestrone sulphotransferase in human breast cancer: potential clinical applications. *Journal of Steroid Biochemistry and Molecular Biology*, 69(1-6):287-292.

Pasqualini, J.R., Chetrite, G. and Nestour, E.L. (1996b). Oestrone sulphatase activities in human breast cancer. *Journal of Endocrinology*, 150:S99-S105.

Pasqualini, J.R., Chetrite, G., Blacker, C., Feinstein, M.C., Delalonde, L., Talbi, M. and Maloche, C. (1996a). Concentrations of oestrone, oestradiol, and oestrone sulphate and evaluation of sulphatase and aromatase activities in pre- and postmenopausal breast cancer patients. *Journal of Clinical Endoccrinology and Metabolism*, 81(4):1460-1464.

Pasqualini, J.R., Cortes-Prieto, J., Chetrite, G., Talbi, M. and Ruiz, A. (1997). Concentrations of oestrone, oestradiol and their sulphatases, and evaluation of sulphatase and aromatase activities in patients with breast fibroadenoma. *International Journal of Cancer*, 70(6):639-643.

Pasqualini, J.R., Gelley, C., Nguyen, B.L. and Vella, C. (1989). Importance of oestrogen sulhates in breast cancer. *Journal of Steroid Biochemistry*, 34(1-6):1-6.

Pathak, R., Dey, S.K., Sarma, A., Khuda-Bukhsh, A.R. (2007). Genotoxic effects in M5 cells and Chinese hamster V79 cells after exposure to ^{7}Li-beam (LET=60keV/mum) and correlation of their survival dynamics to nuclear damages and cell death. *Mutat Res.,* 628(1): 56-66.

Patnaik, B.K., Rout, N.P., Sahu, G., Jena, B., Akhtar, J., Rajesh B.T. (2004). Nature's Chemotherapeutic Agents**.** The Biotech Journal, Online: 16-17. www.biotechjournal.com. 19 November, 2007.

Pauley, R.J. *et al* (1993). Immortal human mammary epithelial cell sublines. US Patent 5,206,165 dated Apr 27.

Paull, K.D., Shoemaker, R.H., Hodes, L., Monks, A., Scudiero, D.A., Rubinstein, L., Plowman, J. and Boyd, M.R. (1989). Display and Analysis of Patterns of Differential Activities of Drugs Against Human Tumour Cell Lines: Development in Mean Graph and Compare Algorithm. *J. Natl. Cancer Inst.,* 81: 1088-1092.

Peltoketo, H., Nokelainen, P., Piao, Y.S., Vihko, P. (1999). Two 17β-hydroxysteroid dehydrogenases (17HSDs) of oestradiol biosynthesis: 17HSD type 1 and type 7. *Journal of Steroid Biochemistry and Molecular Biology*, 69(1-6):431-439.

Perry, M.C. and Yabro, J.W. (eds.) (1984). Toxicity of Chemotherapy. Grune and Stratton Incorporation, New York.

Phillips, R.M., Bibby, M.C., Double, J.A. and Loadman, P.M. (1991). The Relationship Between the *In Vitro* Chemosensitivity of Tumour Cells and Tumour Response *In Vivo* in an Experimental Tumour Model. *International Journal of Cell Cloning*, 9: 144-154.

Plumb, J.A., Milroy, R. and Kaye, S.B. (1989). Effect of the pH Dependence of 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium Bromide-formazan Absorption on Chemosensitivity Determined by a Novel Tetrazolium-based Assay. *Cancer Research*, 49(16): 4435-4440.

Ponten, J. and MacIntyre, E.H. (1968). Long-term culture of normal and neoplastic human glia. *Acta Pathol Microbiol* 74:465-486.

Poutanen, M., Isomaa, V., Lehto, V.P. and Vihko, R. (1992). Immunological analysis ao 17β-hydroxysteroid dehydrogenase in benign and malignant human breast tissue. *International Journal of Cancer*, 50(3):386-390.

Poutanen, M., Isomaa, V., Peltoketo, H. and Vihko, R. (1995). Role of 17β-hydroxysteroid dehydrogenase type 1 in endocrine and intracine oestradiol biosynthesis. *Journal of Steroid Biochemistry and Molecular Biology*, 55(5/6):525-532.

Powis, G., Prough, R. A., eds. (1987). Metabolism and Action of Anticancer Drugs. Taylor & Francis Ltd.

Prasain, J.P., Tezuka, Y., Li, J.X., Tanaka, K., Basnet, P., Dong, H., Namba, T. and Kadota, S. (1997). Six novel diarylheptanoids bearing chalcone or flavone moiety from seeds of *Alpinia blepharocalyx. Tetrahedron,* 53(23): 783-786.

Pratt, W.B., Ruddon, R.W., Ensminger, W.D. and Maybaum, J. (1994). The Anticancer Drugs. 2nd edition. Oxford University Press Inc., New York.

Preston, R.J., W. Au, M.A. Bender, J.G. Brewen, A.V. Carrano, J.A. Heddle, A.F. McFee, S. Wolf, J.S. Wassom (1981). Mammalian *in vivo* and *in vitro* cytogenetic assays. *Mutation Res.* 87: 143-188.

Puck, T.T. (1958). Genetics of somatic mammalian cells III. Long-term cultivation of euploid cells from human and animal subjects. *J. Exp. Med.* 108: 945-956.

Purohit, A., H.J. Tutill, J.M. Day, S.K. Chander, H.R. Lawrence, G.M. Allan, D.S. Fischer, N. Vicker, S.P. Newman, B.V.L. Potter and M.J. Reed (2006). The regulation and inhibition of 17β-hydroxysteroid dehydrogenase in breast cancer. *Molecular and Cellular Endocrinology*, 248(1-2): 199-203.

Raina, K., Agarwal, R. (2007). Combinatorial strategies for cancer eradication by silibinin and cytotoxic agents: efficacy and mechanisms. *Acta Pharmacologica Sinica,* 28(9): 1466-1475.

Ramachandra, S. and Studzinski, G.P. (1995). Morphological and Biochemical Criteria of Apoptosis. *In*: Studzinski, G. P. (ed) Cell Growth and Apoptosis: A Practical Approach. IRL Press, Oxford University Press Inc., New York.

Rashid, K.A., Mullin, C.A. and Mumma, R.O. (1986). Structure-mutagenicity relationships of chalcones and their oxides in the Salmonella assay. *Mutation Research/Genetic Toxicology*, 169(3): 71-79.

Raskin, I., D.M. Ribnicky, S. Komarnysky, N. Ilic, A. Poulev, N. Berisjuk, A. Brinker, D.A. Moreno, C. Ripoll, N. Yakoby, J.M. O'Neal, T. Cornwell, I. Pastor, B. Fridlender (2002). Plants and human health in the twenty-first century. *Trends in Biotech*. 20: 522-541.

Rates, S.M.K. Plants as source of drugs. *Toxicon,* 39 (2001) 603-613.

Reed, J.C. (1997). Bcl-2 family proteins: strategies for overcoming chemoresistance in cancer. *In:* Kaufmann, S. H. (ed). Apoptosis: pharmalogical implications and therapeutic opportunities, vol. 41. Academic Press, New York.

Reed, J.C. (1996a). Balancing Cell Life and Death: Bax, Apoptosis and Breast Cancer. *Journal of Clinical Investigation,* 97: 2403-2404.

Reed, M.J., A. Purohit, L. W. L. Woo, S. P. Newman, and B. V. L. Potter. (2005). Steroid Sulfatase: Molecular Biology, Regulation and Inhibition. *Endocrine Reviews,* 26(2):171–202.

Reed, M.J., Rea, D., Duncan, L.J. and Parker, M.G. (1994) Regulation of estradiol 17b-hydroxysteroid dehydrogenase expression and activity by retinoic acid in T47D breast cancer cells. *Endocrinology*, 135: 4-9.

Richardson, C., Williams, D. A., Amphlett, G., Phillips, B., Allen, J. A. and Chanter, D. O. (1989). Analysis of data from *in vitro* cytogenetic assays. In: Kirkland, D. J., ed. Statistical Evaluation of Mutagenicity Test Data. UKEMS sub-committee

on guidelines for mutagenicity testing. Report Part III. Cambridge University Press, Cambridge.

Riley, K.J.-L., Maher, L.J. (2007). Analysis of p53–RNA interactions in cultured human cells. *Biochemical and Biophysical Research Communications,* 363 (2007) 381–387.

Rister, R. (1999). Japanese herbal medicine: The healing art of Kampo. Avery Publishing Group, New York. 33.

Roberts, R.A., Nebert, D.W., Hickman, J.A., Richburg, J.H. and Goldworthy, T.L. (1997). Pertubation of the Mitosis/ Apoptosis Balance: A Fundamental Mechanism in Toxicology. *Fundamental Applied Toxicology*, 38(2): 107-115.

Ronnett, G.V. *et al* (1993). Human neuronal cell line. US Patent 5,196,315 dated March 23.

Rosenblum, M.G., Cheung, L. and Lyu, M.A. (2006). Targeted polypeptides. United States Patent 20060171919. 8th November, 2007. *http://www.freepatentsonline.com/20060171919.html.*

Rossini, G.P., Pinna, C. and Malaguti, C. (1999). Different sensitivities of p42 mitogen-activated protein kinase to phorbol ester and okadaic acid tumor promoters among cell types. *Biochemical pharmacology,* 58: 279-284.

Rothfels, K.H. and Siminovitch, L. (1958). The Chromosome Complement of the Rhesus Monkey (Macaca mulatta) Determined in Kidney Cells Cultivated *in vitro*. *CHROMOSOMA*, 9: 163-175.

Roy, W.P., Kokileva, L., LeBlanc, J. and Sikorska, M. (1993). Detection of the initial stages of DNA fragmentation in Apoptosis. BioTechniques, 15:1032-1040.

Ruder, H.J., Loriaux, L and Lipsett, M.B. (1972), Oestrone sulphate: production rate and metabolism in man. *Journal of Clinical Investigation*, 51(4):1020-1033.

Rudin, C.M. and Thompson, C.B. (1998). Apoptosis and Cancer. *In*: Vogelstein, B. and Kinzler, K. W. (1998). The Genetic Basis of Human Cancer. McGraw-Hill Inc., New York.

Ryan, J.J., Danish, R., Gottleib, C.A. and Clarke, M.F. (1993). Cell Cycle Analysis of p53-induced Cell Death in Murine Erythroleukaemia Cells. *Molecular Cellular Biology,* 13: 711-719.

Sahtout, A.H., Hassan, M.D. and Shariff, M. (2001). DNA Fragmentation, An Indicator of Apoptosis in Cultured Black Tiger Shrimp *Penaeus monodon* Infected with White Spot Syndrome Virus (WSSV). *Dis Aquat Org,* 44;155-159.

Saibara, T., Himeno, H., Ueda, H., Onishi, S., Yamamoto, Y., Enzan, H., Hara, H., Takehara, Y. and Utsumi, K. (1994). Acute Hepatic Failure with Swollen

Mitochondria and Microvesicular Fatty Degeneration of Hepatocytes Triggered by Free Radical Initiator. *Lab Invest*., 70: 517-524.

Sam, T.W., Chew, S-Y., Matsjeh, S., Gan, E.K., Razak, D. and Mohamed, A.L. (1987). Goniothalamin Oxide: An Embryotoxic Compound from *Goniothalamus macrophyllus* (Annonaceae). *Tetrahydron Letters,* 28: 2541-2544.

Sandberg, F. and Bruhn, J.G. (1979). Screening of Plants for Biologically Active Substances. *In:* Sofowora, A. (ed). American Medical Plants. Obafemi Awolowo University Press.

Santner, S.J., Feil, P.D. and Santen, R.J. (1984). *In situ* oestrogen production via the oestrone sulphatase pathway in breast tumours: relative importance versus the aromatase pathway. *Journal of Clinical Endocrinology and Metabolism*, 59(1):29-33.

Santner, S.J., Ohlsson-Wilhelm, B. and Santen, R.J. (1993). Oestrone sulphate promotes human breast cancer cell replication and nuclear uptake of oestradiol in MCF-7 cell cultures. *International Journal of Cancer*, 54(1):119-124.

Sasano, H., Frost, A.R., Saitoh, R., Harada, N., Poutanen, M., Vihko, R., Bulun, S.E., Siverberg, S.G. and Nagura, H. (1996). Aromatese and 17β-hydroxysteroid dehydrogenase type 1 in human breast carcinoma. *Journal of Clinical Endrocrinology and Metabolism*, 81(11):4042-4046.

Satir, P., Christensen, S.T. (2007). Overview of Structure and Function of Mammalian Cilia. *Annu Rev Physiol*, 69: 377-400.

Savage, J.K.R. (1975). Classification and relationships of induced chromosomal structural changes. *J. Medical Genetics,* 12: 103-122.

Schwartzman, R.A. and Cidlowski, J.A. (1994). Glucocorticoid-induced Apoptosis of lymphoid cells. *Int Arch Allergy Immunol*. 105:347-54.

Schwartzman, R. A. and Cidlowski, J. A. (1993). Apoptosis: The Biochemistry and Molecular Biology of Programmed Cell Death. *Endocrine Reviews* 14(2): 133-151.

Scott, D., B.J. Dean, N.D. Danford, D.J. Kirkland. (1990). Metaphase chromosome aberration assays *in vitro*, in: D.J. Kirkland (Ed.) Basic Mutagenicity Tests: UKEMS Recommended Procedures, UKEMS/University of Cambridge Press, Cambridge.

Scott, W.N., Hawkins, R.A., Killen, E. and Miller, W.R. (1990). Levels of androgens conjugates and oestrone sulohate in patients with breast cryst. *Journal of Steroid Biochemistry*, 35(3/4):399-402.

Scudiero, D.A. *et al* (1988). Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumour cell lines. Cancer Res. 48:4827-4833.

Sharifah S.S., Tri, H.S., Azimahtol, H.L. (2007). Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio. *Cancer Cell Int.,* 7: 4.

Shaw, P., Bovey, R., Tardy, S., Sahli, R., Sordat, B. and Costa, J. (1992). Induction of Apoptosis by Wild Type p53 in a Human Colon Tumour-derived Cell Line. *Proc. Natl. Acad. Sci. USA*, 89: 4495-4499.

Sherr, C.J. (1996). Cancer Cell Cycles. *Science* (Washnigton D. C.), 274: 1672-1677.

Shier, W.T. (1991). Mammalian Cell Culture on $5 a Day: A Laboratory Manual of Low Cost Methods. University of Phillipines, Los Barios College, Laguna.

Shier, W.T. (1983). An Undergraduate Experiment to Demonstrate the Use of Cytotoxic Drugs in Cancer Chemotherapy. *American Journal of Pharmaceutical Education*, 47: 216-220.

Shim, S.J., Seong, J., Lee, I.J., Han, K.H., Chon, C.Y., Ahn, S.H. (2007). Radiation-induced hepatic toxicity after radiotherapy combined with chemotherapy for hepatocellular carcinoma. *Hepatology Research,* 37(11): 906-913.

Shin, D., Kinoshita K, Koyama K, Takahashi K. (2002). Antiemetic principles of Alpinia officinarum. *J Nat Prod.,* 65(9):1315-8.

Shin, J.E., Han, M.J., Song, M.C., Baek, N.I., Kim, D.H. (2004). 5-Hydroxy-7-(4'-hydroxy-3'-methoxyphenyl)-1-phenyl-3-heptanone: a pancreatic lipase inhibitor isolated from Alpinia officinarum. Biol Pharm Bull. 2004 Jan;27(1):138-40.

Shin, J.E., Joo, H.M., Kim, D.H. (2003). 3-Methylethergalangin isolated from Alpinia officinarum inhibits pancreatic lipase. *Biol Pharm Bull.,* 26(6):854-7.

Sinclair, J. (1955). A revision of the Malayan Annonaceae. *The Garden's Bulletin, Singapore,* 14: 149-545.

Singh, R.P., Al-Rubeai, M., Gregory, C.D. and Emery, A.N. (1994). Cell Death in Bioreactors: A Role for Apoptosis. *Biotech. Bioeng.* 44:720.

Singh, R.P., Al-Rubeai, M. and Emery, A.N. (1996a). Apoptosis: Exploiting Novel Pathways to the Improvement of cell culture Processes. *The Genetic Engineer and Biotechnologist* 16:227.

Singh, R.P., Emery, A.N. and Al-Rubeai, M. (1996b). Enhancement of Survivability of Mammalian Cells by Overexpression of the Apoptosis-Suppressor Gene bcl-2. *Biotechnology and Bioengineering,* 52:166-175.251.

Skalko, R.G. (1981). Biochemical mechanisms in developmental toxicology. In: Kimmel, C. A. and Buelke-Sam, J. (eds). Developmental Toxicology, Raven Press, New York.

Skehan, R. (1995). Assays of Cell Growth and Cytotoxicity. *In*: Studzinski, G. P. (ed). Cell Growth and Apoptosis: A Practical Approach. IRL-Oxford University Press Inc., New York.

Sluyser, M. ed. (1996). Apoptosis in Normal Development and Cancer. Taylor & Francis Ltd., London.

Smith, P., Nicholson, L.J., Syed, N., Payne,N., Hiller, N., Garrone, O., Occelli, M., Gasco, M., Crook, T. (2007). Epigenetic Inactivation Implies Independent Functions for Insulin-like Growth Factor Binding Protein (IGFBP)-Related Protein 1 and the Related IGFBPL1 in Inhibiting Breast Cancer Phenotypes. *Clin Cancer Res.,* 13: 4061-4068.

Smith, T. (1993). British Medical Association Family Health Encyclopedia. Colour Library Ltd., London.

Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan.

Sofowora, A. (1982). Medicinal Plants and Traditional Medicine in Africa. John Wiley & Sons Ltd., Chichester.

Solecki, R.S. (1975). Shanidar, IV, A Neanderthal flower burial of Northern Iraq. *Science,* 190: 880.

Soule, H.D., McGrath, C.M. (1986). A simplified method for passage and long-term growth of human mammary epithelial cells. *In Vitro Cell. Dev. Biol.,* 22: 6-12.

Soule, H.D. *et al* (1973). A human cell line from a pleural effusion derived from a breast carcinoma. *J. Natl. Cancer Inst.,* 51: 1409-1416.

Speirs, V., Green, A.R. and Atkin, S.L. (1998). Activity and gene expression of 17β-hydroxysteroid deydrogenase type I in primary cultures of epithelial and stromal cells derived from normal and tumourous human breast tissue: the role of IL-8. *Journal of Steroid Biochemistry and Molecular Biology*, 67(3):267-274.

Stein, C.A., LaRocca, R.V., Thomas, R. McAtee, N. and Myers, C.E. (1989). Suramin: An Anticancer Drug with a Unique Mechanism of Action. *J. Clin. Oncol*., 7: 499-508.

Strassman, R. (2001). DMT: The spirit molecule: A Doctor's revolutionary research into the biology of near-death and mystical experiences. Park Street Press, Rocehster.

Studzinski, G.P. ed. (1995). Cell Growth and Apoptosis: A Practical Approach. IRL-Oxford University Press Inc., New York.

Stute, P., Götte, M., Kiesel, L. (2007). Differential effect of hormone therapy on E_1S-sulfatase activity in non-malignant and cancerous breast cells in vitro. *Breast Cancer Research and Treatment,* 10.1007/s10549-007-9615-7.

Suffness, M. ed. (1995). TAXOL®: Scientific and applications. CRC Press Boca Raton.

Surh, Y.J., Park, K.K., Chun, K.S., Lee, L.J., Lee, E., Lee, S.S. (1999). Anti-tumor-promoting activities of selected pungent phenolic substances present in ginger. *J Environ Pathol Toxicol Oncol*., 18(2):131-9.

Suzuki, T., Miki, Y., Nakamura, Y., Moriya, T., Ito, K, Ohuchi, N., Sasano, H. (2005). Sex-steroid producing enzymes in human breast cancer. *Endocrine-Related Cancer*, 12: 701-720.

Suzuki, T., Moriya, T., Ariga , N., Kaneko, C., Kanazawa, M. And Sasano, H. (2000). 17β-hydroxysteroid dehydrogenase type 1 and type 2 in human breast carcinoma: a correlation to clinicopathological parameters. *British Journal of Cancer*, 82(3):518-523.

Syahida, A., Israf, D.A., Lajis, N.H., Khozirah, S., Habsah, M., Jasril, Permana, D. and Norhadiani, I. (2006). Effect of compounds from Natural Products on IFN-/LPS-Induced Nitric Oxide Production in RAW 246.7 Macrophages. *Pharmaceutical Biology,* 44:1, 50-59.

Tannock, I.F. and Goldenberg, G.J. (1998). Drug Resistance and Experimental Chemotherapy. *In*: Tannock, I. F. and Hill, R. P. (eds.). The Basic Science of Oncology, 3rd edition. The McGraw-Hill Book, Co-Singapore.

Tannock, I.F. and Hill, R.P. (1998). The Basic Science of Oncology, The McGraw-Hill Book, Co-Singapore.

Tannock, I.F. and Hill, R.P. (1992). The Basic Science of Oncology. The McGraw-Hill Companies, New York.

Tao**,** L.T., Wang, Z.-T., Zhu, E.-Y., Lu, Y.-H. and Wei, D.-Z. (2006). HPLC analysis of bioactive flavonoids from the rhizome of *Alpinia officinarum South African Journal of Botany***,** Volume 72, Issue 1, February 2006, Pages 163-166.

Tavares, D.C., Mazzaron, B.G.R, Silva, L.F., Chacon, T.C.C., Bastos, J.K. (2006). Propolis-induced genotoxicity and antigenotoxicity in Chinese hamster ovary cells. *Toxicol In Vitro,* 20(7):1154-8.

Tawata S, Taira S, Kobamoto N, Ishihara M, Toyama S. (1996). Syntheses and biological activities of dihydro-5,6-dehydrokawain derivatives. Biosci Biotechnol Biochem. Oct;60(10):1643-5.

Tawn, E.J. and Holdsworth, D. (1994). The role of cytogenetics in the investigation of mutagen exposure and chromosome instability, in: D.E. Rooney and B.H.Czepulkowski (Eds). Human Cytogenetics: Essential Data, John Wiley & Sons, Ltd, Chichester. 102-110.

Terziyska, A., L. Waltschewa, P. Venkov. (2000). A new sensitive test based on yeast cells for studying environmental pollution. *Environmental Pollution*, 109: 43-52.

Thompson, C.B. (1995). Apoptosis in the Pathogenesis and Treatment of Disease. *Science*. 267(5203):1456-62.

Thomson, G.E. (2007). The Health Benefits of Traditional Chinese Plant Medicines: Weighing the scientific Evidence: A report for the Rural Industries Research and Development Corporation. Rural Industries Research and Development Corporation (RIRDC), BARTON, Australia.

Timbrell, J.A. (1995). Introduction to Toxicology. 2nd ed. Taylor & Francis Ltd., London.

Tomatis, L. (1986). Relation between mutagenesis, carcinogenesis and teratogenesis - experience from the IARC monographs programme, in: C. Ramel, B. Lambert and J. Magnusson (Eds). Genetic Toxicology of Environmental Chemicals. Part B: Genetic Effects and Applied Mutagenesis. Proceedings of the Fourth International Conference on Environmental Mutagens, Stockholm, Sweden, June 24 – 28, 1985. Alan R. Liss, Inc., New York.

Toné, S, Sugimoto, K., Tanda, K., Suda, T., Uehira, K., Kanouchi, H., Samejima, K., Minatogawa, Y. and Earnshaw, W.C. (2007). Three distinct stages of apoptotic nuclear condensation revealed by time-lapse imaging, biochemical and electron microscopy analysis of cell-free apoptosis. *Experimental Cell Research,* 313(16): 3635-3644.

Trukhcheva, E.,Z. Lin, M. Milad, E. Confino and S.E. Bulun (2007). ERβ regulates ERα expression and response to estradiol via specific promoters in endometrium and endometriosis. *Fertility and Sterility,* 88(1): S60.

Twentyman, P.R. and Luscombe, M. (1987) A Study of Some Variables in a Tetrazolium Dye (MTT) Based Assay for Cell Growth and Chemosensitivity. *Br. J. Cancer*, 56: 279-285.

Umar-Tsafe, N., Mohamed-Said, M.-S., Rosli, R. Din, L.B. and Lai, L.C. (2004). Genotoxicity of goniothalamin in CHO cell line. *Mutation Research* 562: 91–102.

Umar-Tsafe, N., Ali, A.M., Stanslas, J., Napis, S., Din, L.B. and Inayat-Hussein, S.H. (2000). Enumeration of Apoptosis-Inducing Potential in Human Leukaemia Cells by Goniothalamin. Extracts from the oral presentation slides - published in the *Proceedings of the 6th National Conference on Medical Sciences,* USM Kubang Krian, Malaysia. Abstract: Page 34.

Utsumi, T., Yashimura, N., Takeuchi, S., MAruta, M., Maeda, K. and Rochefort, H. (1986). Elevated steroid sulphates expression in breast cancers. *Journal of Steroid Biochemistry and Molecular Biology*, 73(3-4):141-145.

Vargas, V.M.F., V.E.P. Motta, A.C. Leitao, J.A.P. Heriques (1990). Mutagenic and genotoxic effects of aqueous extracts of *Achyroline satureoides* in prokaryotic organisms. Mutation Res. 240: 13-18.

Venitt, S. and Parry, J.M. (1984). Background to mutagenicity testing, in: S. Venitt and J.M. Parry (Eds.), Mutagenicity Testing: A Practical Approach, IRL Press, Ltd., Oxford, 1984, pp. 1-24.

Venitt, S. and Parry, J.M. eds. (1984). Mutagenicity Testing: A Practical Approach. IRL Press, Oxford.

Venitt, S., Crofton-Sleigh, C. and Forster, R. (1984). Bacterial Mutation Assays Using Reverse Mutation. *In*: S. Venitt and J. M. Parry (eds). Mutagenicity Testing: A Practical Approach. IRL Press Ltd., Oxford.

Virchow, R. (1971). Cellular Pathology as Based Upon Physiological and Pathological Histology, 2nd edition. Translated by Chance, B. Dover Publications, New York.

Vital Statistics (1998). Department of Statistics, Malaysia. In: Vital Statistics: Disputed Cost of Creating a Drug. Wall Street Journal, November 9.

Vitetta, E.S., Thorpe, P.E. and Uhr, J.W. (1993). Immunotoxins: Magic Bullets or Misguided Missiles. *Trends Pharmacol. Sci*., 14: 148-154.

Vogel, C., Hager, C. and Bastians, H. (2007). Mechanisms of Mitotic Cell Death Induced by Chemotherapy-Mediated G_2 Checkpoint Abrogation. *Cancer Research,* 67: 339-345.

Vogelstein, B. and Kinzler, K.W. (1998). The Genetic Basis of Human Cancer. McGraw-Hill Inc., New York.

Wahid, M.I.A. (2004). Common Cancers: Breast Cancer. Malaysian Oncological Society, *http://www.malaysianoncology.org*. 17 November, 2007.

Walker, N.I., Harmon, B.V., Gobe, G.C., et al (1988). Patterns of Cell Death. *Methods Achiev Exp Pathol.* 13:18-54.

Wallace, R.W. (1999). Pharmacogenomics: The Next Logical Step. *DDT*, 4: 105-107.

Wang, T.H., and Wang, H.S. (1996). p53, Apoptosis, and Human Cancers. *J Formos Med Assoc.* 95:509-522.

Wani, M.C., Talylor, H.L., Wall, M.E., Coggon, P. and McPhail, A.T. (1970). *Journal of American Chemistry Society*, 93: 2325-2327.

Webb, S.J., Harrison, D.J., and Wyllie, A.H. (1997). Apoptosis: An Overview of the Process and its Relevance in Disease. *In:* Kaufmann, S.H. (ed.). Apoptosis:

Pharmacological Implications and Therapeutic Opportunities. Academic Press Ltd., London.

Wei, Q-Y., Ma, J.-P., Cai, Y.-J., Yang, L. and Liu, Z.-L. (2005). Cytotoxic and apoptotic activities of diarylheptanoids and gingerol-related compounds from the rhizome of Chinese ginger. *Journal of Ethnopharmacology,* 102(2): 177-184.

West, C.P.A. (2006). Standard Practice for Indirect Detection of Mycoplasma in Cell Culture by 4-6-Diamidino-2-2 Phenylindole (DAPI) Staining: ASTM International; ASTM Standard Test Method (Reapproved): E 1533-00.

Whitfield, M.L., George, L.K., Grant, G.D., Perou, C.M. (2006). Common markers of proliferation. *Nature Reviews Cancer,* 6: 99 - 106.

Wilkipedia (2007). Wilkipedia Free Encylopedia. Wilkimedia Foundation Inc., USA. (http://en.wikipedia.org/wiki/Alpinia_zerumbet).

Wilson, A.P. (1992). Cytotoxicity and Viability Assays. *In*: Freshney, R. I. (ed) (1992). Animal Cell Culture: A Practical Approach. 2nd edition. Oxford University Press, New York.

Wilson, W.R., Anderson R.F. an Denny, W.A. (1989). Hypoxia-selective Antitumor Agents. 1. Relationships Between Structure, Redox Properties and Hypoxia-selective Cytotoxicity for 4 Substituted Derivatives of Nitracine. *J. Med. Chem.,* 32:23-30.

Wong, S.F., Reimann, K., Lai, L.C. (2001). Effect of Transforming Growth Factor-β1, Insulin-Like Growth Factor-I and Insulin-Like Growth Factor-II on Cell Growth and Oestrogen Metabolism in Human Breast Cancer Cell Lines *Pathology*, 33(4): 454 – 459.

Wong, S.F. (2002). Roles of transforming growth factor-beta, insulin-like growth factors and proteases in human breast cancer. MSc Thesis. Universiti Putra Malaysia.

Working, P.K. (1989). Toxicology of the Male and Female Reproductive Systems. Hemisphere Publishing Corporation - A Member of the Taylor and Francis Group, London.

Wyllie, A.H., Kerr, J.F.R., Currie, A.R. (1973a). Cell death in the normal neonatal rat adrenal cortex. *J. Pathol.,* 111, 255-261.

Wyllie, A.H., Kerr, J.F.R., Macaskill, I.A.M., Currie, A.R. (1973b). Adrenocortical cell deletion: the role of ACTH. *J. Pathol.,* 111, 85-94.

Wyllie, A.H. (1997b). Clues in the p53 Murder Mystery. *Nature,* 389: 237-238.

Wyllie, A.H. (1997a). Apoptosis: An Overview. *Br Med Bull*., 53(3):451-65.

Wyllie, A.H. (1995). The Genetic Regulation of Apoptosis. *Current Opinion in Genetics and Development,* 5: 97-104.

Wyllie, A.H. (1992). Apoptosis and the Regulation of Cell Numbers in Normal and Neoplastic Tissues: an Overview. *Cancer Metastasis Rev.* 11:95-103.

Wyllie, A.H. (1987) Apoptosis: cell death in tissue regulation. *J Pathol,* 153:313-316.

Wyllie, A.H. (1985). The Biology of Cell Death in Tumours. *Anticancer Research,* 5: 131-136.

Wyllie, A.H., Morris, R.G., Smith, A.L. and Dunlop, D. (1984) Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. *J Pathol*., 142:67-77.

Wyllie, A.H., Kerr, J.F.R. and Curie, and R. (1980). Cell Death: The Significance of Apoptosis. *Int Rev Cytology*, 68: 251-306.

Xu, Z.-X., Liang, J., Haridas, V. Gaikwad, A., Connolly, F.P., Mills, G.B. and Gutterman, J.U. (2007). A plant triterpenoid, avicin D, induces autophagy by activation of AMP-activated protein kinase. *Cell Death Differ,* 14: 1948-1957.

Yadav, P.N., Liu Z, Rafi, M.M. (2003). A diarylheptanoid from lesser galangal (Alpinia officinarum) inhibits proinflammatory mediators via inhibition of mitogen-activated protein kinase, p44/42, and transcription factor nuclear factor-kappa B. *J Pharmacol Exp Ther.,* 305(3):925-31.

Yang, E. and Korsmeyer, S.J. (1996). Molecular Thanatopsis: A discourse on the BCL-2 family and cell death. *Blood*, 1996;88:386-401.

Yasumura, Y., Kawakita, Y. (1963). Studies on SV40 in tissue culture - preliminary step for cancer research in vitro. *Nihon Rinsho* 21: 1201-1215.

Ye, Y. and Li, B. (2006). 19S-19-Acetoxychavicol acetate isolated from Alpinia galanga inhibits human immunodeficiency virus type 1 replication by blocking Rev transport. *Journal of General Virology,* (2006), 87, 2047–2053.

Yonish-Rouach, E., Resnitsky, D., Lotem, J., Sachs, L., Kimchi, A. and Oren, M. (1991). Wild-type p53 Induces Apoptosis of Myeloid Leukaemic cells that is Inhibited by Interleukin-6. *Nature*, 352: 345-347.

Young, B.D. and Monard, S. (1996). Chromosome Analysis and Sorting by Flow Cytometry. *In*: Ormerod, M. G. (ed.). Flow Cytometry: A Pratical Approach, 2nd edition. Oxford University Press, Inc. New York.

Yue, W., Wang, J.P., Hamilton, C.J., Demers, L.M. and Santen, R.J. (1998). *In situ* aromatization enhances breast tumour oestradiol levels and cellular proliferation. *Cancer Research*, 58(5):927-932.

Zachary, I. (2003). Determination of Cell Number, in Hughes, D. and Mehmet, H. eds. Cell Proliferation and Apoptosis. BIOS Scientific Publishers, Oxford. Pages 13-36.

Zhang, Y., Zhao, W., Zhang, H.J., Domann, F.E. and Oberly, L.W. (2002). Over-expression of copper zinc superoxide dismutase suppresses human glioma cell growth. *Cancer Res.,* 62: 1205-1212.

Zhou, B.N., Baj, N.J., Glass, T.E., Malone, S., Werkhoven, M.C., van Troon, F., David, W.J.H., Kingston, D.G. (1997). Bioactive labdane diterpenoids from Renealmia alpinia collected in the Suriname rainforest. *J Nat Prod.,* 60(12):1287-93.

Zimmerman, F.K. (1975). Procedures used in the induction of mitotic recombination and mutation in the yeast *Saccharomyces cerevisiae. Mutation Res.,* 31: 71-86.