CHAPTER II

REVIEW OF LITERATURE

1. General knowledge of anticancer

Recently, many cancer researches have been focused on the use of natural products since most of the synthetic chemotherapeutic agents cause several unavoidable problems. One problem is the high toxicity owing to the non-specificity and non-selectivity of the drugs. Pure cytotoxic substances from natural products are more advantages than some other synthetic cytotoxic drugs because being naturally occurring and probably possessing less toxicity with more potent anticancer activity.

Many investigations have been focused on using active extracts of toxins from natural products, a variety of natural products have been investigated for cancer therapy. Numerous plants have been reported to possess active cytotoxic and anticancer agents (32). It was figured that at least 2-4% of plant species synthesize such cytotoxic substances (33). These natural products are a great promise to be explored for their medical potential application in cancer treatment. A large number of anticancer agents have been obtained from plants. So far, more than 300 cytotoxic compounds from known higher plants have been isolated and chemically characterized. According to their chemistry, they are categorized as terpenoids, steroids, quinones, flavones, alkaloids, amides and other non-nitrogenous products (34). In China, traditional medicines have been used mostly in treating or managing carcinomas of the esophagus or gastric cardia (35, 38).The effect of a treatment with the mistletoe preparation, iscador (ISC), a plant extract on the survival of patients with liver metastases and patients with inoperable colorectal tumors has been investigated (36-37).

Camptothecin is a plant alkaloid present in wood, bark, and fruit of the Asian tree *Camptotheca acuminate*, isolated and characterized for the first time by Wall *et*

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al. (1966) (39). Members of the Icacinaceae, Olacaceae, Rubiaceae, and Apocynacee families were also reported to produce camptothecin. CPT analogues and derivatives were a novel class of effective anticancer agents that exert their action against DNA topoisomerese I (39). The worldwide market size of camptothecin derivatives (*e.g.*topotecan and irinotecan) reached 1.5 billion US dollars in 2002 (40). Due to the cytotoxicity of camptothecin itself, the CPT derivatives, irinotecan and topotecan, were used throughout the world for the treatment of various cancers, and over a dozen or more CPT analogues were currently at various stages of clinical development (41). However, they were synthesized from natural camptothecin which was extracted from plants. The use of CPTs as inhibitors of replication, transcription, and packing of double stranded DNA containing adenoviruses, papovaviruses, and the single stranded DNA-containing autonomous parvoviruses has been studied (42). It appeared that CPTs could be powerful antiviral drugs for several DNA viruses, which were causative agents for a large number of diseases. Since 1994, CPT has been in use clinically in Japan for the treatment of lung, ovarian and uterine cancer (43). It has also proved useful as an insect chemosterilant and plant growth regulator and as an inhibitor of herpes virus (44). The therapeutic values of CPT derivatives were highlighted against colon cancer (45), AIDS (46), uterine cervical and ovarian cancer (47), and falciparum malaria (48).

The genus *Ophiorrhiza* belongs to the family Rubiaceae, The roots of *Ophiorrhiza* species, *O. mungos* and *O. pumila* have been reported as the sources of CPT and 10-methoxycamptothecin (49-52). The *Ophiorrhiza* spp. was also used to provide remedies for ulcers, helminthiasis, snake poison, poisonous wounds, gastropathy, leprosy, and hydrophobia (53). *O. prostrata* D. Don was an herbaceous perennial medicinal plant, exploited for the production of camptothecin, which is accumulated mainly in the roots. A comparative study of camptothecin content in *Nothapodytes foetida, O. mungos* and *O. rugosa* indicated highest yields of camptothecin and 9-methoxy camptothecin in *N. foetida* (54).

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1.1 History of camptothecin

The US National Cancer Institute screening programmed identified camptothecin as a drug with potential antitumor activity in 1966 (55). Promising preclinical results were seen in mouse L1210 leukaemia and rat Walker carcinosarcoma models. However, the drug was poorly soluble, a problem that greatly hampered its initial clinical development (20, 55-56). Preclinical data showing camptothecin activity in tumours of both colonic and gastric origin, and toxic effects of the drug to the digestive tract, led to phase 1 trials focusing largely on gastrointestinal malignancies. In these initial trials, myelosuppression was identified as the primary dose-limiting toxic effect. Haemorrhagic cystitis also developed unpredictably in a few patients, and was occasionally severe. In retrospect, haemorrhagic cystitis can most probably be explained by the in-vivo chemistry of camptothecin.

Camptothecin has a five-ring structure **(figure 1.1.1)**. Early assessments identified the importance of the 20*S* chiral carbon for activity, and also noted a dynamic equilibrium between the closed ring lactone and open-ring carboxylic acid forms **(figure 1.1.2)**. The closed ring lactone has most cytotoxic activity. At neutral and alkaline pH, equilibrium favours the essentially inactive carboxy-acid form (20, 40).

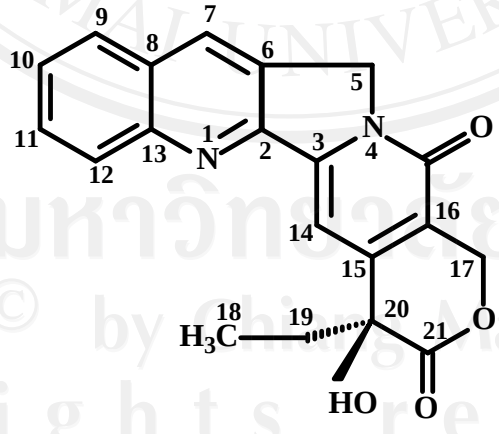

Figure 1.1.1 The Camptothecin skeleton and its numbering system

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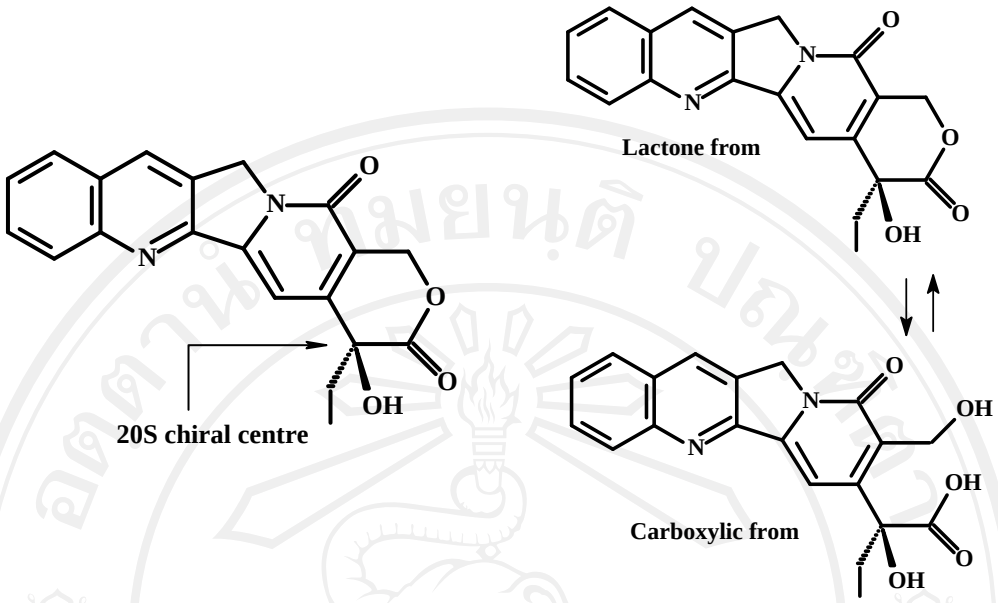

Figure 1.1.2 The 20*S* chiral carbon and a dynamic equilibrium between the closed ring lactone and open-ring carboxylic acid forms

However, the importance of the intact lactone ring on activity was not fully appreciated initially. To deal with insolubility of the parent compound, the sodium salt of camptothecin was studied. This resulted in irreversible opening of the lactone ring, so that very high concentrations of the drug were required for clinical activity. This outcome could have contributed to the unpredictable nature of the toxic effects initially seen, since equilibrium between the carboxylic acid and lactone forms is largely dictated by ambient pH. For example, acidity of the bladder would favour dissociation of the sodium salt. These conditions also facilitate spontaneous closure of the lactone ring, thus potentially producing large amounts of active drug in the urine. Variability of the collecting system might have further contributed to unpredictability of hemorrhagic cystitis. Despite great toxic effects, some activity was seen, leading to phase 2 trials.

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In 1972, workers on a phase 2 study looked at efficacy and safety in 61 patients with adenocarcinoma of gastrointestinal origin (57). Again, hematological toxic effects seemed to be dose-limiting. With only three of 61 patients obtaining an objective response and toxic effects being severe and unpredictable, further development of camptothecins was abandoned.

Although discovered in the 1970s, the role of topoisomerase 1 as an important enzyme in DNA replication was not fully appreciated until the 1980s (20, 58-60). DNA normally exists as a supercoiled double helix. During replication, it unwinds, with single strands serving as a template for synthesis of new strands (78). To relieve the torsional stress that develops ahead of the replication fork, transient cleavage of one or both strands of DNA was needed (20, 60). Topoisomerases facilitate this process. Topoisomerase 2 caused transient double-stranded breaks, whereas topoisomerase 1 caused single-strand breaks. This action allowed for rotation of the broken strand around the intact strand. Topoisomerase 1 then re-ligated the broken strand to restore integrity of doublestranded DNA (20).

In 1985, topoisomerase 1 was found to be the target of camptothecin (58-59). The drug reversibly induced single-strand breaks, thereby affecting the cell's capacity to replicate. Camptothecin stabilises the so-called cleavable complex between topoisomerase 1 and DNA. These stabilized breaks were fully reversible and non-lethal (61). However, when a DNA replication fork collided with the cleavable complex, single-strand breaks were converted to irreversible double-strand breaks (58-59). Apoptotic cell death was then mediated by caspase activation. Inhibition of caspase activation shifts the cells from apoptosis to transient G1 arrest followed by cell necrosis (63). Thus, the mechanisms of cell death needed active DNA replication to be happening, resulting in cytotoxic effects from camptothecin that was S-phase-specific. Indeed, cells in S-phase in vitro have been shown to be 100–1000 times more sensitive to camptothecin than cells in G1 or G2 (64). Results of early experiments showed camptothecin sensitivity in cancer cell lines was directly correlated to topoisomerase 1 concentrations (65). Other investigators reported topoisomerase 1 to be over expressed in tumour tissue of patients with adenocarcinoma of the colon (66). Further investigation noted amplified concentrations of the enzyme in other tumours (67). Identification of topoisomerase 1 as a viable target for antineoplastic treatment,

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and elucidation of its inhibition as the mechanism of action of camptothecin, led to renewed efforts to develop soluble analogues of camptothecin.

Topotecan (9-[(dimethylamino) methyl]-10-hydroxycamptothecin) was the first camptothecin analogue to be approved for clinical use by the US Food and Drug Administration (FDA). It is water-soluble because of its side-chain at carbon 9 of the A ring **(figure 1.1.3)**. Results of preclinical studies suggested topotecan to have excellent antitumour activity in vitro (68). Tumour xenograft models showed activity in many tumour types, including adenocarcinomas of the ovary and colon, tumours of the central nervous system, and sarcomas (69-71). Workers on initial phase 1 trials assessed treatment with topotecan as a daily 30 min infusion for 5 consecutive days repeated every 3 or 4 weeks (72-73). In each of these studies, the dose limiting toxic effect was myelosuppression. In other phase 1 trials, continuous or protracted infusions were assessed (74-75). Although phase 1 studies seemed to suggest a higher response rate with extended infusions or frequent dosing, phase 2 studies in unrespectable and metastatic non-small-cell lung cancer and in advanced breast cancer did not confirm this apparent advantage (76-77). Early phase 2 studies mainly focused on colorectal and gastric cancer; however very little evidence of activity was seen, and toxic effects were considerable (78-80). Indications of activity in ovarian and small-cell lung cancer in phase 1 trials led to further investigation of topotecan in these areas. An oral formulation of topotecan also continues to be studied. The pivotal study in the 1996 FDA approval of topotecan for relapsed ovarian cancer (81). Clinicians use this drug routinely, and thus clearly feel that it is beneficial to their patients; however, no randomised trial has shown a survival advantage with topotecan.

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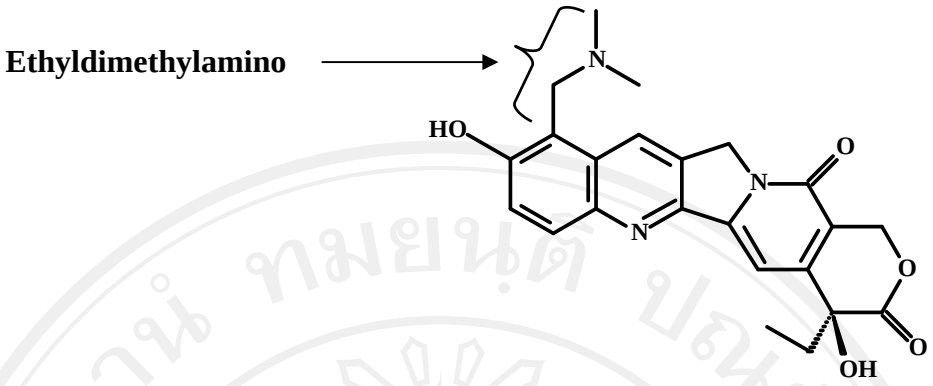

Figure 1.1.3 Topotecan, the side-chain at carbon 9 of the A ring was found water-soluble.

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycampto thecin) was the first water-soluble semisynthetic derivative of camptothecin to enter clinical trials. After initial development in Japan, the drug has been licensed to several different pharmaceutical firms and has undergone a truly global development plan. Investigation began in the late 1980s, with phase 1 study in Japan assessing a bolus infusion every week and a 5-day continuous infusion (82-83). Irinotecan became commercially available in Japan in 1994, where it's approved indications were cancers of the lung (small-cell and non-small-cell), cervix and ovaries. Irinotecan was approved in Europe in 1995 as a second-line agent for colon cancer, 1 year before European approval of topotecan. Irinotecan was approved in the USA in 1996 for treatment of advanced colorectal cancer refractory to fluorouracil **(Figure 1.1.4)**.

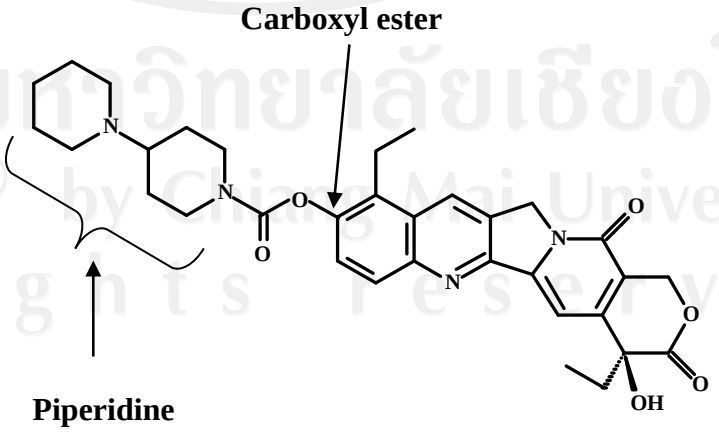

Figure 1.1.4 Structure of irinotecan

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1.2 Camptothecin Biosynthesis

Several investigators have recognized plant cell cultures as a tool to unravel the biosynthetic pathway that leads to camptothecin (CPT) formation in plants and as a potential system for its production. The first tissue cluture study of *C. acuminate* was reported by Sakato *et al.* (1974). Later reports of in vitro CPT production by *C. acuminate* in cell suspensions in callus cultures (86).

All TIAs, including CPT are derived from the common precursor strictosidine, which is the product of a condensation reaction between the indole tryptamine and the terpenoid secologanin catalyzed by the enzyme strictosidine synthase (*SSS*) (87) **(Figure 1.2.1.)**. Early feeding experiments with radiolabeled precursors confirmed that the tryptamine moiety is completely incorporated into the CPT molecule (88). Strictosidine is then converted to strictosamide via intermolecular cyclization and this compound is a precursor of CPT as proven by incorporation of the radiolabeled precursor.

Trp biosynthesis begins with the conversion of chorismate to anthranilate by anthranilate synthase. After production of the intermediates 5- phosphoribosylanthranilate and indole glycerol phosphate, the a-subunit of Trp synthase (TSA) produces indole, which is then condensed with Ser by the b-subunit of Trp synthase (TSB) to form the final product **(Figure 1.2.1.)**.

The formation of isopentenyl diphosphate (IPP), the precursor of terpenoid biosynthesis and the mevalonate (MVA) pathway have been known since the 1950s. For many years the MVA route was thought to be the only source of building blocks for all plant isoprenoids. Recently, the 2C-methyl-D-erythritol- 4-phosphate (MEP) pathway, in which IPP is formed from 1-deoxy-D-xylulose-5-phosphate by condensation of glyceraldehyde-3-phosphate and pyruvate, was found to be present in many eubacteria, green algae, and plastids of plants. Both isoprenoid pathways are operative simultaneously in higher plants. The enzymes of the MVA route are located in the cytoplasm, where they supply precursors of triterpenes, sesquiterpenes, and sterols. The enzymes of the MEP pathway carry putative plastid targeting signals and are believed to be located in plastids where they produce precursors for monoterpenes, some sesquiterpenes, diterpenes and carotenoids (89).

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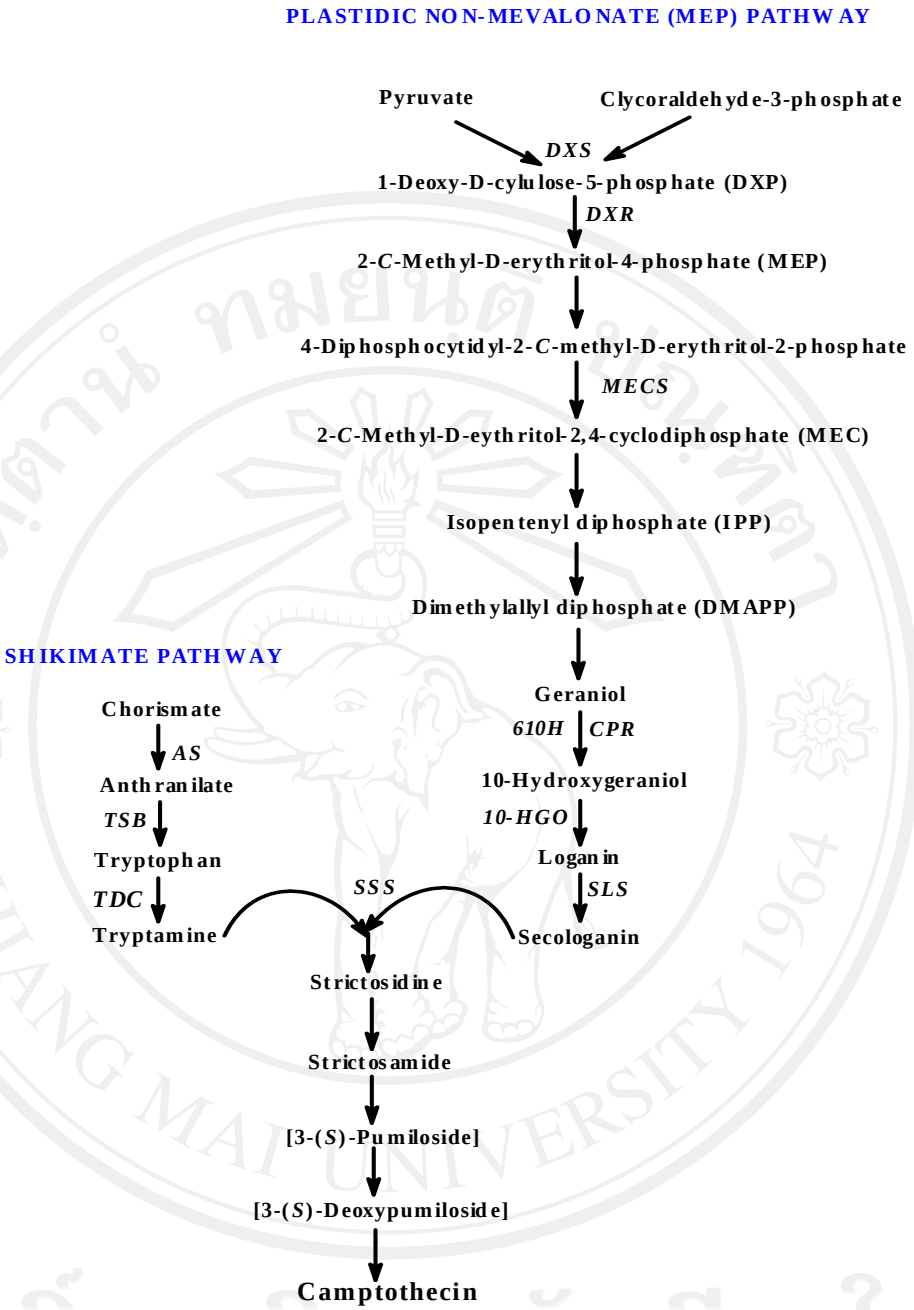

Figure 1.2.1. Biosynthetic pathway for TIAs in CPT-producing plants. Multiple arrows indicate multiple steps between intermediates. The enzymes that have been cloned and characterized in either *C. acuminata* or *O. pumila* are shown in bold: TSB (b-subunit of tryptophan synthase), TDC (tryptophan decarboxylase), SSS (strictosidine synthase), and 10-HGO (10-hydroxygeraniol oxidoreductase). TSB is abundant in vascular tissues (cambium, primary xylem and primary phloem). Other enzymes involved in this pathway already cloned in *C. roseus*, the best characterized TIAs-producing model, are also shown: 1-deoxy-D-xylulose-5-phosphate (DXP) synthase (DXS); DXP reductoisomerase (DXR); 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (MECS); geraniol-10-hydroxylase (G10H), and secologanin synthase (SLS). In C. roseus, dxs, dxr, mecs, and g10h are expressed in the internal phloem parenchyma, while sls, tdc and sss are expressed in the epidermis.

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Strictosidine synthase (*SSS*) is the enzyme that mediates the condensation of tryptamine with the iridoid glucoside secologanin to yield strictosidine.

CPT-producing plants are necessary to gain a better understanding of the regulation of the pathway(s) leading to the formation of this important alkaloid. CPT has been localized in the mesophyll and sub-palisade layers of young leaves and in the palisade and one cell layer beneath the palisade layer in older Camptotheca leaves. At the subcellular level, CPT has been localized in the vacuoles of young and older leaves. The precise mechanism of transport and storage of CPT and its precursors remains unclear. One possible mechanism of transport of such an insoluble compound is its conversion into a more water soluble form such as a glucoside, e.g. chaboside and transport to other parts of the plant (90).

2. Antioxidants and its mechanism

Antioxidant is defined as any substance the when present at low concentration compared to those of an oxidizable substrate (e.g. lipid, proteins and DNA) significantly delays of presents oxidation of that substrate (91, 93-95). These definitions therefore includes not only chain-breaking antioxidant e.g. ascorbic acid, tocophero, uric acid, glutathione (95-96, 103-104) and proteins used to sequester metals capable to OH production e.g. transferring, ferritin, ceruloplasmin, nemopexin, haptoglobin and albumin (101-102).

2.1 Free radicals and active oxygen species

Free radicals are chemical species, which have unpaired electrons (96-97, 105). Electrons are present generally in pairs, under certain conditions; molecules have unpaired electrons and as such are called free radicals (106). Free radicals, generally, always occur as intermediates in metabolic and physiological processes and also are as old entities as life itself (99, 103-104). Active oxygen species denote oxygen-containing molecules, which are more active than the triplet oxygen molecule present in air. Superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical, and singlet oxygen ($^1O_2$) are accepted as typical active oxygen species, but in broader sense, other species such as alkoxyl radical, peroxyl radical ($LO_2^{\bullet}$), nitrogen dioxide

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($NO_2^{\bullet}$), lipid hydroperoxide (LOOH), protein hydroperoxide, and hypochlorite (HOCl) are also considered as active oxygen species (105). However, under certain conditions such as temperature, light, air diet, alcohol aging etc. (92, 97) the efficiency of such protecting system decrease, resulting in disturbances of the redox equilibrium established under health conditions. Over-production of free radicals initiates an uncontrolled chain reaction that lead to oxidation stress, damaging in cell membranes, proteins in tissue or enzymes carbohydrates and DNA resulting in aging and several degenerative diseases (98, 101, 103-104).

Table 2.1.1 Active Oxygen and Related Species

Radicals		**Non- radicals**	
$\mathbf{O_2^{\cdot-}}$	superoxide	$\mathbf{H_2O_2}$	hydrogen peroxide
$\mathbf{HO^{\cdot}}$	hydorxyl radical	$\mathbf{^1O_2}$	singlet oxygen
$\mathbf{HO_2^{\cdot}}$	hydroperoxyl radical	**LOOH**	lipid hydroperoxide
$\mathbf{L^{\cdot}}$	lipid radical	**Fe=O**	iron-oxygen complexes
$\mathbf{LO_2^{\cdot}}$	lipid peroxyl radical	**HOCl**	hypochlorite
$\mathbf{LO^{\cdot}}$	lipid alkoxyl radical		
$\mathbf{NO_2^{\cdot}}$	nitrogen dioxide		
$\mathbf{^{\cdot}NO}$	nitric oxide		
$\mathbf{RS^{\cdot}}$	thiyl radical		
$\mathbf{P^{\cdot}}$	protein radical		

2.2. Mechanism of Antioxidants

Generally, free radical- generated reactions take place in mitochondria, which is considered to be the energy storehouse of living cell. The oxygen-mediated radicals can be countered by the body's natural defense using various types of radical scavengers, here call endogenous antioxidants such as glutathione peroxidase, superoxide dismutase (SOD), catalase and melatonin However, different external factors as mentioned in previous paragraph initiate imbalances of these systems, thus lead to oxidative stress, resulting in various diseases. Therefore, dietary antioxidants such as ascorbic acid, carotenoids and flavonoids are need for diminishing the cumulative effects of oxidative damage over the life spans. These external

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antioxidants can protect oxidative stress induced by various active oxygen species by abstract the lone electron from free radical molecules and help humans to keep control on these injurious species. The preventive mechanisms of antioxidant action consist of the steps. The first line defense is suppressing the formation of free radicals and active oxygen species. The radicals scavenging antioxidants are responsible in the second defense line and inhibit chain initiations and or/break the propagation. Then, the antioxidant enzymes such as phospholipase, proteases, DNA repair enzymes and transferases act as the third line defense. In addition, the appropriate antioxidant is generated and transfer to right site at the right time with the right concentration when oxidative stress occur. This adaptation mechanism is also important in the total defense system (101, 103).

As be acknowledged, oxidation is essential to many living organisms for production of energy to fuel biological processes. However, an imbalance caused by excess oxidants, particularly Reactive Oxygen Species (ROS) e.g. hydroxyl radicals (**HO**$^{\cdot}$), superoxide (**$O_2^{\cdot-}$**), peroxyl radicals (**ROO**$^{\cdot}$). These toxic substsnces are capable oxidizing protein cells, nucleic acid and lipids, thus lead to pathophysiological conditions and/or diseases. Among the free-radical reactions, lipid peroxidation is important, for example, food deterioration and oxidative modification of low density lipoprotein (LDL) which is accepted as a key initial event in the progression of atherosclerosis (92, 103). The chain reaction can be summarized as shown in **Fig.2.2.1**. The organic substrate by RH and the carbon-centred radical derived from it by removal of a hydrogen atom by **R**$^{\cdot}$, then the overall process can be represented by toe following 4 elementary chemical reactions.

Initiation	**Production of $R^{\cdot}$ (Rate = R_i)**	**(1)**
Propagation	**$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$**	**(2)**
	$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$	**(3)**
Termination	**$ROO^{\cdot} + ROO^{\cdot} \longrightarrow$ Molecular products**	**(4)**
	or (Ketone + alcohol + O_2)	

Fig.2.2.1 Chain reaction of Lipidperoxidation

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The first step for each chain involves the production of the radical **R**• from some molecular precursor. Such chain initiation is generally caused by the action of heat, light, or ionizing radiation on the system. In the presence of oxygen, the carbon-centred radical **R**• will rapidly be converted to a peroxyl radical, **ROO**•. This step (reaction 2) is a diffusion-controlled process, i.e. it occurs on every encounter between **R**• and an oxygen molecule. In a subsequent and much slower step the peroxyl radical attacks the organic substrate forming a molecule of hydroperoxide, **ROOH**, and generating a new radical **R**• (i.e. reaction 3). This radical adds O_2 and so continues the chain, many molecules of RH being oxidized before the chain is broken (i.e. terminated) when 2 of the chain carrying peroxyl radicals react together to give molecular products (reaction 4).

Living organisms are exposed to much more severe oxidative stresses than is food in a refrigerator. Nevertheless, they do not become rancid until they, in turn, become food, i.e. until after they die. The specific materials in the organism that most require this protection are the polyunsaturated fatty acids. Like other fatty acids, these form a part of various lipid materials within the organism including in particular the organism's membranes. Autoxidation of a biological membrane will breach the integrity of the membrane. This can have disastrous consequences for the organism since one of the principle functions of a biological membrane is to act as a dividing wall, compartmentalizing biochemical processes so that they take place in specific regions, i.e. in specific cells and even in specific parts of a cell. Autoxidation of a biomembrane will lead first to an increase in its permeability and eventually to its complete destruction.

2.3 Radical-scavenging methods

Radical-scavenging is main mechanism by which antioxidant act in foods. Several methods have been developed in which the antioxidant activity is assessed by the scavenging of the synthetic radicals in polar organic solvents, e.g. methanol, at room temperature. The reactional mechanism between the antioxidant and DPPH• depends on the structural conformation of the antioxidant. Some compounds react very quickly with DPPH•, reducing a number of DPPH• molecules equal to their number of available hydroxyl groups (106).

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In the DPPH$^{\bullet}$ test, the scavenging of DPPH$^{\bullet}$ radicals is followed by monitoring the decrease in absorbance at 515 nm with occur due to reduction by antioxidant or reaction with a radical species (107).

$$DPPH^{\bullet} + RH \longrightarrow DPPH\text{-}H + R^{\bullet}$$
$$DPPH^{\bullet} + R^{\bullet} \longrightarrow DPPH - R$$

Fast reaction of DPPH$^{\bullet}$ radicals occurs with some phenolics e.g. α-tocopherol, but slow secondary reactions may cause a progressive decrease in absorbance, so that the steady state may not be reached for several hours. Most papers in which the DPPH$^{\bullet}$ method has been used report the scavenging after 15 or 30 mins reaction time. The data is commonly reported as IC_{50}, which in the concentration of antioxidant required for 50% scavenging of DPPH$^{\bullet}$ radical in the specified time period (106-107).

These methods may be useful for screening antioxidants. But antioxidant effectiveness in food must always be studies by other methods because their activity in food is dependent on a variety of factors including polarity, solubility and metal-chelating activity.

3. Antimicrobial

Anti-infective chemotherapy is the science of administering chemical agents to treat infectious diseases. This practice has proven to be one of the most successful of all pharmaceutical studies. Historically, the use of anti-infective agents can be credited with saving more human lives than any other area of medicinal therapy discovered to date. Humanity has enjoyed a tremendous increase in life expectance in the past century. A better understanding of infectious disease pathogenesis and the importance of sanitation are contributing factors. Regardless, most individuals will become infected with a microbial pathogen many times throughout their lives and in developed countries, anti-infective chemotherapy will be periodically administered. Antibacterials account for the majority of anti-infective agents in comparison to antifungals, antivirals and antiparasitic agents.

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3.1 Antibacterial

The origin of most pharmaceutical drug can be traced to naturally occurring sources. Microbial organisms and plants dominate in this suspect, a large percentage of compounds and structural leads are derived from these sources. The β-lactam, tetracycline, aminoglycoside, erthyromycin and macrolide families of antibacterial agents, as well as a host of anti-infective polypeptides, glucopeptides and polyethers have all resulted from initial discoveries of active, naturally occurring substance. Currently, many antibacterial agents are obtained, directly or indirectly, from a microbial source. Often, these biosynthetic factories have been made more productive by some modification of the fermentation process that either increases the yield of the product, of allows for an improved isolation and/or purification (108). For example, all of these advances were monumental in the development of the penicillin antibiotics.

The classification is based on whether the organisms do or do not stain with Gram's stain, but is has a significance far beyond that of an empirical staining reaction. Gram-positive and Gram-negative organisms are different in several respects, not least in the structure of the cell wall, which has implications for the action of antibiotics (109).

3.2 Antifungal

Many of the fungi that can cause infections live in association with humans as commensals or are present in the environment. But until recently, serious superficial infections were relatively uncommon and systemic infections very uncommon in deed at least in cool and temperate climatic zones. In these zones, a fungal infection usually meant athlete is foot or oral or vaginal thrush, which cause discomfort but were hardly life-threatening.

Since the 1970s, there has been a steady increase in the incidence of serious secondary systemic fungal infections. One of the factors aiding the spread of fungal disease has been the widespread use of broad-spectrum antibiotics, which eliminate or decrease the non-pathogenic bacterial populations that normally compete with fungi (109). Another has been the increased number of individuals with reduced immune responses caused by the acquired immunodeficiency syndrome (AIDS) or by the

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action of immunosuppressant drugs or cancer chemotherapy agents; this has led to an increased prevalence of opportunistic infections i.e. infections with fungi that rarely cause disease in healthy individuals (109).

Fungal infections are termed mycoses and, in general, can be divided into superficial infection such as affecting skin, nails, scalp or mucous membranes and systemic infections such as affecting deeper tissues and organs. In the UK, the commonest systemic fungal disease is systemic candidiasis an infection with a yeast-like organism. In other parts of the world, the commonest systemic fungal infections are blastomycosis, histoplasmosis, coccidiomycosis and paracoccidiomycosis; these are often primary infections, i.e. they are not secondary to reduced immunological function or altered commensal microorganisms (109).

However, choices of medicinal plants are an important therapeutic aid for various ailments. Scientific experiments of the antimicrobial properties of plant components were first documented in the late 19^{th} century (110). In India, from ancient times, different parts of medicinal plants have been used to cure specific ailment. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compare with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derive from plants, animal tissues or microorganisms (111). The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants (112). To determine the potential and promote the use of herbal medicine, it is essential intensify the study of medicinal plants that final place in folklore.

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