CHAPTER 2

Literature review

Cancer is a severe disease and is the main leading cause of death worldwide. The human body has various types of cells, which can become uncontrolled and differentiate into cancer cell through cell hyper-proliferation, cell differentiation and loss of apoptosis or programmed cell death. Cancer occurs as a result of an alteration or mutation of genes that control cell function. Abnormalities of genetic materials are caused by carcinogens such as cigarettes, smoke, radiation, chemicals or pathogen. Genes related to cancer may occur without specificity during DNA duplication or may be transmitted genetically. Since, cancer cell can metastasize into vital organs such as intestines, lungs, brain or liver, the short-term survival of the cancer patient is not surprising. According to data from the World Health Organization (2008), the number of colorectal cancer patients increases annually worldwide with 60,000 people dead from this disease each year while the rate of risks for having this disease throughout a person's lifetime has increased from 1:25 (30 years ago) to 1:20 with gradual upward trends. In Thailand, the latest statistics showed that, colorectal cancer was the third highest cause of death among patients who died from cancer after lung cancer and liver cancer (Attasara and Buasom, 2011). Nevertheless, cancer can be prevented and cured if diagnosed at an early stage.

2.1 Colorectal cancer

Colorectal cancer is the severe pathological features of epithelial cells lining the large intestinal tract caused by various factors such as environmental and genetic factors. The large intestine and section between rectum and sigmoid colon are the sensitive areas for cancer pathogenesis (Grady, 2004; Krysko *et al*., 2008).

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Although colorectal cancer is a severe type of cancer, this cancer can also be completely cured as evidence that the chances of surviving more than five years after surgery are 60% and will increase to 80% if cancer can be diagnosed quickly. However, the treatments of colorectal cancer remain a debatable issue in the medical field because the treatment capacity depends significantly on its cost.

2.1.1 Colorectal Cancer Symptoms

- Insidious onset of symptom is encountered at approximately 70-80%. This group of patients has gradual symptoms such as gradually increased in abnormal excretions. The factor with significant influence on symptoms is the location of the cancer (Markowitz *et al*., 2002).

- Acute intestinal obstruction is encountered at approximately 10-20%. This group of patients is found to have gradually occurring symptoms. However, because of patients' negligence or inaccurate diagnosis with gastrointestinal diseases or dysentery and treatment by decreasing bloating which may help to improve patients' symptoms.

- Perforation and peritonitis is encountered at approximately 5-10%. Patients with these symptoms have severe abdominal infections which can be fatal. In addition, the cancer may metastasize to the abdominal cavity. (Winawer *et al*., 1997; Regine *et al*., 2004)

2.1.2 Stages of Colorectal Cancer

Classifying colorectal cancer into stages has tremendous benefits for planning treatment after diagnosis. Classification of the stages of the disease according to the metastasis of cancer is divided into stages 0, 1, 2, 3 and 4 as follows: (Figure 1) (Fadok *et al*., 2000; Edinger and Thompson, 2004)

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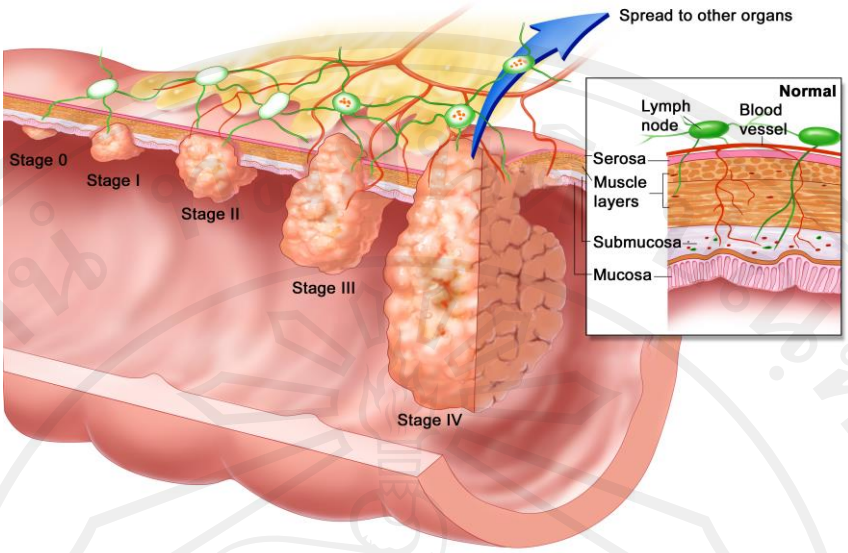

Figure 1 Changes of colorectal cancer cells at various stages

Source: (MetroHealth, 2013)

Stage 0 is the earliest stage of colorectal cancer with the cancer existing only on the epithelial tissue of the intestine.

Stage 1 is the stage where the cancer is at the intestinal walls and has not spread outside the intestines.

Stage 2 is the stage where the cancer has spread outside the intestines but has not reached the lymph node.

Stage 3 is the stage where the cancer has spread to nearby lymph node but has not reached other organs.

Stage 4 is the stage where the cancer has spread to other organs, mostly the liver and lungs.

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Recurrence is when the cancer recurs after treatment.

Stage 3 and Stage 4 of colorectal cancer patients are considered to be at metastasized stages due to an ineffective in the treatment as well as the tumors were incompletely removed. These reasons result in a high chance for recurrence. The study found that, although cancer has spread, a group of patients responded very well to chemotherapy after surgery. The chemotherapy drug mainly composed of irinotecan which a single use or combination with 5-flourouracil (5-FU). Nevertheless, the difficulties in planning treatment for colorectal cancer patients in the metastasis stages is due to the doctors were previously unaware of how to identify patients in the group that will respond to chemotherapy and patients who will not be affected. At present, however, new knowledge has revealed the amount of DNA in cancer cells to help in predicting treatment outcomes in the aforementioned cases (Butler *et al*., 1999; Acehan *et al*., 2002; Goldstein and Kroemer, 2007).

2.2.3 Colorectal Cancer Etiology

1. Food: Food low in fiber with high protein and fat content are risk factors for colorectal cancer. Persons who consume meat higher than fibrous foods such as vegetables and fruits are found to be at higher risk for having colorectal cancer than persons who consume fibrous foods. On the contrary, persons who regularly consume fibrous foods are found to be capable of reducing colorectal cancer incidence rates. Concerning this factor, Thailand is believed to be accepting more consumption cultures from the Western world, especially when it comes to meats, foods with high fat content and various types of fast foods. Hence, the incidence of colorectal cancer is gradually increasing.

2. Genetics: If parents and siblings are found to have colorectal cancer, family members will have higher detection rates than ordinary persons.

3. Polyp: Polyp usually occur at the large intestine walls or colon. Polyps causing cancer are called malignant polyps.

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4. Crohn's Disease: Ulcerative Colitis: Patients with Crohn's Disease or ulcerative colitis will have thirty time higher cancer incidence rates than ordinary persons.

Mechanisms involved in the occurrence of cancer are the alteration from normal cells into cancer cells, which can be caused by a number of factors such as random mutations, gene rearrangements, chemical stimulation or induction such as polycyclic aromatic hydrocarbon, tar and aromatic amine, etc. At each stage, the abnormalities of various genes have been found, e.g. APC gene, K-ras gene, DCC gene and P53 gene (Zamzami *et al*., 1995; Gordon 1999). The physical factors also causes of cancer such as radiation and viral infection, especially oncogenic viruses divided into DNA and RNA viruses. DNA viruses compose of papovaviruses, herpesviruses and adenoviruses. RNA viruses consist of retroviruses, which have reverse transcriptase enzymes capable of converting RNA into DNA (Cecconi and Gruss, 2001; Devasagayam *et al*., 2004; de Bruin and Medema, 2008) .

2.2 Current colon cancer treatments

Bevacizumab is classified as the first anti-cancer drug in the angiogenesis inhibitors'group, or anti-angiogenesis drugs, which can act to inhibit angiogennesis. This mechanism is different from older generation of anti-cancer drugs. The drug enhances the effectiveness of drug chemotherapy. Bevacizumab was found to be capable of reducing angiogenesis and inhibiting the spread of cancer cells in colorectal cancer cells implanted nude mice. Bevacizumab was shown to be effective in preventing cancer cell growth in experimental animals when administered with Cisplatin or Trastuzumab at doses lower than doses with effective treatment (Hurwitz *et al*., 2004).

This drug prevents cell damage caused by the reactive agents created by the drug metabolizing enzymes. Enzymes capable in creation of highly reactive radicals are phase I enzymes such as CYP1A1, CYP1A2, CYP2A6, and CYP2A13, etc. (Guengerich, 2001; Jalas *et al*., 2003; Park *et al*., 2005) and phase II drug metabolizing enzymes such as arylamine N-acetyltransferase-1 (NAT1) and NAT-2 (Hein *et al*.,

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1992). These enzymes convert substances and are related to certain types of cancer. Therefore, the inhibition of the function of these enzymes suppresses the creation of carcinogens in the body. Phase II drug metabolizing enzymes usually play a role in detoxification and excretion of reactive agents. Phase II metabolizing enzyme include glutathione S-transferase (GST), UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT), epoxide hydrolase and NADPH-quinone oxidoreductase (NQO1). The enzymes conjugate with various receptors such as glutathione, glucuronate, sulfate, which add the water molecule (trans-addition) and reduce quinines with two electrons, respectively. An increase in function of these enzymes reduces the chance of carcinogen reaction (Chen and Kong 2004; Kwak *et al*., 2004). Furthermore, this also increases the solubility and accelerates the elimination from the body. Carcinogen suppression can also be performed by increasing antioxidant system such as antioxidant enzymes consisting of glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione reductase, glutamy-cystein ligase (GCL), heme oxygenase-1 (HO-1) and non-enzymes such as ferritin and bilirubin (Fridovich, 1995; Mates and Sanchez-Jimenez, 1999). Chemotherapy drug acts only on divided cells and has no effects on resting cells while some drugs are only effective at some stages of cell division such as mitosis or synthesis stages. Therefore, understanding about the cell cycle is useful in selecting chemotherapy drugs and proper frequency because the chemotherapy drugs destroy the dividing cells without distinguishing to normal dividing cells such as hair follicle cells or epithelial cells in the gastrointestinal tract and proliferating cancer cells (McDonnell, 1993). This unidentified activity of chemotherapy could lead to various side effects. Therefore, the consideration in the use of chemotherapy drug should regard the benefits from controlling cancer and reducing the side-effects of chemotherapy drugs on patients.

Therefore, the use of medicinal plants is an alternative for treating cancer. Many chemicals, especially polyphenols and coumarins, the natural substances and substances with sulfur atoms such as isothiocyanate can stimulate the expression and function of phase II drug metabolizing enzymes and antioxidative enzymes. These substances are found in vegetables and fruits. The most studied substances include curcumin found in curcuma which suppresses liver cancer (Chuang *et al.,* 2000), epigallo-catechin gallate (EGCG) which prevent skin, liver, colorectal and forestomach cancer in rats (Park and Surh, 2004) and other catechins, the flavanol group found in green tea, quercetin and

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rutin (Chen and Chan, 1996; Surh, 2003) found in citruses (oranges and limes) , mulberry leaves and guava leaves. Sulforaphane, a substance in the sulfur group has been found to be capable of suppressing breast and skin cancer (Tseng *et al.*, 2004), while phenethyl isothiocyanate (PEITC) is found in broccoli, cabbage and other vegetables. The mechanisms of these substances in combatting cancer are similar. They may have direct antioxidation activity due to the presence of polyphenolics or chelating metals, inhibition of the Fenton reaction (Lopes *et al*., 1999; Middleton *et al*., 2000), phase II enzymes stimulation, phase I enzyme suppression (Muto *et al*., 2001) and stimulation of the antioxidative enzymes (Chen and Kong, 2004; Kwak *et al*., 2004). Substances stimulated phase II enzymes are called "Monofunctional inducers" while substances stimulated CYP (β -naphthoflavone and indole-3-carbinol) are called "Bifunctional inducers". Although CYP1A1 stimulation may have negative effects by enhancing the effects from the alteration of carcinogens, high increases in the function of phase II enzymes and antioxidantive enzymes caused these chemicals to become protective chemicals (Enari *et al.*, 1998).

2.3 Oxidative stress and antioxidants

2.3.1 Free radicals from oxygen

Reactive oxygen species (ROS) occurred during cellular respiration which are frequently encountered in higher amounts than other types of oxidants are divided into two groups consisting of reactive oxygen species comprising of superoxide ($O_2^{\bullet-}$) (Halliwell and Gutteridge, 1985; Bagchi and Puri, 1998; Beckman and Ames, 1998; Leiris, 2003) and hydroxyl radical (°OH) while non ROS radicals consist of hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Leiris, 2003) (Figure 2).

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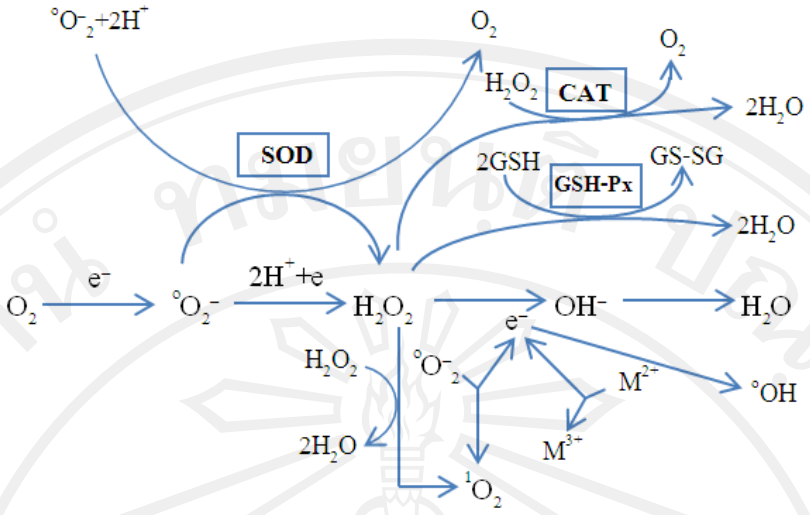

Figure 2 Formation and destruction of Reactive Oxygen Species

Source: Leiris (2003)

Superoxide, hydrogen peroxide, peroxide and hydroxyl radicals are classified as the group of primary reactive oxygen species (Wu and Cederbaum, 2003). Cellular respiration involves four times of the oxygen reduction until the generation of water and oxygen and the complete electron transport is not found in all reactions. It was found that superoxide is regularly created with the use of every oxygen molecule (Beckman and Ames, 1998). When oxygen changes into a reactive oxygen species or reactive oxygen metabolites (ROM), the chemical characteristics of reactive oxygen species can be found in either atoms or molecules with unpaired electrons at the outer orbitals. Single and independent electrons have high reactive capabilities. Superoxide reduction can continuously occur through the catalysis of superoxide dismutase (SOD) (Beckman and Ames, 1998; Leiris, 2003) or SOD-like compounds. This reaction generates hydrogen peroxide (Leiris, 2003). The formation of superoxide and hydrogen peroxide is estimated to occur at approximately 1-2% of all oxygen passing through the electron transport chain (Beckman and Ames, 1998). Hydrogen peroxide is reduced as follows:

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1) by catalase enzymes (CAT) or glutathione peroxidase (GSH-Px)

2) by peroxidase enzymes and yield singlet oxygen ($^1O_2$)

3) to generate hydroxyl radicals through the Fenton reaction using the catalysis of transition metals (M) such as iron or copper (Leiris, 2003; Beckman and Ames, 1998). Hydroxyl radical (°OH) is hypothesized to be the oxidant causing oxidative stress and destructing various molecules in the cells (Beckman and Ames, 1998).

4) by reacting with superoxide in creating singlet oxygen ($^1O_2$) (Leiris, 2003; Beckman and Ames, 1998). Singlet oxygen has molecular formula as $^1\Delta gO_2$ and $^1\Sigma g^+O_2$, respectively.

The reduction of one molecule of oxygen results in one molecule of superoxide. Superoxide has been found to have a half-life of 10^{-15} seconds if the superoxide dismutase enzymes already presented (Leiris, 2003) (Figure2). Furthermore, the interaction between superoxide and nitric oxide (NO) can generate peroxynitrite ($ONOO^-$) (Beckman and Ames, 1998; Nguyen *et al*., 2004).

Superoxide is mostly generated in the mitochondria and a rate of generation depends on the amount of oxygen used in this organelle. In general, superoxide half-lives are short. However, this radical can react with all of the molecules or atoms presented in the body (Halliwell and Gutteridge, 1985). Superoxide is necessary for producing hydroxyl radical after conversion into hydrogen peroxide by superoxide dismutase enzymes. The half-life of hydrogen peroxide is approximately 10^{-3} seconds and 10^{-8} seconds if there are catalase enzymes as a continuing reaction from the formation of superoxide. There are two types of superoxide dismutase in mammal' cells, manganese-centered SOD (Mm-SOD) found in mitochondria and copper-zinc-centered SOD (Cu/Zn-SOD) found in cytoplasm. Generally, hydrogen peroxide is a highly reactive radical in the reaction which slowly reacts with every types of organic matter. With the presence of iron and copper in the Fenton reaction, hydrogen peroxide is rapidly oxidized by superoxide dismutase to generate hydroxyl radical, which is a reactive oxygen species with higher reactive capacity than superoxide and hydrogen peroxide. Hydroxyl radical can react with all biological substances. Moreover, hydroxyl

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radical can react with all substance molecules surrounding areas with this radical creation. Furthermore, hydroxyl radical can be created from Haber - Weiss reaction (Figure3).

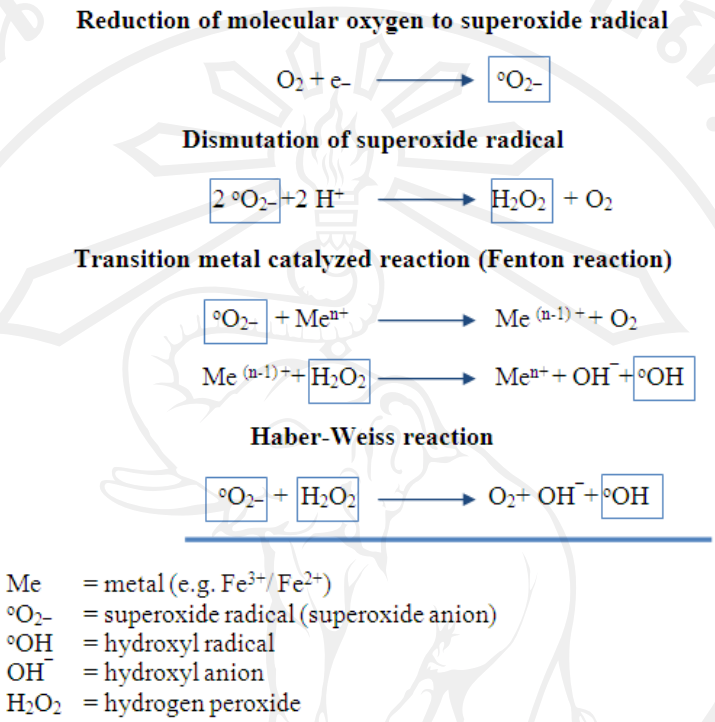

Figure 3 The formation and destruction of radical generated from oxygen and Fenton and the Haber-Weiss Reaction

Source: Leiris (2003)

The interaction between superoxide and hydrogen peroxide can generate two molecules of hydroxyl radical. Moreover, this radical can be produced by the reaction between superoxide and nitric oxide. Singlet oxygen ($^1O_2$) is one form of oxygen molecules without paired electrons with the half-life of approximately 2.10^{-6} seconds. Singlet oxygen is formed in cells by the substitution reaction in the reaction of hyper chloride and hydrogen dioxide formation (Leiris, 2003). Hydrogen peroxide can be produced in animal bodies by numerous reactions. This radical can be converted into hydroxyl radical or water by glutathione peroxidase (Alessio and Blasi, 1997). In addition to production in the mitochondria, free radicals can be produced in peroxisome

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from β-oxidation of fatty acids, which produces hydrogen peroxide as a by-product. Nevertheless, the oxidation in the peroxisome have been found to have a large numbers of catalase enzymes. Therefore, it is uncertain whether hydrogen peroxide will leak from peroxisome and cause oxidative stress in cells under normal conditions (Klauning *et al*., 1998; Wei *et al*., 2000). However, during the induction of rat liver cancer, the amount of peroxisomes in the liver was found to have increased significantly causing oxidative stress. Therefore, it is possible that more radicals will be produced during the regeneration of hepatocytes. The function of cytochrome P450 is related to NADPH. Cytochrome P450 reduces oxygen to superoxide and potentially causing oxidative stress (Figure 4) . Moreover, phagocytes can generate free radical such as superoxide, hydrogen peroxide, nitric oxide and hypochlorite (Degenhardt *et al*., 2002). While, chronic inflammation has been found to be a significant source of free radicals in the body (Beaudeux *et al*., 1996; Beckman and Ames, 1998).

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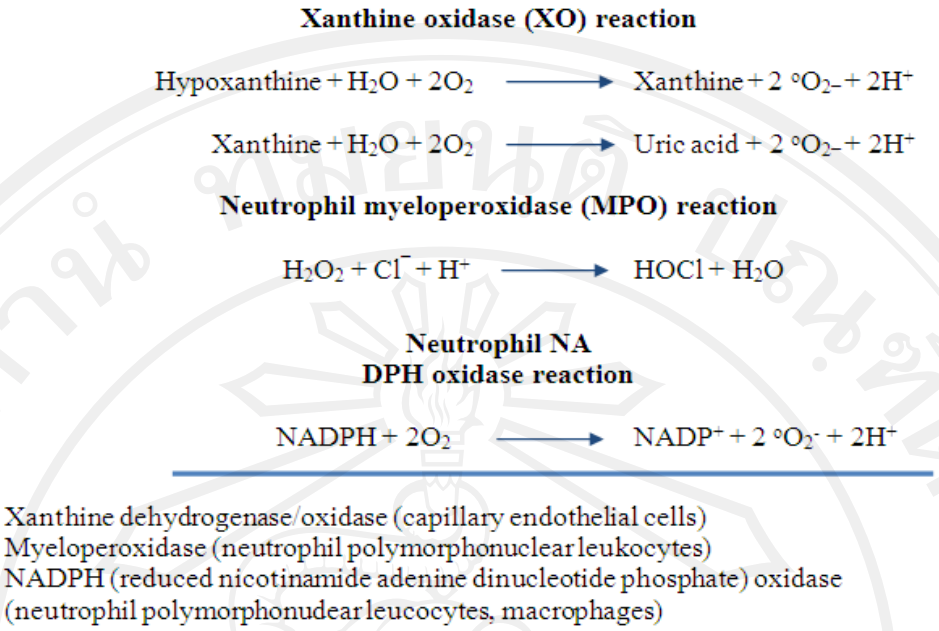

Figure 4 Formation of oxygen radicals catalyzed by xanthine oxidase, myeloperoxidase and NADPH oxidase.

Source: Leiris (2003)

2.3.2 Free radicals produced by Nitrogen

Important free radicals in the nitrogen group consist of nitric oxide (Bagchi and Puri, 1998) and peroxynitrite produced by the reactions of superoxide ($O^{-}_{2}\cdot$) and nitric oxide (NO·), which are highly reactive free radicals. Furthermore, peroxynitrite can occur in the body as a result of reactions of superoxide and nitric oxide by stimulating reactions with glutathione, cysteine and deoxyribose (Sikka, 1996)

2.3.3 The role of free radicals on body balance

Most free radicals are unstable and have high reactive capabilities. In addition, all of compounds can be damaged by these free radicals (Leiris, 2003). When oxidative stress occurs, it causes lipid peroxidation, particularly in phospholipids of the cell membranes, which compose of unsaturated fatty acid susceptible to destruction by unstable molecules (Thomas and Aust, 1986; Aust *et al*., 1993; Wu and Cederbaum,

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2003). The damages of cell membrane can lead to cell death. In addition, free radicals also cause enzymes dysfunction especially in enzyme composed of sulfhydryl groups which are important targets of reactive oxygen species. The damages of proteins channels can lead to the loss of function in ion channel regulation. Free radicals give various adverse effects to proteins, such as; denaturing and fragmenting while these denatured proteins are destroyed and eliminated outside the cells. Furthermore, many types of enzymes such as catalase, glyceraldehydes-3-phosphate, dehydrogenase, glutathione peroxidase, adenylate cyclase, myofibrillar ATPase and creatine kinase (Leiris, 2003) can be denatured by reactive oxygen species. In addition to the effects on fat and protein, free radical also damage DNA, a genetic material in the cells, by causing fragmentation of structures or nucleotides with impacts on DNA sequence (Thomas and Aust, 1986; Aust *et al*., 1993; Wu and Cederbaum, 2003). In recently, it is accepted that, excessive free radicals production in the body is a cause of many oxidative stress-related diseases involved with inflammation (Canavese *et al*., 1987), respiratory distress syndrome (Bostex, 1989), organ transplantation, neurologic diseases, gastrointestinal diseases and cancer (Floyd, 1990; Leiris, 2003).

2.3.4 Antioxidants

The scavenging system of free radicals in mammals required oxygen in cellular respiration is divided into two forms; enzyme and non-enzyme free radical scavengers (Yu, 1994). The enzymes involved in enzymatic mechanisms compose of superoxide dismutase (SODs), catalase (Canavese *et al*., 1987; Bostex, 1989; Cross and Jones, 1991; Wu and Cederbaum, 2003) and glutathione peroxidase (Canavese *et al*., 1987; Wu and Cederbaum, 2003). The types of superoxide dismutase in mammals are found to differ according to sites in cells and ions of metals which are key components in function such as copper-zinc superoxide dismutase is found in cytosol, the space between outer and inner membranes of mitochondria. Furthermore, manganese superoxide dismutase is found in mitochondrial matrix. Both types of enzymes help prevent toxicity of free radical produced in cells. In addition to superoxide dismutase, catalase and glutathione peroxidase enzyme systems help eliminate hydrogen peroxide. Catalase enzyme catalyses the breakdown of two molecules of hydrogen peroxide into water and oxygen or supports hydrogen peroxide to react with compounds that can

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donate a hydrogen ions, which will produce water molecules. Glutathione peroxidase enzyme systems are composed of glutathione peroxidase, glutathione reductase, glutathione (GSH) and nicotnamide adenosine dinucleotide phosphate (NADPH). The function of these enzymes is to eliminate hydrogen peroxide. Furthermore, glutathione has been found to react directly to destroy free radicals. Since glutathione is not normally synthesized in the mitochondria, it is transported from cytosol in to mitochondria by using the mitochondrial membrane transport proteins (Wu and Cederbaum, 2003). Non-enzymatic mechanisms are related to vitamin E (α-tocopherol) and vitamin C (Johnson, 1979; Basaga, 1990; Wu and Cederbaum, 2003). Vitamin E is an antioxidant found in large amounts in cell membranes and vitamin E is an important substance in preventing lipid peroxidation (Packer *et al*., 1979; Devasagayam *et al*., 2003). The aforementioned vitamins can also function with glutathione and vitamin C (Wu and Cederbaum, 2003). Furthermore, secondary minerals such as selenium, zinc, vitamin A and beta-carotene can help to eliminate free radicals. The current bio-markers used to check oxidative stress levels in the blood or tissues are as follows; isoprostane (8-iso-$PGE_{2\alpha}$) malondialdehyde (MDA), glutathione (GSH), glutathione oxidized, glutathione reduced, glutathione peroxidase (GSHPx activity), superoxide dismutase (SOD activity), catalase activity, selenium, zinc (Zn), vitamin C, vitamin E, β-carotene and vitamin A (Wood *et al*., 2003).

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2.4 Antioxidants from plants

Most antioxidants from plants are phenolic compounds. This group of compounds contains various types such as tocopherol, tocotrienol, γ- oryzanol, oryzanols (Lai *et al*., 2007), caffeic acid, syring acid, rutin, (-)-epicatechin, (+)-catechin, gallic acid, vanilic acid, p-coumaric acid, feruric acid and quercetin (Que *et al*., 2007) including alkaloid, glycoside, tannin, steroid, saponin, stigmasterol, flavonoid (Ramamoorthy and Bono, 2007; Vichitphan *et al*., 2007). Phenolic compounds have a variety of pharmacological effects depending on different chemical structures. For example, some types have properties in antiallergic, anti-cancer, anti-inflammation, anti-viruses, preventing heart diseases, reducing blood cholesterol levels and antioxidation (Phan *et al*., 2005). These compounds are found in many plant species and they exhibit antioxidant properties in both *in vitro* and *in vivo* studies.

Antioxidants are intimately involved in the prevention of cellular damage, the common pathway for a variety of diseases, including cancer. It is believed that plant-based diets contain substances, possibly antioxidants, which protect against the development of cancer. A number of plant species have been reported to contain different kinds of antioxidants. *Nigella saliva* L., *Platycodon grandiflorum* A., *Cuscuta reflexa* Roxb. and *Tribulus terrestris* L. are known as sources of antioxidant enzyme such as catalase, superoxide dismutase (SOD) and peroxidases (Canavese *et al*.,1987; Wu and Cederbaum, 1989), while tea, *Pomegranate*, guava leaf, grape, *Gynostemma pentaphyllum* (Thunb.) and *Emblica officinalis* Gaertn. are the promising sources of non-enzyme antioxidants such as phenolic compounds, carotenoids, vitamin C, and vitamin E (Johnson, 1979; Kaur and Kapoor, 2001). Aside from the direct measurement of antioxidant activities in plant extracts, studies in animal models have also helped to shed light on the capacity of such those plant extracts to reduce free radicals. A significant decrease in malondialdehyde, the end product of cellular lipid peroxidation and an increase in oxidative free radical scavenging enzymes such as superoxide dismutase, catalase and glutathione peroxidase have been reported in rats fed with extracts from *Magnifera indica*, *Emblica officinalis* (Anila and Vijayalakshmi, 2003) and *Centella asiatica* (Kumar and Gupta, 2003).

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In recent years, *Moringa oleifera* Lam. and *Pseuderathemum palatiferum* (Nees) Radlk. have become the popular medicinal plants in Thailand. The use of these two plant species covers a broad spectrum from common maladies to chronic diseases. Besides being used for curing disease, they are also consumed by healthy people for gaining health benefits. Although *M. oleifera* and *P. palatiferum* have been suggested for colon cancer therapy, there has not been any scientific evidence supporting this application. Leaves of these two plants recently become a widespread form of products and are promoted commercially as having anticancer and antioxidant properties. Nevertheless, there are no laboratory results to confirm or support the possibility for consumer use. Thus, the antioxidant property and the capacity in treating colon cancer of these two plant species are still challenged.

2.5 Plants used in this study

***Moringa oleifera* Lam.** (Siddhuraju and Becker, 2003)

Kingdom Plantae

Class Magnoliopsida

Order Brassicales

Family Moringaceae

Genus *Moringa*

Species *M.oleifera*

Binomial Name *Moringa oleifera* Lam.

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Figure 5 Characteristics of *Moringa oleifera* Lam. leaves.

Moringa is a herbal tree from Moringaceae family with the scientific name of *Moringa oleifera* Lam and is used as food in many countries. Moringa is native to the western and southern Himalayas, India, Pakistan, Asia Minor, Africa and Arabia (Somali *et al*., 1984; Mughal *et al*., 1999) and has spread to the Philippines, Cambodia, Thailand, Central America, North America, South America and the Caribbean islands (Morton, 1991; Ghasi *et al*., 2000). Moringa is also known by various other names such as “drum stick tree”, “horse radish tree” or “kelor tree”. Moringa trees reach a height of five to ten meters and are perennial plant that grows well in humid or dry climates with good endurance against drought (Adebayo *et al*., 2011). Moringa trees have thick grey bark, white fragrant flowers and long green fruits with edible parts consisting of leaves, fruits, flowers and young fruits (Cajuday and Pocsidio, 2010). In Thailand, so called “Ma-Room” in Thai, is known in every Thai kitchen as almost all parts of it are commonly used in many dishes such as Kaengsom and Kaengom (Figure 5). In terms of pharmacological uses, every part of *M. oleifera* has been found to have medicinal properties. In South Asia, the parts used are root, bark, gum leaves, pods, flowers, seed and seed oil. Every parts of the *M. oleifera* are edible with nutrition and medicinal

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values (Fuglie, 1999; Pocsidio, 2008; Atawodi *et al*., 2010). Ghasi *et al.* (2000) reported the use of the crude extract from the leaves of *M. oleifera* at a concentration of 200 mg/ml to be able to reduce serum cholesterol levels of rats fed high fat diet. In this experiment, *M. oleifera* crude extracts at that concentration was found to have no impact on blood protein levels. Thereafter, studies were conducted on the effects of *M. oleifera* seed extracts to inhibit the incidence of tumors and skin cancer. Various compounds such as 4a-L-rhamnosyloxy.-benzyl isothiocyanate, niazimicin, niazirin, *O*-ethyl-4-a-L- rhamnosyloxy benzyl, carbamate, b-sitosterol, glycerol-1-9-octadecanoate, 3-*O*-6X-*O*-oleoyl-b-D-glucopyranosyl -b-sitosterol and b-sitosterol-3-*O*-b-D-glucopyranoside were isolated from aqueous and ethanolic extracts of *M. oleifera* seeds by using gas chromatography. All these compounds were found to be able to inhibit tumor and skin cancer, while niazimicin showed the highest inhibitory capabilities (Guevara *et al*., 1999). Afterwards, the ability to reduce blood glucose levels of diabetic rats induced by streptozotocin was noted. *M. oleifera* extracts at the dose of 200 mg/kg BW have ability to reduce blood glucose levels in diabetic induced rats (Kumar *et al*., 2009). Similarly, Alaaeldin (2010) reported that ethanolic extract of *M. oleifera* seed extracts at a dose of 1 g/kg BW could reduce cirrhosis induced by carbon tetrachloride in rats after eight week of the treatment period. Furthermore, the ethanolic extracts from *M. oleifera* leaves could inhibit free radicals by increasing catalase level and reducing lipid peroxidation. *M. oleifera* extract at a dose of 100 mg/kg BW has been found to be capable of suppressing free radical equal to 50 mg of vitamin E (Verma *et al.*, 2009).

Although every part of the *M. oleifera* has many benefits, the leaves were found to be more popular than other parts due to a variety of benefits and its simplicity to be prepared as food. Key substances and various pharmacological effects of *M. oleifera* leaves are shown in Table 1.

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Table 1 Summary of chemical components and pharmacological effects of various parts of the *Moringa oleifera* Lam.

Tree part	Chemical components	Pharmacological effects	References
Leaves	ascorbic acid, flavonoid, phenolics, carotenoids, estrogenic compounds and beta-sitosterol, iron, calcium, phosphorus, vitamin A, vitamin B, vitamin C, alpha-tocopherol, riboflavin, protein and essential amino acid such as methionine, cysteine, tryptophan, lysine, nitrile, mustard oil glycosides, thiocarbamate glycosides	Reduces blood pressure	Makkar and Becker, (1996) Faizi *et al*. (1998); Gilani *et al*. (2006)
		Cholesterol-lowering activity	Mehta *et al.* (2003); Anwar *et al*. (2007); Chumark *et al*. (2008)
		Antioxidant activities	Kumar and Pari, (2003); Chumark *et al.* (2008); Yammuenart *et al.* (2008); Paliwal *et al*, (2011)
		Antispasmodics activity	
		Hepato-protective activities	Pari and Kumar, (2002)
		Antibacterial and antifungal activities	Bennett *et al*. (2003); Kumar and Pari, (2003); Pal *et al.* (2006)
		Anticancer and antitumor activities	Nadro *et al.* (2005)
		Control of thyroid hormone levels	Berger *et al*. (1984)
		Hypoglycemic activities	Manjari *et al.* (2007)

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Table 1 Summary of chemical components and pharmacological effects of various parts of the *Moringa oleifera* Lam. (Continued)

Tree part	Chemical components	Pharmacological effects	References
Flowers	Thiamine, riboflavine, nicotinic acid, folic acid, pyridoxine, ascorbic acid, alpha-tocopherol, beta-carotene, D-mannose, D-glucose, unidentified monosaccharides, protein and nine types of amino acids, potassium, cacium flavonoid pigment	Diuretic activity Antibacterial and antifungal activities	Caceres *et al*. (1992) Faizi *et al*. (1998)
Fruits	Thiocarbamate, isothiocyanate glycosides, O-(2'-hydroxy-3'-(2"-heptenyloxy)) propylundecanote, O-ethyl-4-(alpha-L-rhamnosyloxy) benzyl carbamate, methyl p-hydroxy benzoate, beta-sitosterol, p-hydroxybenzaldehyde, beta-sitosterol, cytokinins, niaziridin, niazirin, niazidin	Cholesterol-lowering activity Hepato-protective activities	Anwar *et al*. (2007) Bharali *et al*. (2003); Pal *et al*. (2006)

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Table 1 Summary of chemical components and pharmacological effects of various parts of the *Moringa oleifera* Lam. (Continued)

Tree part	Chemical components	Pharmacological effects	References
Seeds	O-ethyl-4-(alpha-L-rhamnosyloxy) benzyl carbamate , 4-(alpha-L-rhamnosyloxy)-benzyl isothiocyanate, niazimicin, niazirin, beta-sitosterol, glycerol-1-(9-octadecanoate), 3-O-(6-O-oleoyl-beta-D-glucopyranosyl)-beta-sitosterol,beta-sitosterol-3-O-beta-D glucopyranoside, niazirin	Antioxidant activities Antispasmodics activity Anti-cancer and anti-tumor activities	Lalas and Tsaknis, (2002) Olsen, (1987); Caceres *et al*. (1992) Guevara *et al*. (1999); Tsaknis *et al*, (1999); Gupta *et al*. (2007)
Bark	vanillin, beta-sitosterol, octacosanoic acid, hydroxyproline, Leucodelphinidin-3-O-beta-D-galactopyranosyl-O-beta-D-glucopyranoside	Antibacterial and antifungal activities Reduce renal calculi Diuretic activity	Karadi *et al*. (2005) Anwar *et al*. (2007) Yammuenart *et al*. (2008)
Roots	Pterygospermin, 4-(alpha-L-rhamnosyloxy) benzyl isothiocyanate, beta-sitosterol-3-O-beta-D-glucopyranoside	Antioxidant activities Hepato-protective activities Antibacterial activity	Anwar *et al*. (2007); Fakurazi *et al*. (2008); Kumar *et al*. (2010) Nadro *et al*. (2005)

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***Pseuderanthemum palatiferum* (Nees) Radlk.** (Khanh, 1997)

Kingdom Plantae

Class Magnoliosida

Order Scrophulariales

Family Acanthacea

Genus *Pseuderanthemum*

Species *P. palatiferum*

Binomial Name *Pseuderanthemum palatiferum* (Nees) Radlk.

Figure 6 Characteristics of *Pseuderanthemum palatiferum* (Nees) Radlk. Leaves

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Hwan-Ngoc (Vietnamese) or Payawanorn (Thai) is an herb belonging to the Acanthaceae family with the scientific name of *Pseuderanthemum palatiferum* (Nees) Radlk. *P. palatiferum* was discovered in northern Vietnam in 1990 (Cuong and Quynh, 1999; Bac and Oanh, 2003). The plant is a shrub reaching heights of approximately one to two meters. Young branches are green in color and change to brown or dark green once branches grow old (Dieu *et al*., 2005; Kankamon, 2008). The leaves are single green leaves alternately place opposite from one another and have smooth edges (Ho, 2002). Leaf lines branch out from both sides of the central line and the leaves are feather-shaped with a length of approximately 12.0-17.0 centimeters and a width of 3.5-5.0 centimeters (Oanh *et al*., 2000). *P. palatiferum* root systems are composed of fibrous roots and the plant is bred by cuttings (Cuong and Quynh, 1999; Khanh, 1998; Oanh, 1999). The plant is easy to cultivate and breed with rapid growth when planted in shaded or shady areas (Hung *et al*., 2004; Padee and Nualkeaw, 2009). *P. palatiferum* plants can yield as many as 700-1,000 leaves per tree when fully grown (Figure 6).

P. palatiferum leaves are widely used in the traditional medicine of Vietnam and neighboring countries due to enormous benefits in treating diseases such as hypertension, diarrhea, arthritis, hemorrhoids, gastrointestinal diseases, tumor, inflammatory bowel diseases, common cold, colorectal cancer, nephritis, diabetes (Oanh, 1998; Dieu and Hoa, 2003; Hung *et al*., 2003) Apart from therapeutic use, *P. palatiferum* is also used in health and immune promotion. The requirement of *P. palatiferum* is subsequently increased. Several researches on pharmacological property and mechanism of action are therefore carried out. Phytochemical isolation using thin layer chromatography (TLC) of extract from the leaves of *P. palatiferum* showed various bioactive components such as flavonoids, phenolic, terpenoids (Peungyicha *et al*., 2011). Piglets fed with crude extract from *P. palatiferum* leaves at 5 mg/kg BW for 30 consecutive days revealed a decrease in diarrhea and mortality rates while growth rate was increased (Dieu *et al*., 2006). Thereafter, Padee *et al*. (2009) reported that the ethanolic extract from *P. palatiferum* leaves was found to have no toxicity to Vero cell (African green monkey kidney). In addition, aqueous extract from the leaves of *P. palatiferum* could reduce blood glucose levels of streptozotocin induced diabetes in rats and the extract at a dose of 250 mg/kg BW was found to be the most suitable dosage in the experiment (Padee *et al*., 2010). Moreover, aqueous extract from

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P. palatiferum leaves at a concentration of 5 mg/ml has been reported to be capable of reducing arterial pressure in rats (Khonsung *et al*., 2011). During the same year, a study of the effects of aqueous extract from *P. palatiferum* leaves to reduce blood glucose levels in diabetes rats induced with streptozotocin and nicotinamide found that aqueous extract at the doses of 0.25 and 0.50 g/kg BW to be capable of reducing blood glucose levels within 30 minutes. There is no different in efficiency between the gliben clamide, a diabetes drug and *P. palatiferum* extract (Chayarop *et al*., 2011). Furthermore, aqueous ethanol, methanol and acetone extracts of *P. palatiferum* have been found to have antioxidant effects. These extracts with various solvents have been found to have phenolics and flavonoids with all extracts showing high DPPH scavenging activity while ethanolic *P. palatiferum* extracts showed highest efficiency (Nguyen and Eun, 2011).

P. palatiferum contains flavonoids and other substances with antioxidant, antibacterial and antifungal effects such as β-Sitosterol and apigenin as shown on Table 2.

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Table 2 Chemical components of *Pseuderanthemum palatiferum* (Nees) Radlk leaves.

Substances	Phytochemical effects
Flavonoids	Flavonoids are chemicals with various pharmacological effects depending on different chemical structures. For example, some types have antioxidant, anti-allergy, anti-cancer, anti-inflammatory, and antiviral properties, ability to prevent heart disease and reduce blood cholesterol levels (Phan, 2 0 0 5). According to research reports, the leaves of this plant contain high amounts of flavonoids with no reports indicating the type of flavonoids (Giang *et al*., 2003; Ferguson *et al*., 2004)
β-Sitosterol ($C_{29}H_{50}O$)	Properties for reducing blood cholesterol levels, preventing hair loss, reducing risks of prostate cancer, colorectal cancer, ovarian cancer, stomach cancer, breast cancer (Young *et al*., 2004), and uterine cancer (Ashok *et al*., 2006).
Stigmasterol ($C_{29}H_{48}O$)	Antioxidant, anti-inflammation ant anti-cancer properties (Huynh and Tran, 2003)
Apigenin ($C_{15}H_{10}O_5$)	Natural diterpenes alcohol with properties in treating arthritis (Sang *et al*., 2008)
Phytol ($C_{20}H_{40}O$)	Phytol is a saponin with triterpene as components in the molecule. It has qualities for reducing blood cholesterol levels, blood glucose levels, antioxidant, anti-cancer and anti-inflammatory properties (Young *et al*., 2004; Giang *et al*., 2005)
Triterpenoid Saponin	Controls weight, balances acid-base in blood, support immune system, reduces osteoporosis and reduces risks for heart disease (Feng *et al*., 2007)

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