2.0 Literature Review

2.1 Antioxidants

2.1.1 Oxidants and antioxidants

An oxidant is defined as a substance which oxidizes any other substance or molecule in the presence of oxygen or other reducing agents. On the contrary, an antioxidant is a reducing agent which stabilizes oxidants by donating electrons or protons towards the oxidant. The Food and Nutrition board, Washington defines antioxidants or dietary antioxidants as substances in food which helps to decrease or minimize the adverse effects of reactive oxygen species (ROS) and/or reactive nitrogen species on human normal physiological functions (Cornelli, 2009).

ROS is a free radical or non-radical reactive molecule which contains oxygen atoms in the formation of the molecule. Some examples of ROS are hydroxyl, peroxyl, alcoxyl, and superoxide (Table 2.1). These molecules are highly reactive due to the presence of unpaired electrons on the outermost shell of the oxygen atom. In order to form the most stable state of an atom based on octet rule, ROS tends to oxidize other molecules so that the oxygen atom consists of eight electrons on the valence shell. This causes the formation of a chain reaction in which the molecule which losses the electrons after being oxidized by ROS tends to oxidize another molecule to stabilize itself. This chain reaction can be halted when there are substances such as antioxidants or other unpaired molecules that can be coupled with the ROS. These ROS are produced in the body or generated by the environment.

Table 2.1: List of reactive oxygen species (Cornelli, 2009)

Free radical/ Non-radical	Formula
Oxygen	$O_2^{\cdot}$
Superoxide	$O_2^{\cdot-}$
Hydroxyl	$OH^{\cdot}$
Peroxyl	$RO_2^{\cdot}$
Alkoxyl	$RO^{\cdot}$
Organic peroxide	ROOH
Hydrogen peroxide	H_2O_2

R= major chain

In living organisms, ROS is produced and accumulated through cellular metabolic processes. During cellular metabolism, superoxide anions being generated through the mitochondrial respiratory transport chain will be released into the mitochondrial matrix. In the presence of certain stimuli, the generation of superoxide anions will be increased. For example, serum deprivation, apoptosis, and tumor necrosis factor-alpha (TNFα) will induce the generation of superoxide anions in the mitochondria. These anions can be converted to hydrogen peroxide by superoxide dismutase and later to water and oxygen by catalase (Figure 2.1) (Han *et al.*, 2001; Novo and Parola, 2008). Apart from that, ROS can also be generated by the environment, for example by exposure to ultraviolet radiation (UV).

Oxidative effect of ROS tends to oxidize unsaturated fatty acids which will lead to lipid peroxidation. Apart from that, DNA damage caused by ROS is a factor which contributes to aging and disease. As a response towards the presence of ROS, our body's defense system will produce endogenous antioxidative enzymes such as catalase, superoxide dismutase and glutathione peroxidase to combat and reduce the accumulated ROS (Manian *et al.,* 2008). When the accumulated ROS overcomes antioxidants, a situation which is known as oxidative stress is formed. This oxidative stress will eventually lead to several pathological conditions such as inflammation, cardiovascular disease, carcinogenesis, neurodegenerative diseases and atherosclerosis. The presence of accumulated oxidative stress in our body can be evaluated through several clinical tests which includes determination of blood antioxidant capability. This involves the determination of blood uric acid, oxygen radical absorbance capacity and ferric reducing ability (Cemeli *et al.*, 2009; Cornelli, 2009).

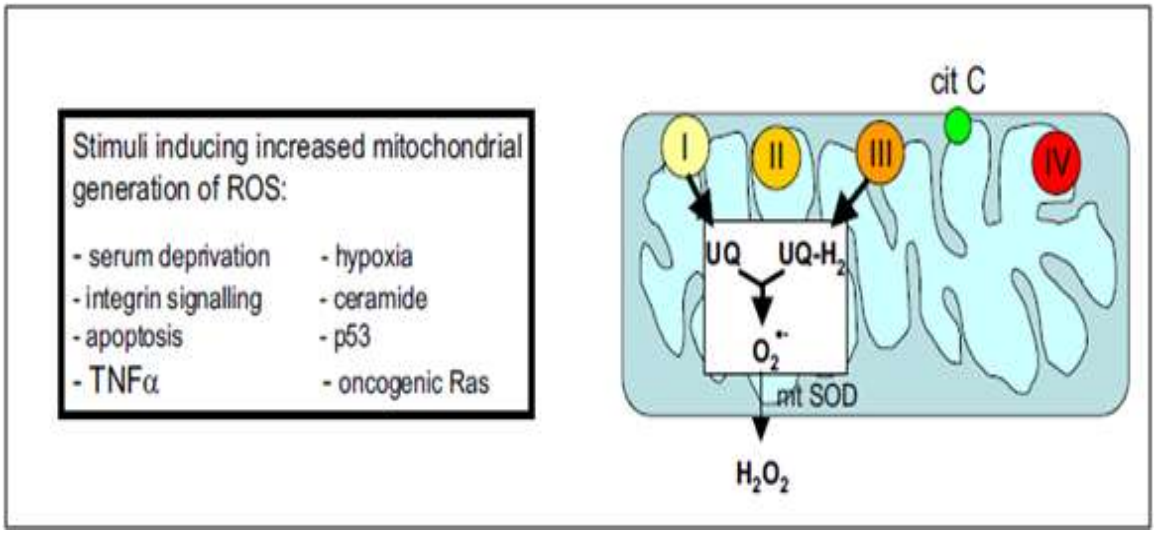

Figure 2.1: Generation of ROS in the mitochondria of living organism (Novo and Parola, 2008)

Electron transport chain (I, II, III, and IV) caused the production of hydrogen peroxide. UQ: ubiquinone, mt: mitochondria, cit: cytochrome, TNFα: tumor necrosis factor-alpha, p53: tumor protein 53.

2.1.2 Types of antioxidants

Antioxidants can be cathegorized as either endogenous or exogenous. Endogenous antioxidants (Table 2.2) are produced by our body while exogenous antioxidants are derived from food source which can be naturally obtained or synthetically produced. These antioxidants can be classified into four groups based on their localization: membrane antioxidants, circulating antioxidants, cytosol antioxidants and system antioxidants (Cemeli *et al.*, 2009; Cornelli, 2009).

Table 2.2: Types of endogenous and exogenous dietary antioxidants

Antioxidants	Types	Examples
Endogenous	Enzymes	Superoxide dismutase Glutathione peroxidase Catalase
	Hormones	Melatonin Oestrogen
	Other molecules in blood	Albumin Transferrin Lactotransferrin Uric acid
Exogenous	Vitamins	Vitamin C Vitamin E
	Polyphenols	Flavonoids (Quercetin, Flavones, Anthocyanins) Phenolic acids (Gallic acid, Salicylic acid) Tannins
	Synthetic compounds	Butylated hydroxyanisole (BHA) Butylated hydroxytoluene (BHT)

2.1.2.1 Endogenous antioxidants

Endogenous antioxidants can either be an enzyme, a hormone or a shock adsorber. Antioxidant enzymes which work as our first line of defense are produced naturally in the system to neutralize unstable oxidative species present as a result of cellular metabolic processes. Among the cellular antioxidant enzymes, the three most common are superoxide dismutase, glutathione peroxidase and catalase. The main activity of these enzymes is to convert toxic oxidative species into end products which are less or non-toxic. Superoxide dismutase; an enzyme found in the cytosol, can be divided into a few types depending on the ion coupled to it. The major superoxide dismutases which can be found in our body are copper-zinc superoxide dismutase (Cu-Zn SOD) and manganese superoxide dismutase (Mn SOD). The Cu-Zn SOD is present in the lysosomes and nucleus while Mn SOD in the mitochondria. SOD works as an enzyme which converts oxidative molecules such as superoxide anions into oxygen and hydrogen peroxide. Glutathione peroxidase can be found in tissues such as liver and kidney. It functions as a good antioxidant towards lipid peroxidation as it will reduce lipid hydroperoxide to alcohol. It will also reduce hydrogen peroxide into water and oxygen. Catalase which is present in most organs is highly active in the peroxisome. This enzyme catalyzes the reduction of hydrogen peroxide to water and oxygen. It is considered to be the most important endogenous enzyme as it has the highest turnover value. A molecule of catalase can convert millions of hydrogen peroxide molecules into water and oxygen in a second (Cemeli *et al.*, 2009).

Apart from enzymes, hormones such as melatonin and oestrogen can also act as endogenous antioxidants. Melatonin, unlike antioxidant enzymes; does not undergo redox reaction. It has free radical scavenging capability as it can capture up to ten ROS by donating hydrogen atoms from its amine group. Furthermore, melatonin functions in

the regulation of antioxidant enzymes and cellular mRNA expression of these enzymes (Rodriguez *et al.*, 2004). Oestrogen on the other hand, has a cardio protective effect. It helps to lower the level of interleukin-6 (IL-6) which is related to diseases such as cancer, atherosclerosis and diabetes. It also functions as an antioxidant by reducing oxidative stress and inhibiting the formation of superoxide anions and hydrogen peroxide (Wei *et al.*, 2001; Wilson *et al.*, 2006).

Apart from enzymes and hormones, other proteins and molecules can also function as antioxidants. Plasma proteins such as albumin, transferrin and lactoferrin have the ability to scavenge ROS. Albumin, a major protein in plasma has the capability to act as an antioxidant either through specific or non-specific binding. In specific binding, albumin binds to the free copper found in the physiological system. Free copper catalyzes the production of free radicals. The binding of albumin to copper will stop the reaction. In non-specific binding, albumin scavenges ROS in the presence of thiol groups (Kouoh *et al.*, 1999). Transferrin is a major iron transporting protein. It functions as a cell surface receptor which regulates cellular iron uptake. Cellular iron homeostasis will lead to cellular oxidative damage by ROS (Kotamraju *et al.*, 2002). Lactoferrin is an iron-binding protein that functions in protection from cell lysis. Iron can initiate the process of lipid peroxidation that causes breakage of membrane especially in erythrocytes. Binding of lactoferrin to erythrocytes reduce the occurrence of erythrocytes membrane lipid peroxidation as unsaturated lactoferrin still possess iron-binding capacity (Manevaa *et al.*, 2003). Other than proteins, uric acid in serum is also an important antioxidant. Even though high concentrations of uric acid is associated with increased cardiovascular risk, uric acid is shown to be effective in free radical scavenging. It can prevent lipid peroxidation as uric acid can effectively capture superoxide, hydroxyl and peroxynitrite radicals (Waring, 2002).

2.1.2.2 Exogenous dietary antioxidants

Dietary antioxidants can be in the form of vitamins, polyphenols or even synthetic antioxidants. Vitamins, such as vitamin C (a water soluble antioxidant) and E (a lipid soluble antioxidant) which can easily be obtained from dietary supplements are a good source of exogenous antioxidants. Even though the vitamins dissolve in different sources, both are powerful antioxidants. Vitamin C serves as an electron donor in the prevention of protein, lipid and DNA oxidation while vitamin E prevents the production of ROS during lipid oxidation. Both have the ability to prevent cardiovascular diseases, cancer and stroke (Kris-Etherton *et al.*, 2004; Padayatty *et al.*, 2003).

Phenolic compounds are a natural source of antioxidants and are widely found in plants. They can be divided into four classes which are flavonoids, phenolic acids, hydroxycinnamic acid derivatives and lignans. These simple compounds has been shown to possess the ability to combat or lower the incidence of several pathological conditions caused by oxidative stress such as cardiovascular disease, inflammation, neurodegenerative disease. Quercetin which is classified under flavonoids was proven to have the ability to reduce blood pressure of hypertensive subjects (Edwards *et al.*, 2007). Phenolic acids which are commonly found in wheat bran and berries are strong antioxidants against free radicals (Kim *et al.*, 2006). Apart from that, tannins which were either soluble or insoluble in water has been shown to have atheroprotective effects (Zargham and Zargham, 2008).

Apart from obtaining antioxidants from natural sources, synthetic antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) are widely used as antioxidants in the food industry. However, these synthetic antioxidants has been shown to possess toxic effects (Anagnostopoulou *et al.*, 2006), volatile and are easily

decomposed at high temperature (Hinneburg *et al.*, 2006). Due to these reasons, consumption of antioxidants from natural sources such as plants has become crucial.

2.1.3 Detection of exogenous and endogenous antioxidants

There are a few tests that had been used widely in the detection of the presence of exogenous and endogenous antioxidants. For exogenous antioxidants, there are varieties of tests available depending on the targeted function or compounds that are going to be quantified. Exogenous antioxidants can be quantified through total phenolic assay, DPPH assay and lipid peroxidation assay. Endogenous antioxidants such as catalase, glutathione peroxidase and superoxide dismutase can be quantified through chemical reactions based on their mechanisms in the body.

Total phenolic assay is the most basic assay to quantify phenolic compounds present in a mixture. Total phenolic content in a sample can be measured through Folin-Ciocalteu method. The Folin-Ciocalteu reagent consists of a mixture of phosphomolybdate and phosphotungstate. This assay depends on the oxidation-reduction reaction of Folin-Ciocalteu reagent with hydroxyl (-OH) groups or reducing agents present in the sample (Folin-Ciocalteu reagent). In the presence of reducing agents, the yellow colored reagent will be reduced to a blue colored solution.

DPPH is a stable free radical. In DPPH assay, it detects the scavenging ability of antioxidants present in samples towards DPPH are detected. As this assay has antiradical activity, it is also known as free radical scavenging assay. DPPH radical which absorb strongly at 517 nm will be reduced upon reduction by antioxidant. The kinetic reactions of the antiradical measurement depend on the nature and amount of

antioxidants present (William *et al.*, 1995). The scavenging activity of DPPH by antioxidant is shown in the equation below:

$$DPPH^{\cdot} + AO \rightarrow DPPH\text{-}H + AO^{\cdot}$$

*AO stands for antioxidant

Lipid peroxidation assay is an indirect measurement of antioxidants present in a sample as it does not quantify the antioxidative compounds but depends on the formation of the end product, malondialdehyde (MDA). Free radical will oxidize polyunsaturated lipid or fatty acids to form MDA which is toxic. MDA will react with thiobarbituric acid (TBA) to form a colored TBA-MDA complex (Figure 2.2). In the presence of antioxidants, it will terminate or inhibit the chain reaction leading to the formation of MDA. The formation of MDA is inversely proportionate to antioxidant availability.

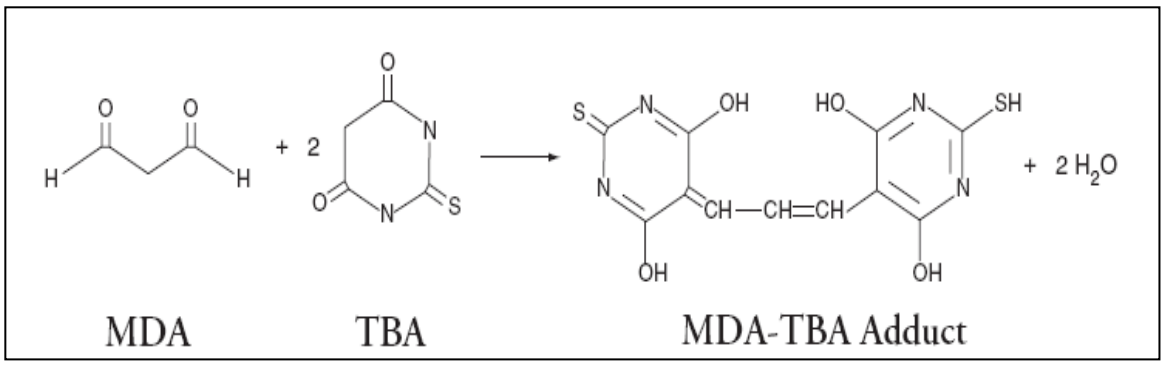

Figure 2.2: Formation of MDA-TBA complex in lipid peroxidation assay (TBARS Assay Kit, 2009)

Catalase will convert hydrogen peroxide into water and oxygen. As direct measurement of catalase activity based on hydrogen peroxide depletion is not stable, an indirect way to measure catalase activity is based on the reaction of potassium permanganate with

hydrogen peroxide remain after reacting with sample. It is based on the chemical equation below:

$$5\ H_2O_2 + 2\ KMnO_4 + 3\ H_2SO_4 \longrightarrow 2\ MnSO_4 + K_2SO_4 + 5\ O_2 + 8\ H_2O$$

When more catalase is present in the sample, more hydrogen peroxide will react with it, thus reducing the amount of hydrogen peroxide available. The lower concentration of hydrogen peroxide present in the mixture causes less decolorization of the purple color of potassium permanganate which absorbs stongly at 490 nm.

The measurement of glutathione peroxidase enzyme activity involves two consecutive steps. Firstly, in the presence of peroxide (R-OOH), reduced glutathione (GSH) will be oxidized forming oxidized glutathione (GSSG) in the presence of glutathione peroxidase (GPx). This is followed by conversion of GSSG back to GSH with the utilization of glutathione reductase (GR) and reduced β-nicotinamide adenine dinucleotide phosphate (NADPH).

$$\text{R-OOH} + 2\ \text{GSH} \xrightarrow{\text{GPx}} \text{R-OH} + \text{GSSG} + 2\ H_2O$$

$$\text{GSSG} + \text{NADPH} + H^+ \xrightarrow{\text{GR}} 2\ \text{GSH} + NADP^+$$

GPx determination can be considered an indirect measurement as it depends on the conversion of NADPH which absorbs at 340 nm in the presence of GPx . In the reaction, GSSG produced is proportionate to GPx present in lysate. Thus, a high level of GPx will eventually lead to faster decreament in NADPH measurement.

The activity of superoxide dismutase (SOD) can be measured indirectly based on the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide radicals. The process can be divided into two steps. First, in the presence of oxygen and water, xanthine will be converted to uric acid and superoxide radical. In the second step, SOD will convert the radical to oxygen and hydrogen peroxide. NBT will also convert superoxide radicals into oxygen by reduction of NBT to NBT-formazan which is blue in color.

$$\text{Xanthine} + O_2 + H_2O \xrightarrow{\text{XOD}} \text{Uric acid} + O_2^{\cdot} + H^+$$

$$2\,O_2^{\cdot} + 2\,H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

OR

$$\text{NBT} + 2\,O_2^{\cdot} \longrightarrow \text{NBT-formazan} + 2\,O_2$$

SOD will then compete with NBT in the conversion of superoxide radical into oxygen. Thus, the NBT-formazan produced is inversely proportionate to SOD present in the lysate.

2.2 Cancer

2.2.1 Formation of cancer

Cancer can be classified based on their origin, histology or staging. The origin refers to the type of tissue where the cancer is being developed. For example, carcinoma is derived from epithelial tissue; sarcoma from connective tissue or mesenchymal cells while leukemia originates from the hematopoietic cells (Waldum *et al.*, 2008). Apart from that, it can be classified based on their histology; study of cells and tissue by microscopic anatomy. Through this study, cancer can be categorized into four grades. At higher grade, the cells are highly abnormal and poorly differentiated. Grade 1 is the stage at which the cells are slightly abnormal but well differentiated. During grade 4, the cells are immature and undifferentiated. Besides the two types of classification, cancer can be grouped depending on their stage. In stage 0 or 1, cancer is usually localised to a certain area. At the highest stage (Stage IV), the cancer has spread to distal tissue or organ.

Cancer occurs when there is an uncontrolled growth or proliferation of cells. This can be due to the contribution and accumulation of oxidative stress in our body. Exogenous oxidative stress is accumulated when our body is exposed to carcinogenic chemicals, radiation, or even viral infections. Some examples of carcinogenic chemicals are tobacco tar, nitrosamines, polycyclic aromatic hydrocarbon, benzene and benzo[a]pyrene. Endogenous oxidative stress will be accumulated if our endogenous antioxidative enzymes do not neutralize ROS produced through metabolic processes. Oxidative stress has effects towards the DNA and signal transduction pathways. This will either initiate cell transformation or promote synthesis of growth factors (Mariete, 2000). As an example, when oxidative stress affects the DNA, it can cause changes or mutations in genes which regulate the synthesis of normal cells. The deregulation of

oncogenes, tumor-suppressor genes and microRNA genes will then lead to tumor formation as the cells lose the ability to detect terminal differentiation or undergo apoptosis (Croce, 2008).

Cancer formation can be divided into three stages which are: initiation, promotion and progression (Figure 2.3). Exposure to chemicals and radiation may irreversibly alter genes and cause DNA mutations. These mutations can either inactivate tumor-suppressor genes or activate oncogenes. If the mutated genes are able to replicate and survive throughout the cell cycle process, the cells will continuously divide and tumor formation will be initiated. The tumor promotion step is when the mutated gene is continuously replicated. This will lead to mutated cells being accumulated and it depends on the cell cycle process. When growth-related genes are being stimulated, cells proliferate by undergoing four phases cell cycle: G1, S, G2 and M phase. Uncontrolled cell proliferation will lead to the formation of a benign tumor or preneoplastic lesion. This occurs when the cells are resistant to cytotoxic compounds, not able to terminate cell differentiation, cannot undergo apoptosis and is undergoing uncontrolled growth. In the progression step, the malignant phenotype will be expressed and metastasis may occur (Bast *et al.*, 2000; Croce, 2008).

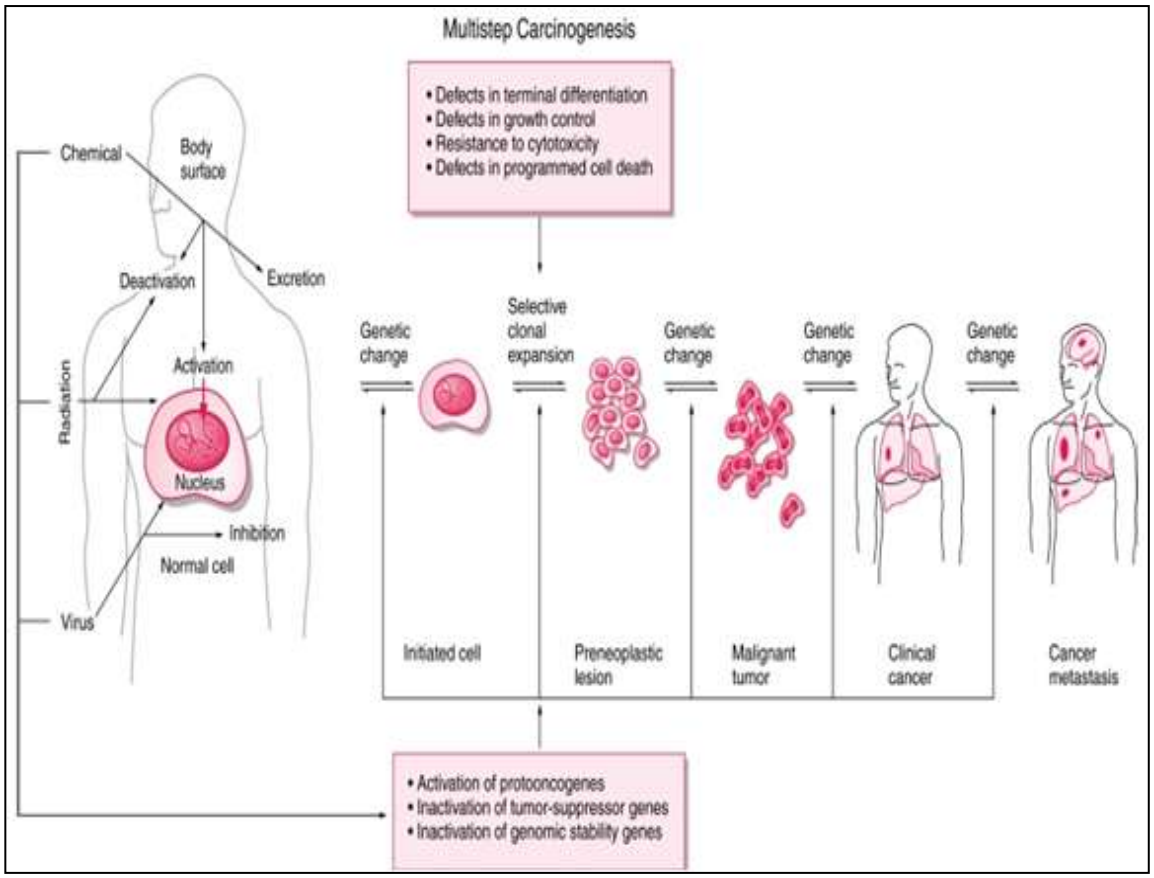

Figure 2.3: Cancer formation processes (Bast *et al.*, 2000)

2.2.2 Cancer statistics

Based on World Health Statistics 2009 by the World Health Organization (WHO), the average mortality rate due to cancer in the year 2004 is 1.29 per 1000 populations. The African region has the highest occurrence with a value of 1.47 per 1000 populations while the Eastern Mediterranean shows the lowest with 1.01 per 1000 populations. For the South-east Asia region, death rate is 1.07 per 1000 populations which is lower than the average value. However, in Malaysia there are 1.37 deaths per 1000 population due to cancer. These statistics also revealed that those from the higher income group have a slightly higher incidence compared to the lower income group (World Health Statistics 2009).

The *Malaysian Cancer Statistics–Data and Figure, Peninsular Malaysia 2006* retrieved from Majlis Kanser Nasional showed that there were a total of 21,773 cancer cases diagnosed in Malaysia during the year 2006. The five most diagnosed were cancers of the breast, colorectal, lung, cervix and nasophrynx. The occurrence of cancer incidents can be categorised based on sex, race and age. Statistics showed that females make up 54.19% of total cancer cases. Females are more prone towards breast cancer followed by colorectal and cervix, while colorectal cancer was shown to be the most prominent in males. Among all the races, the Chinese were the higher risk group to be diagnosed with cancer compared to Malays and Indians. Youngsters have a higher probability of developing leukemia compared to older individuals as the latter dominate the top five cancers diagnosed (Zainal *et al.*, 2006).

2.2.3 Current treatment for cancer

Cancer treatment is generally either through surgery, radiation, chemotherapy, hormonal treatment, and/or targeted treatments depending on the stage of the cancer tumor and the health status of the patient. In the case of benign tumors, the most common treatment is to remove the tumor by surgery. As for malignant tumors, radiation and chemotherapy are usually performed after the removal of the tumor. However, radiation and chemotherapy have the same weakness. Both will unselectively kill healthy cells along with cancer cells. Chemotherapy uses drugs which interfere with the cell cycle causing them to undergo apoptosis. These drugs will either promote the up-regulation of proapoptotic molecules or down-regulation of antiapoptotic molecules. The list of molecules that may be regulated depending on treatment is shown in Table 2.3.

The use of targeted or biological therapy may work as a better option in cancer treatment. These treatments are more specific compared to other treatments that are not able to identify and target only cancerous cells. Targeted therapy functions by targeting specific proteins or pathways that cause cell proliferation in cancer cells or activate the apoptosis program (Solary *et al.*, 2002). Current research on gene therapy and the usage of stem cells which are still in clinical trial could be useful in cancer treatments. Gene therapy is a way to prevent or combat cancer by altering a person's genetic material (Dass and Choong, 2006) while stem cells can be used to replenish the cells after radiotherapy or chemotherapy (Sagar *et al.*, 2007). It is hoped that in the future, more treatment methods will be developed through better understanding of the mechanism of cell death. The research in natural product may be a way in the search of new drugs for cancer treatment.

Table 2.3: List of antiapoptotic and proapoptotic molecules (Solary, 2002)

Type of modulator	Antiapoptotic	Proapoptotic
Bcl-2-family of proteins	Bcl-2, Bcl-X_L, Mcl-1	Bax, Bak, Bcl-X_s, Bim, Bid, Bad
Caspases	Caspase isoforms (2S,9b) Nitrosylated caspase-3 Phosphorylated caspase-9	Initiator caspases (2L, 8, 9, 10) Effector caspases (3, 6, 7)
Heat shock proteins	Hsp27, Hsp70	Hsp60, Hsp90
Cell cycle	$p21^{CIPI/WAF1}$, $p27^{Kip1}$	p53
Lipid signals	Ceramide	Diacylglycerol
Kinases	Phosphatidylinositol 3'-kinase (PI-3K) Protein kinase C (PKC) Mitogen activated protein kinase (MAPK)	c-Jun N-terminal kinase (JNK)
Other modulators	Inhibitors of apoptosis (IAPs) FLICE inhibitory protein (FLIP) Nuclear factor-kappa B (NF-κB)	Death receptor

2.2.4 Detection of anticancer agents

The trend of cancer treatment nowadays is to focus on the more natural and specific or targeted pathway. These drugs will work faster, safer and more specific compared to what we have in the market at the moment. Unlike synthetic drugs, drugs which obtained from natural product have lesser or no side effect towards normal cells as they can serve as our daily consumption. There are several ways to determine the effectiveness of these agent and their pathways.

Cell survival rate after treatment with natural product lead compounds can be determined by 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. MTT is a yellow colored tetrazolium salt which can be reduced by succinate dehydrogenase in the respiratory chain to form purple colored formazon. As it occurs in living cells, the quantity of insoluble formazon precipitated is directly proportional to the amount of living cells present after a certain treatment. MTT has been used as a method to determine the cytotoxic effect of samples towards cells (Bernharda *et al.*, 2003). In this assay, it is important to ensure whether the treatment samples possess reducing capability as it might give false negative results. If the samples have reducing properties, it has to be removed prior to the addition of MTT (Peng *et al.*, 2005; Shoemaker *et al.*, 2004).

The most common way to eliminate unwanted cells would be directing the cells to undergo apoptosis. The easiest method to detect apoptosis after certain treatment would be conducting DNA fragmentation assay. In this assay, DNA will be fragmented into oligonucleosomal size with intervals of 180-200bp. This phenomenon is due to the presence of DNA fragmentation factor (DFF) which acts as the apoptotic endonuclease. DFF is a heterodimeric protein which consists of DFF40 and DFF45. During apoptosis,

DFF45 which acts as an inhibitor of DFF40 will dissociate from DFF by the cleavage of activated caspase 3. Free or activated DFF40 which possess intrinsic nuclease activity will then cleave chromosomal DNA at the internucleosomal site (Zhang and Ming, 2000). The fragmented DNA can be separated through agarose gel electrophoresis. In apoptosis cells, it will form a DNA ladder with approximately 180bp intervals (Daniel *et al.*, 1999).

2.3 *Ficus* spp.

2.3.1 *Ficus* taxonomy

The taxonomy of *Ficus deltoidea* is classified by United States Department of Agriculture (USDA Plant Database) as follows:

Kingdom:	Plantae (plant)
Subkingdom:	Tracheodionta (vascular plant)
Superdivision:	Spermatophyta (seed plant)
Division:	Magnoliophyta (flowering plant)
Class:	Magnoliopsida (dicotyledon)
Subclass:	Hamamelididae
Order:	Urticales
Family:	Moraceae (Mulberry family)
Genus:	Ficus L. (fig)
Species:	*Ficus deltoidea*

Ficus or commonly known as figs are from the Moraceae family. This genus covers approximately 800 species of woody tree, shrubs, vines, epiphytes and hemiepiphyte. They are usually found in the tropical and sub-tropical regions of the world. The flowers of *Ficus* are very tiny while the fruits are big. Due to this, *Ficus* is known as "no flower fruit" in Chinese. Each *Ficus* species depends on its fig wasp for pollination (Lansky *et al.*, 2008). It is often cultivated as an ornament and is widely used in landscaping (Wong, 2007).

In ancient times, *Ficus carica* was used as a medication for tumors, swelling and ulcers while the bark of *Ficus racemosa* was used in the treatment of diabetes, rheumatism and eczema (Lansky *et al.*, 2008). In modern times, *Ficus* has been shown to have the

ability to be used for treatment of certain diseases. *Ficus pumila* L. which was used by the Japanese to treat diabetes and high blood pressure also possess high antioxidant activity (Abraham *et al.*, 2008). Research done by Ao *et al*. showed that the extracts of *Ficus microcarpa* L. have antioxidant and antibacterial activities (Ao *et al.*, 2008).

2.3.2 *Ficus deltoidea*

Ficus deltoidea (Figure 2.4) is a shrub herb from the Moraceae family. It is grown in Malaysia, Indonesia and Africa with different botanical names. *F. deltoidea* is known as 'Mas Cotek', 'Telinga Beruk' or 'Serapat Angin' by the Malays.

This plant is classified into male and female plants with different varieties. The most obvious characteristic of the plant is the morphology of their leaves (Figure 2.5). Male plants have smaller leaves with red spots at the underside of the leaves while female plants are bigger in size and have black spots rather than red. Even though *F. deltoidea* is generally classified by gender, each gender can be further divided into different types. For example, there are at least two types of female plant depending on the size of the leaves, medium type leaves and large type leaves. Besides the leaves, the size of the fruits can be also a way to differentiate *F. deltoidea*. The fruit of male plants are smaller compared to female plants.

Traditionally, this plant has been used in the treatment of a large variety of ailments and symptoms such as irregular blood sugar level, high blood pressure, nerve and joint pains, and migraine. It is also claimed to be effective in delaying menopause, improving blood circulation, and tightening the uterus wall after giving birth.

Figure 2.4: *Ficus deltoidea* plant with fruits

Figure 2.5: *Ficus deltoidea* female (left) and male (right) leaves

F. deltoidea extract has been shown to contain secondary metabolites such as flavonoids, tannins, triterpenoids, proanthocyanins and phenols. These compounds act as antioxidants and anti-inflammatory agents help in prevention and treatment of certain diseases (Desaku). Zunoliza *et al.* who worked on antioxidant activities and secondary metabolites showed a correlation between antioxidant activities and polyphenolic compounds (Zunoliza *et al.*, 2009). The non-enzymatic and enzymatic antioxidant levels of *F. deltoidea* has been reported by Hakiman and Maziah (Hakiman and Maziah, 2009). Based on their reports, female leaves possess better antioxidant effects compared to male leaves in most of the antioxidant assays conducted.

Work by Adam *et al*. provides significant scientific evidence towards antidiabetic properties of *F. deltoidea* as claimed traditionally. They showed that the plant extract has the ability to enhance either basal or insulin-stimulated glucose uptake into Chang liver cell line (Adam *et al.*, 2009). Besides antidiabetic effect, *F. deltoidea's* water extracts has been proven to have anti-ulcer (Zahra *et al.*, 2009) and antinociceptive effects (Sulaiman *et al.*, 2008). In anti-ulcer effect, treatment of *F. deltoidea's* extract prior to absolute ethanol-lesion induction reduced the ulcer area compared to the untreated ulcer. While for antinociceptive effect, the presence of flavonoid and tannins could be the possible explanation since they are proven for their anti-inflammatory activity.

2.4 Genomics and Proteomics

Genomics studies which started abundantly in the early 70's enable us to understand the genetic materials of an organism. For example, the human genome project which was completed in 2003 gave us a greater understanding towards human gene organization. As the gene might be mutated and the protein translated could be different from the expected protein, it caused some limitations in only studying the gene itself. Due to this reason, proteomics studies became a new aspect in the late 90's. Proteomics studies can potentially elucidate the properties, structures, and functions of proteins. The combination of both genomics and proteomics studies permits thorough inspection of pathways involved in particular processes.

2.4.1 Genomics

Genomics is a comprehensive study of genetic material of an organism. The study is important as each individual carry their own genetic materials which determine the growth, appearance, diseases throughout their life time. DNA sequencing is the most basic technique used to understand the gene as it will give us the exact order of the bases which build up DNA.

2.4.1.1 Reverse transcription-polymerase chain reaction (RT-PCR)

The exact expression of genes can be visualized through the study of their transcriptomes (RNA). RT-PCR can be employed to analyze the expression of a specific gene. The understanding of the gene expressed can provide us a better inspection towards the complex regulatory network and biological processes. Besides, it can be used as a technique to quantify the copies of mRNA present in certain environment.

2.4.2 Proteomics

Proteomics studies consist of two contrasting but complementary approaches. Cell-mapping proteomics aims to elucidate the protein-protein interactions in complex signaling pathways. Protein expression proteomics on the other hand, monitors global expression of large numbers of proteins within a sample, and quantitatively identifies patterns of expression change under different circumstances (Anderson *et al.*, 2000; Simpson and Dorow, 2001). The most basic way to show a protein profile is the application of sodium dodecyl sulphate (SDS) gel electrophoresis. Besides that, the elucidation of protein profile can also be done with high performance liquid chromatography (HPLC), two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), matrix assisted laser desorption/ionization (MALDI), surface-enhanced laser desorption/ionization (SELDI), liquid chromatography- mass spectrometry (LC-MS). In this study, 2D-PAGE and MALDI were incorporated.

2.4.2.1 Two dimensional polyacrylamide gel electrophoresis (2-D PAGE)

2-D PAGE study is composed of two steps; a first dimension isoelectric focusing (IEF) step followed by a second dimension sodium dodecyl sulphate PAGE (SDS-PAGE). In first dimension electrophoresis, the proteins are separated based on charge (pI) while the second dimension electrophoresis separates the proteins based on molecular mass (M_r). It is an effective way to elucidate the proteome map of protein mixtures as it is unlikely for proteins to share similar properties in both charge and molecular mass. The advantage of this technique is the ability to resolve post-translationally modified (PTM) proteins into multiple spots (Oh-Ishi and Maeda, 2007). This enables us to identify the changes by comparing the spots with its actual properties.

2.4.2.2 Matrix assisted laser desorption/ionization (MALDI)

MALDI is a technique used in mass spectrometry to analyse biomolecules such as peptides, nucleotides and oligosaccharides. These biomolecules will be fragmented when ionised. MALDI TOF/TOF will elucidate the spectrum by detecting the masses of the molecules based on their mass to charge (m/z) ratios and later identified based on the comparison with the database available. Those with lighter mass travel faster and were detected earlier by the sensor. TOF/TOF (time of flight) indicate that the peptide undergo two times of TOF acceleration. During the first detection, it will select, isolate and fragment precursor ion of interest. It will then followed by reacceleration of the precursor ion and fragments, and again the mass and intensity of the fragmented ion will be detected. This was done with the help of low molecular weight, UV absorbing matrix. Ionised matrix will collide with neutral samples producing ionized samples which will be quantified based on their mass and charge of the samples.