CHAPTER 2

Literature Reviews

2.1 Skin Anatomy

The skin consists of three layers including the epidermis, dermis and subcutaneous layers (Figure 2.1)

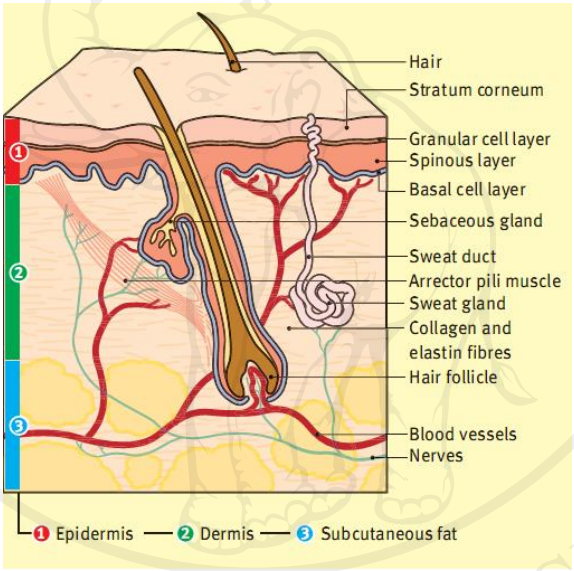

Figure 2.1 Skin structure (Quinn, 2004)

2.1.1 Epidermis

Epidermis is stratified squamous epithelium. The epidermis varies in thickness from 0.05 -1.5 mm. The main cells of the epidermis are the keratinocytes, which synthesize protein keratin and protein bridges called desmosomes connect the keratinocytes. The keratinocytes are in a constant state of transition from the deeper layers to the superficial. The four separate layers of the epidermis are formed by the different stages of keratin maturation (Quinn, 2004; Venus *et al.*, 2010).

1) Stratum basale or germinativum cell layer or basal layer

The cells of basal layer are similar to those of other tissues within the body. This contains the typical organelles, mitochondria and ribosome.

2) Stratum spinosum or spinous or prickle cell layer

The stratum spinosum is found on the top of basal layer between the stratum granulosum and stratum basale. They compose of langerhans cells, which are found on all epidermal surfaces but mainly located in the middle of this layer. They play a significant role in immune reactions of the skin by acting as antigen-presenting cells.

3) Stratum granulosum or granular cell layer

Stratum granulosum is a thin layer of cells in the epidermis. Keratinocytes migrating from the underlying stratum spinosum are known as granular cells in this layer. The cells lose their nuclei and their cytoplasm appears granular at this level. These cells contain keratohyalin granules, which are filled with proteins that promote hydration and cross linking of keratin.

4) Stratum corneum or horny layer

The stratum corneum is generated from epidermis cell differentiation, and it is often viewed as a separate membrane in typical and transdermal drug delivery study. The stratum corneum comprises only 10 to 15 cell layers and it is around 10 µm thick.

2.1.2 Dermis

The dermis varies in thickness ranging from 0.6 - 3 mm and composes of the connective tissue. It is the major component of the human skin and found below the epidermis. Moreover, the dermis has numerous embedded structures such as blood and lymphatic vessels, nerve endings, pilosebaceous units (hair follicle and sebaceous glands), and sweet glands (Quinn, 2004; Venus *et al.*, 2010).

2.1.3 Subcutaneous tissue

The subcutaneous fatty layer or hypodermis bridge is between the overlying dermis and underlying body constituents. In most areas of the body, this layer is

relatively thick with several millimeters. However, there are areas in the body in that the subcutaneous fat layer is absent, such as eyelids. This layer of adipose tissue principally serves as body insulation and mechanical protection against physical shock. The subcutaneous fatty layer can also provide a readily available supply of high-energy molecules, whilst the principle blood vessels and nerves are carried to the skin in this layer (Quinn, 2004; Venus *et al.,* 2010).

2.2 Normal microbial flora of the skin

The normal flora of the skin consists of either resident or transient populations of bacteria and fungi. The most common and stable resident microorganisms are Gram positive bacteria that generally restrict to a few genera, including *Streptococcus, Staphylococcus, Corynebacterium* and *Propionibacterium* especially *Propionibacterium acnes*, which causes acne. Moreover, gram negative bacteria are occasional constituents of normal skin because intestinal organisms such as *Escherichia coli* may be inoculated to the skin by fecal contamination. *Malassezia spp.* is the most common fungi found on the skin. Many types of yeast such as *Candida* sometimes grow and cause serious skin infection (Madigan *et al.,* 2009).

2.3 Common bacterial skin infectious diseases

The description of common bacterial skin infection is shown in Table 2.1.

Table 2.1 Description of six common bacterial skin diseases (Guay, 2003)

Diseases	**Description**
Impetigo	Large vesicles and/or honey crusted lesions
Erysipelas	Fiery, red, painful infection of superficial skin with sharply demarcated borders
Cellulitis	Painful, erythematous infection of deep skin with poorly demarcated borders
Folliculitis	Popular/pustular inflammation of hair follicle
Carbuncle	A network of furuncles connected by sinus tracts

2.3.1 Cellulitis

Cellulitis is a bacterial infection of dermis and subcutaneous tissue with severe inflammation of the skin. It presents as a localized area of tenderness, warmth, edema, and erythema of the skin, which may advance rapidly. More severe diseases may include fever, chills, and malaise. Cellulitis commonly occurs near breaks of the skin such as surgical wound, trauma, tinea infections or ulceration. The most common bacteria that cause cellulitis are *Staphylococcus aureus* and group A streptococcus, *Haemophilus influenza* and *Pseudomonas aeruginosa*. These bacteria may be either normal flora or transient microorganisms of the skin. In addition, infection may be acquired through contact with soil or water, or may arise from nosocomial pathogens (Allen *et al.*, 2004; Stulberg *et al.*, 2002).

2.3.2 Erysipelas

Erysipelas is superficial skin infectious diseases that is characterized by erythematous, warm, tender plaque with raised, sharply borders, peel orange texture of the involved skin, and occasional large tense bullae or vesicles at the borders of the lesion (Allen *et al.*, 2004). Erysipelas is usually caused by β-hemolytic streptococcus and thus can be treated by penicillin (Stulberg *et al.*, 2002).

2.3.3 Impetigo

Impetigo is the common skin infection in children and is classified as bullous and nonbullous. Both forms involve only the most superficial layers of the skin. Nonbullous impetigo is primary caused by *S. aureus* and β-hemolytic streptococcus. It is the most commonly found in summer and warm climates. The symptom appears initially as erythematous maculopapules that evolves over a period of 2 to 3 weeks and turns into vesicles, then pustules that rupture to form the classic thick and honey colored crusted plaques. These lesions are usually less than 2 cm in diameter but may coalesce to form larger tenderness and lesions. Bullous impetigo presents as a large thin wall bulla containing yellow fluid. There are commonly caused by *S. aureus* (Allen *et al.*, 2004). .

2.3.4 Folliculitis

Folliculitis is the inflammation of the hair follicle by physical injury, chemical irritation, or infection. The most common pathogen of folliculitis is Staphylococci, which will occasionally invade the deeper portion of the follicle, causing swelling and erythema with or without a pustule at the skin surface. These lesions are painful and may cause a scar. This inflammation of the entire follicle or the deeper portion of the hair follicle is called deep folliculitis. Moreover, there are also caused by gram negative bacteria including *Klebsiella*, *Enterobacter*, *Pseudomonas* and *Proteus* species in the patient who used antibiotic for long term such as acne treatment (Stulberg *et al.*, 2002).

2.3.5 Furuncle and Carbuncle

Furuncles and carbuncles occur as a progressive follicular infection into deeper skin and extend out of the follicle. The infection is commonly known as an abscess or boils. A furuncle is a tender, erythematous, firm or fluctuant mass of walled-off purulent material which is arising from the hair follicle. The pathogen is usually caused by *S. aureus.* Carbuncles are an aggregation of infected hair follicles that form broad, swollen, erythematous, deep, and painful masses. The symptoms of carbuncles are fever and malaise (Stulberg *et al.*, 2002).

2.4 Mode of action of antibiotic agents

Several mode of action of antimicrobial agents are shown in Figure 2.2.

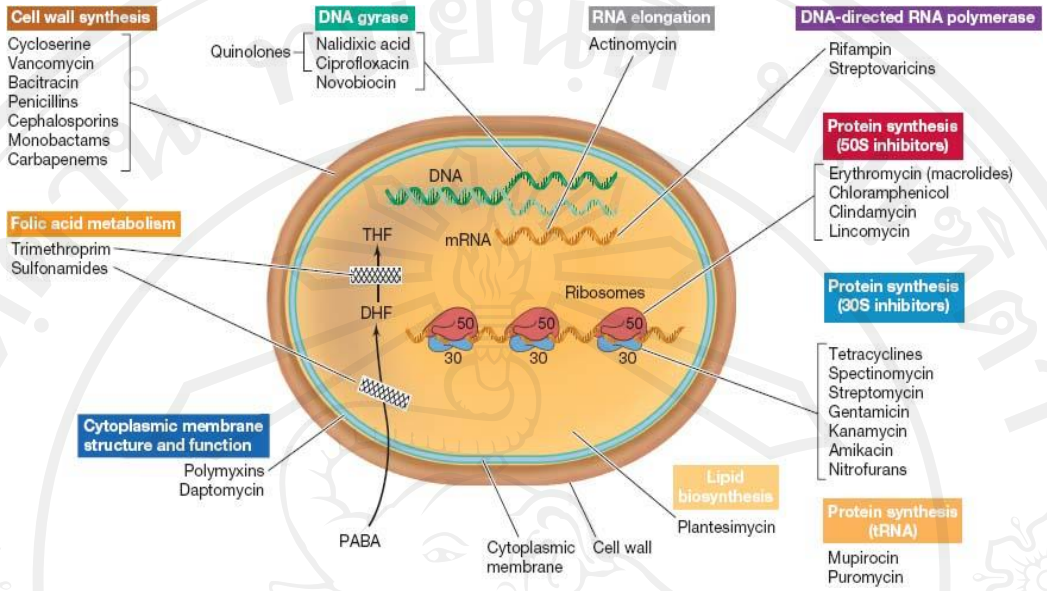

Figure 2.2 Modes of action of some major antimicrobial chemotherapeutic agents (Madigan *et al.*, 2009).

2.4.1 Inhibition of cell wall synthesis

B-lactam antibiotics such as penicillins and cephalosporins are example of antibiotics that interrupt peptidoglycan synthesis. Moreover, glycopeptides such as vancomycin, teicoplanin and oritavancin target bacterial cell wall by binding to the D-alanyl-D-alanine terminal of the peptidoglycan chain, thereby preventing the cross-linking steps. Telavancin, rapidly bactericidal lipoglycopeptide could inhibit peptidoglycan biosynthesis through transglycosylation (Benton *et al.*, 2007).

2.4.2 Inhibition of protein synthesis

The ribosomes are important organelles that are responsible for protein synthesis. Antibiotics that interfere with protein synthesis include the tetracyclines aminoglycosides, and macrolides (Hills, 2010). The aminoglycosides such as gentamicin are bactericidal agent. It causes misreading of the code on mRNA and results in the dysfunctional bacterial proteins. Tetracyclines such as oxytetracycline, doxycycline, minocycline and tetracycline inhibit protein synthesis by blocking a

transfer RNA that transports amino acids to produce polypeptide. The macrolides such as erythromycin and clarithromycin can bind to one of the ribosomal subunits and inhibit the ribosomes from their function (Kaufman, 2011).

2.4.3 Interference with nucleic acid synthesis

DNA replication and cell division are fundamental of the production of new bacterial cells, and some antibiotics can inhibit the DNA replication. Rifampicin interferes with RNA polymerase. Quinolones disrupt DNA synthesis by interference with type II topoisomerases, DNA gyrase and topoisomerase IV during DNA replication and cause double strand breaks (Strohl, 1997).

2.4.4 Inhibition of metabolic pathway

Important antibiotics that inhibit folate synthesis include trimethoprim and sulfonamides. The sulfonamides such as sulfamethoxazole and trimethoprim can block the key steps in folate synthesis. Folate is a cofactor in the biosynthesis of nucleotides, which are the building blocks of DNA and RNA (Strohl, 1997).

2.4.5 Interference with cell membrane integrity

In general, the primary site of antibiotic action is the cytoplasmic membrane of Gram positive bacteria, or the inner membrane of Gram negative bacteria. The antibiotic, polymyxins exert their inhibitory effects by increasing bacterial membrane permeability and cause leakage of bacterial content. The cyclic lipopeptide daptomycin displays rapid bactericidal activity by binding to the cytoplasmic membrane in a calcium dependent manner. The oligomerizing of drug in the membrane leads to an efflux of potassium from the bacterial cell and cell death (Tenover, 2006; Leach *et al.*, 2007).

2.5 Mechanism of antibiotic resistance in bacteria

Bacteria exhibit various mechanisms to protect themselves from antibiotics.

2.5.1 Antibiotic inactivation

The defense mechanisms within the category of antibiotic inactivation include the production of enzymes that degrade or modify the drug itself. Biochemical strategies are hydrolysis, group transfer, and redox mechanisms. Some bacteria produce modified enzyme to destroy antibiotic activity by targeting and cleavaging the chemical bonds (e.g. esters and amides). These enzymes can be often excreted by the bacteria and inactivated antibiotics before they reach their target within the bacteria. The classical hydrolytic amidases are the β-lactamases that cleave the β- lactam ring of the penicillin and cephalosporin antibiotics (Poole, 2004). The most diverse family of resistant enzymes is the group of transferases. These enzymes inactivate antibiotics (aminoglycosides, chloramphenicol, streptogramin, macrolides or rifampicin) by chemical substitution. Adenylyl, phosphoryl or acetyl groups are added to the periphery of the antibiotic molecule. The oxidation or reduction of antibiotics has been infrequently exploited by pathogenic bacteria. Lyases are enzymes that cleave C-C, C-O, C-N and C-S bonds by non-hydrolytic or non-oxidative routes. These reactions frequently result in double bond formation or ring closure (Wright, 2005).

2.5.2 Target modification

The second major resistant mechanism is the modification target sites of antimicrobials that prevent drug binding or action. This is a common mechanism of resistance. Target site changes often result from spontaneous mutation of a bacterial gene on the chromosome and selection in the presence of the antimicrobial agent (Lambert, 2005). In some cases, the modifications in target structure for resistance require other changes in the cell to compensate for the altered characteristics of the target. The best known example of this mechanism is probably the alternative penicillin binding protein (PBP2a), which is produced by MRSA in addition to the "normal" penicillin binding proteins. The protein is encoded by the *mecA* gene, and PBP2a is not inhibited by antibiotics such as methicillin and the β- lactam antibiotics. The resistant

bacterial cell continues to synthesize peptidoglycan, and hence has a structurally around cell wall (Leski and Tomasz, 2005).

2.5.3 Interference of protein synthesis

A wide range of antibiotics interfere with protein synthesis on different levels of protein metabolism. The resistance to antibiotics that interfere with protein synthesis (aminoglycosides, tetracyclines, macrolides, chloramphenicol, fusidic acid, mupirocin, streptogramins and oxazolidinones) or transcription via RNA polymerase (the rifamycins) is achieved by modification of the specific target (Bockstael and Aerschot, 2009).

2.5.4 Interference of DNA synthesis

Fluoroquinolones interact with the DNA gyrase and topoisomerase IV enzymes and prevent DNA replication and transcription. Resistance is conferred by mutations in specific regions of the structural genes that sufficiently alters these enzymes and prevent the binding of antibiotics (Ince *et al.*, 2002). The most common mutations in this region cause resistance through decreased drug affinity (Eliopoulos, 2004).

2.5.5 Interference of efflux pumps and outer membrane (OM) permeability

The efflux pumps are the membrane proteins that export the antibiotics out of the cell and keep its intracellular concentrations at low levels. Reduction of outer membrane permeability results in reduction of antibiotic uptake. The reduction of uptake and active efflux induce low level resistance in many clinically important bacteria (Nikaido, 1994).

1) Efflux pumps

Increasing the efflux also plays a role, especially with hydrophobic compounds that presumably enter the cell via diffusion. At the same speed where these antimicrobial agents enter the cell, efflux mechanisms pump them out before they reach their target. Efflux pumps affect all classes of antibiotics especially the macrolides, tetracyclines and fluoroquinolones. A mutation resulting in a multidrug resistant efflux

pump leads to resistance to a wide variety of structurally unrelated antimicrobial agents (Wise, 1999).

2) Outer membrane permeability changes

Gram negative bacteria possess an outer membrane that consist of an inner layer containing phospholipids and an outer layer containing the lipid A moiety of lipopolysaccharides (LPS). This composition of the outer membrane slows down drug penetration and transportation of drug across the outer membrane by porin proteins. Antibacterial compounds that are transported in this way may be subject to resistance by loss of non-essential transporters, lack of porin or mutations. These are able to modify the structure of these channels and thus decrease the influx (Yoneyama and Katsumata, 2006). Some microbes possess impermeable cell membranes that prevent drug influx as exemplified by *Ps. aeruginosa*. Furthermore, many large molecules of antimicrobial agents are naturally inactive against certain groups of bacteria because they simply cannot pass into the bacterial cell. Mechanisms of bacterial resistance to antibiotics are shown in Table 2.2 (Rachakonda and Cartee, 2004).

Table 2.2 Mechanisms of bacterial resistance to antibiotics (Madigan *et al.*, 2009)

Resistance mechanism	**Antibiotic example**	**Genetic basis of resistance**	**Mechanism present in**
Reduce permeability	Penicillin	Chromosome	*P.aeruginosa* Enteric Bacteria
Inactivation of antibiotic (for example, penicillinase; modifying enzyme such as methylase, acetylase, phosphorylase	Penicillin	Plasmid and chromosome	*S. aureus* Enteric Bacteria *Neisseria gonorrhoeae*
	Chloramphenicol	Plasmid and chromosome	*S. aureus* Enteric Bacteria *S. aureus*
	Aminoglycosides	Plasmid	*S. aureus*
Alteration of target (for example, RNA polymerase, rifamycin; ribosome, erythromycin, and streptomycin; DNA gyrase, quinolones	Erythromycin	Chromosome	*S. aureus*
	Rifamycin		Enteric Bacteria
	Streptomycin		Enteric Bacteria
	Norfloxacin		Enteric Bacteria *S. aureus*
Development of resistant biochemical pathway	Sulfonamides	Chromosome	Enteric Bacteria *S. aureus*
Efflux (pumping out of cell)	Tetracyclines	Plasmid	Enteric Bacteria
	Chloramphenicol	Chromosome	*S. aureus* *B. subtilis*
	Erythromycin	Chromosome	*Staphylococcus* spp.

2.6 Skin disease causing bacteria

2.6.1 *Escherichia coli*

Escherichia coli is Gram negative and facultative bacteria. The bacteria can motile, capsule producing and non-spore forming. Cells are typically rod shape, 1.1-1.5 µm x 2.0-6.0 µm and occur singly or in pair. *E. coli* is commonly found in the lower intestine of warmed blood organism. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning. The harmless strains are part of the normal flora of the gut. The strains can benefit their hosts by producing vitamin K_2, and preventing the establishment of some pathogenic bacteria within the intestine. However, these bacteria can cause disease through water or food consumption such as gastroenteritis, urinary tract infections, neonatal meningitis, septicemia and pneumonia. Moreover, several reports indicated that *E. coli* was found to be the causative agent of neonatal omphalitis, cellulitis localized to lower or upper limbs, necrotizing fasciitis and surgical site infections (Krieg and Holt, 1984; Petkovšek *et al.*, 2009).

2.6.2 *Pseudomonas aeruginaosa*

Pseudomonas aeruginosa is Gram negative, aerobic bacteria and can motile by one or several polar flagella. Cells are typically straight or slightly curved rod approximately 0.5-1.0 µm x 1.5-5.0 µm. *Ps. aeruginosa* is the most common bacteria that cause both community-acquired and hospital-acquired infection especially immunocompromised patients. Nosocomial infection caused by *Ps. aeruginosa* includes pneumonia, endocarditis, meningitis, osteomyelitis, diarrhea, enteritis, urinary tract infection, blood stream infection, surgical site infection and skin infection such as deep abscesses, cellulitis, and fascilitis (Krieg and Holt, 1984; Driscoll *et al.*, 2007).

2.6.3 *Propionibacterium acnes*

Propionibacterium acnes is Gram positive, rod shape, non-spore forming and typically aerotolerant anaerobic bacteria. This bacterium is normal flora typically found in skin, oral cavity, large intestine, the conjunctiva and the external ear canal. It uses sebum, cellular debris and metabolic byproducts from the surrounding skin tissue as their primary sources of energy and nutrients. However, *P. acnes* can lead to common skin disorder such as acnes, papules and folliculitis. Moreover, the damage caused by

P. acnes can affect to tissue and susceptible to colonization more than opportunistic bacteria, such as *S. aureus and S. epidermidis*. The infection by *P. acnes* results in various diseases including chronic endophthalmitis following cataract surgery and rarely infects heart valves leading to endocarditis (Sneath *et al.*, 1986; Perry and Lambert, 2011).

2.6.4 *Streptococcus pyogenes*

Streptococcus pyogenes is Gram positive, facultative anaerobic, non-motile and non-spore forming. Cells are typically spherical or ovoid shape approximately 0.5-2.0 µm in diameters. It occurs as long chain of cocci and occasionally in pairs. *St. pyogenes* bacteria are classified into group A streptococci which display streptococcal group A antigen in their cell wall and also had strong β-hemolysis. *St. pyogenes* is one of skin normal flora and usually pathogenic results to many disorders. They are the most common cause of bacterial pharyngitis, rheumatic fever, impetigo and also infect deep layer of skin resulting to erysipelas and cellulitis (Sneath *et al.*, 1986; Cunningham, 2000).

2.6.5 *Staphylococcus epidermidis*

Staphylococcus epidermidis is Gram positive, non-motile and non-spore forming, which are arranged in grape-like cluster. The size of *S. epidermidis* is approximately 0.5 -1.5 µm in diameters. Cells occur predominantly in pairs and tetrads and sometimes as single cell. *S. epidermidis* is the part of normal human flora, typically the skin flora, and less common of mucosal flora. Although *S. epidermidis* is not usually pathogenic, patients with compromised immune systems are at risk of developing infection. The infection of *S. epidermidis* can cause various diseases including food poisoning, pneumonia, osteomyetis, endocartitis, toxic shock syndrome and skin infection such as folliculitis, furuncles, impetigo, wound infections and scalded skin syndrome (Sneath *et al.*, 1986).

2.6.6 *Staphylococcus aureus*

Staphylococcus aureus is Gram positive facultative bacteria. Cells are cocci and typically occur singly or in pairs. Division may be more than one plane giving rise to irregular clusters. Some uncommon strains produce capsule or pseudocapsule result in

producing virulence factor over uncapsulated strains. *S. aureus* is the most important normal flora bacteria found on skin and respiratory tract. Although, *S. aureus* is non pathogenic but sometimes it can be pathogen that cause of skin, respiratory and food poisoning diseases. The most common diseases caused by *S. aureus* include pimples, impetigo, furuncles, cellulitis, folliculitis, carbuncle, scaled skin syndrome, abscesses, pneumonia, meningitis, endocarditis, osteomyelitis, toxic shock syndrome (TSS), bacteremia, and sepsis (Sneath *et al.*, 1986; Plata *et al.*, 2009).

2.7 Virulence factor of *Staphylococcus aureus*

S. aureus is a pathogen expressing multiple virulence factors that mediate host colonization, invasion of damaged skin and mucosa, dissemination through the body and evasion of host defense mechanisms (Ferry *et al.*, 2005; Chanda *et al.*, 2010). Some virulence factors of *S. aureus* were described in Figure 2.3.

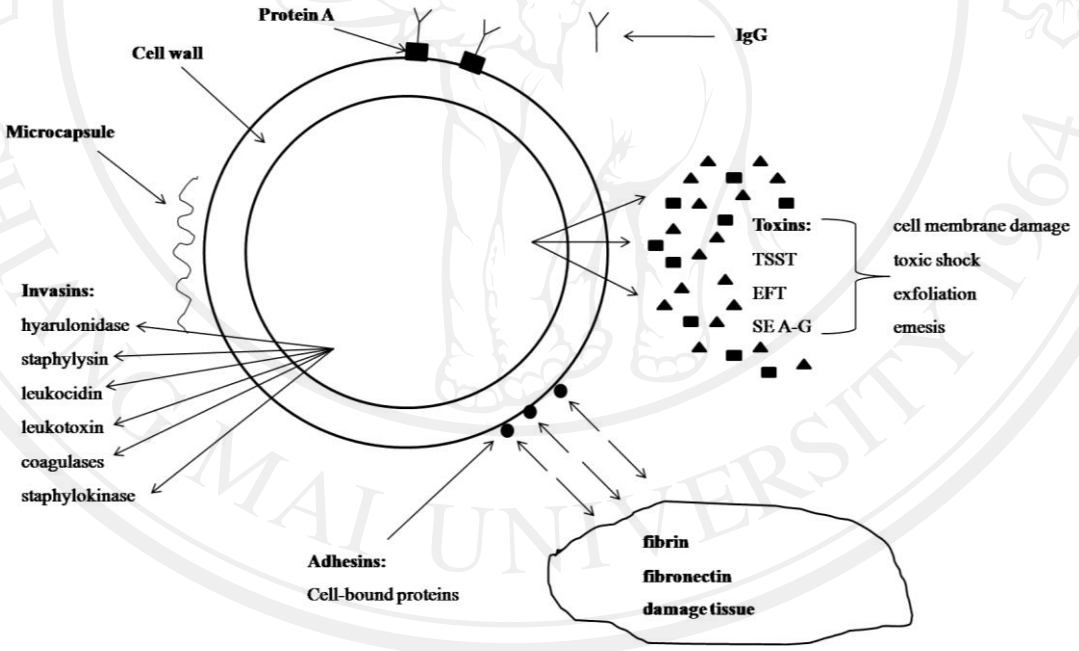

Figure 2.3 Virulence factors of *S. aureus*

2.7.1 Adherence factors

Attachment of *S. aureus* to the host cell surface is mediated by several adhesins. One major class of *S. aureus* adhesins comprises of proteins covalently anchored to cell peptidoglycans. These molecules recognize the most prominent components of the extracellular matrix or blood plasma, including fibrinogen, fibronectin, and collagens.

These plasma proteins coat indwelling medical devices and the ability of the bacteria to adhere to the deposited proteins is believed to be an important factor in the pathogenesis of wound and foreign body infections (Foster and Höök, 1998).

The adhesins are surface attached proteins that allow the bacteria to attach to a wide variety of human tissues. In *S. aureus*, the adhesins are divided into two groups known as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and secreted expandedrepertoire adhesive molecules (SERAMs) (Clarke and Foster 2006). MSCRAMMs are covalently bound to the peptidoglycan and function in helping bacteria adhere to different host extracellular matrices such as collagen, fibrinogen and fibronectin. This group of adhesins include protein A (SpA), collagen adhesin (Cna), clumping factors (ClfA and ClfB), and fibronectin binding proteins (FnBPs). The second group of adhesins, SERAMs, is secreted but partially bound to the cell wall. SERAMs include the extracellular adhesive protein (Eap) and extracellular fibrinogen-binding protein (Efb), and extracellular matrix protein (Emp), and their main function is to modulate the host immune system (Lowy, 1998; Foster and Höök, 1998).

2.7.2 Secreted factors

The secreted virulence factors of *S. aureus* play active roles in disarming host immunity by disrupting host cells and tissues, and interfering with the host immune system to release nutrients and facilitate bacteria dissemination. The toxins are secreted proteins that cause tissue damage and generate pus in abscesses which is believed to facilitate transmission between hosts (Collins *et al*., 2010). The staphylococcal secreted toxins include cytotoxins, superantigens, proteases, lipase and coagulase. Cytotoxins are a group of toxins that can lyses host cells including α-toxin (*hla*), β-toxin (*hlb*), γ-toxin (*hld*), and leukocidins (LukF-PV). These toxins do not only lyse host cells, but also alter the host immune response such as inducing caspase dependent and caspase independent apoptosis (Haslinger *et al.*, 2003). Superantigens are a class of protein toxins that can cause nonspecific T-cell activation and massive cytokines release. *S. aureus* superantigens include several enterotoxins (SEs), toxic shock toxin-1 (TSST-1) and exfoliative toxins (ETs) (Fraser and Proft, 2008).

2.8 Antibiotic resistance in *S. aureus*

The Gram positive bacterium, *Staphylococcus aureus* is a leading cause of hospital and community associated infections. Moreover, *S. aureus* is the most frequent cause of lower respiratory tract and cardiovascular infections, and surgical infection in immunocompromised patients. Furthermore, it also cause of pneumonia and blood stream infection (Loffler and Macdougall, 2007). In the past few years, β-lactam antibiotics are a good choice for treatment staphylococcal infection, however, there are increasing of the bacteria resistance to these antibiotics. *S. aureus* has shown a unique ability to quickly respond to each new challenge with the development of a new resistance mechanism. Resistance mechanisms include enzymatic inactivation of the antibiotic such as penicillinase and aminoglycoside modification enzymes. Alteration of the target with decreased affinity for the penicillin-binding protein 2a (PBP2a) of methicillin-resistant *S. aureus* and D-Ala-D-Lac of peptidoglycan precursors of vancomycin-resistant strains was found. Interference to efflux pumps was occurred in fluoroquinolones and tetracycline (Pantosti *et al.*, 2007).

2.9 Gene involved in methicillin resistance *S. aureus* (MRSA)

The cell wall is a key structural component comprises the outmost layer of cell wall in Gram positive bacteria, whereas in Gram negative bacteria the cell wall lies underneath an addition layer known as the outer membrane. At the molecular level, the cell wall is meshwork of glycan (polysaccharide) chains that are interconnected by cross-links, known as peptidoglycan (Vollmer *et al.*, 2008). The biosynthesis of the peptidoglycan is accomplished by the membrane bound enzymes known as penicillin binding proteins (PBPs). PBPs are localized to the extracellular surface of the cytoplasmic membrane. This biosynthesis may catalyze in both a glycosyltransferase activity for elongation of glycan strands and transpeptidase activity for the interconnection of the glycan strands by peptide cross linking (Heijenoort, 2001).

β- lactam antibiotics are structural analogues of D-alanyl-D-alanine residues and bind the intrinsic staphylococcal PBPs thus, inhibiting the transpeptidase function and interfering with the cross linking reaction (Tipper and Strominger, 1965). Therefore, the cell wall becomes mechanically weak and some of the cytoplasmic contents are released

and the cell dies. However, *S. aureus* renders methicillin ineffective by the production of an alternative PBP (PBP2a), which has reduced binding affinity for β- lactams. PBP2a is unique peptidoglycan residues, but does not bind to β-lactam antibiotics at the concentration beyond that achievable pharmacology. In addition, PBP2a is encoded by the structural gene *mecA* on the chromosome, which has been detected in methicillin resistant srains of staphylococcal species. The *mecA* gene is a component of a large DNA fragment designated *mec* DNA, which is located at the specific site of the *S. aureus* chromosome (Brown and Reynolds, 1980).

Expression of PBP2a is controlled by two regulator genes on *mec* DNA, *mecI* and *mecR1*, located upstream of *mecA*, which encode *mecA* repressor protein and signal transducer protein, respectively. *mecR1* is required for the induction of PBP 2a production. Upon contact with β-lactam, which is equivalent to an MRSA inducing factor, *mecR1* is activated, and its signal binds to the promoter region of *mecA* and is transduced to *mecI*, thereby suppressing transcription. However, *mecR1* and *mecI* have high protein sequence homology with the protein BlaR1 and BlaI, respectively which induced the expression of β-lactamase gene, *blaZ. BlaR1-BlaI* systems are similar to *mecR1-mecI* system in term of gene arrangement that might be suggested that *mecA* may have acquired the regulatory genes from *blaZ* system (Song *et al.*, 1987). The regulation of PBP2a expression is performed by the repression of MecI induced the transcription of *mecA* and *mecR1-mecI* (Figure 2.4). The DNA binding protein, MecI binds to the sensor transducer domain of MecR1resulting in automatically cleavage of the sensor transducer. Therefore, the intracellular zinc peptidase domain is activated which interns cleavage the MecI repressor into inactive fragments allowing transcription in both *mecA* and *mecR1-mecI* (Berger-Bächi and Rohrer, 2002). Moreover, some genes are also discovered in *S. aureus* includes the factor of essential methicillin gene, namely *femABX*. The *femAB* operon encodes two proteins required for the formation of the pentaglycine interpeptide bridge that serves as the cross link of peptidoglycan. *FemA* was the first described in the family, and was linked to high level resistance in MRSA, because there resistance level decreased when *femA* was inactive. The exact relation between *femA* and high level resistance to methicillin is universally present in all MRSA isolates. However, *femA* has a regulating gene, and regulation of expression of the gene is not known (Hegde and Shrader, 2001). The

femA gene product, a 48-kDa protein, has been implicated in cell wall metabolism and is found in large amounts in actively growing cultures (Johnson *et al.*, 1995).

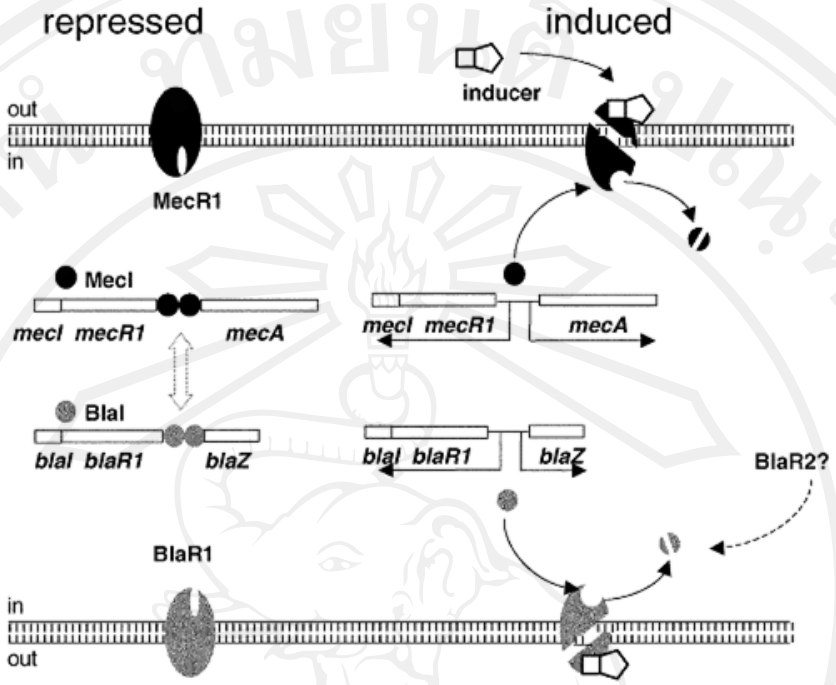

Figure 2.4 The induction of PBP2a and penicillinase control by regulatory system of *mecA* and *BlaZ*, respectively (Berger-Bächi and Rohrer, 2002).

2.10 Free Radicals

Free radicals are atom or molecule containing at least one unpaired electron in their orbital and can be taken one electron from other molecules and terminate the chain reaction. These uncoupled electrons are very reactive with adjacent molecules such as lipids, proteins, and carbohydrates and can cause cellular damage (Kuhn, 2003). Free radicals can also be produced by many cells as a protective mechanism. Neutrophils produce free radicals to attack and destroy pathogens, while a liver uses free radicals for detoxification (Lunec *et al.*, 2002). However, the presence of free radicals within the body can also has a significance role in the development and progression of many disease processes like heart disease, congestive heart failure, hypertension, cerebrovascular diseases and diabetic complications. The details of some free radicals are shown in Table 2.3.

Table 2.3 Examples of biological oxidants formed

Reactive oxygen species		Reactive nitrogen species		Miscellaneous	
Hydroxyl	$^{\bullet}OH$	Free radicals		Thiyl	RS•
Superoxide	$O2^{\bullet-}$	Nitric oxide	$NO^{\bullet}$	Hydrogen atom	$H^{\bullet}$
Alkoxyl	$LO^{\bullet}$	Nitrogen dioxide	$NO_2^{\bullet}$	Carbon-centered radicals e.g.	$CCl_3^{\bullet}$
Hydroperoxyl	$HO_2^{\bullet}$				
Peroxyl	$LO_2^{\bullet}$	Non radicals		Thiol	RSH
Hydrogen peroxide	H_2O_2	Dinitrogen dioxide	N_2O_3		
Singlet oxygen	$^1\Delta G$ O_2	Dinitrogen tetroxide	N_2O_4		
Lipid peroxides	LO_2	Dinitrogen pentoxide	N_2O_5		
Ozone	O_3	Peroxynitriles	$ONO_2^{\bullet-}$		
		Alkyl peroxynitriles	$LO_2NO^{\bullet-}$		
		Nitrocarbonate	O_2NOCO		

2.10.1 Reactive oxygen species (ROS)

ROS includes both oxygen radicals and certain non radicals that are oxidizing agents or are easily converted into radical (Figure 2.5). Radicals derived from oxygen represent the most important class of radical species generated in living systems. Excessive generation of ROS has pathological consequences including damage of proteins, DNA, lipids and membranes. ROS can be produced from both endogenous and exogenous substances. Potential endogenous sources include mitochondria, cytochrome P450 metabolism, peroxisomes and inflammatory cell activation (Inoue *et al.*, 2003).

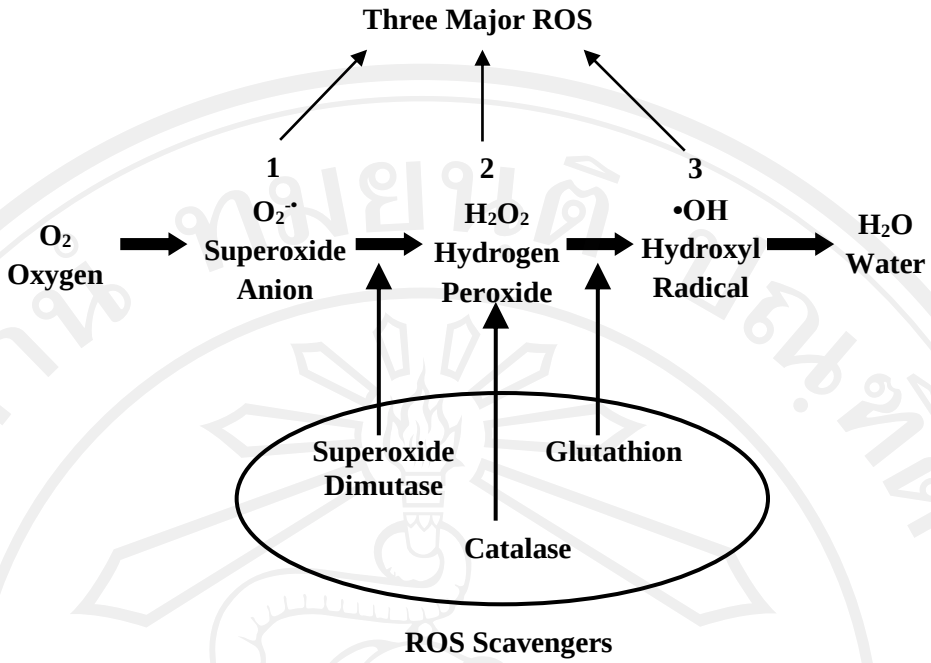

Figure 2.5 The process of formation of reactive oxygen species (ROS) (Scheibmeir *et al.*, 2005)

1) The hydroxyl free radical (˙OH)

The hydroxyl free radical is one of the strong radicals found in the body with almost every molecule in the living cell including DNA, lipids, proteins and carbohydrate. Hydroxyl radical is generally generated by two principle mechanism; hemolytic fission of water molecule by ionizing radiation (ultraviolet, gamma, microwave, X-rays, etc.) or the breakdown of hydrogen peroxide with metals:

$$Metal^{n} + H_2O_2 \rightarrow Metal^{(n+1)} + {}^{\bullet}OH + HO^{-}$$

Several metals can react in this way (iron, copper, chromium, vanadium, etc.), but the most common is Fe^{2+}. In the Fenton reaction, Fe^{2+} is likely generate from Fe^{3+} by cellular oxidants (reducing agents) such as ascorbate. The resulting ascorbyl free radical is much less reactive and probably dissipates by dismutation into ascorbic acid and dehydroascorbic acid. Similar reaction, the Haber-Weiss reaction (or $O2^{\bullet-}$ Fenton reaction), $O_2^{\bullet-}$ reacts with Fe^{3+} to generate Fe^{2+} (and O_2), thereby permitting the Fenton reaction to proceed more effectively. As discuss later, ˙OH also can be produced from peroxynitrous acid and from the reaction between $O_2^{\bullet}$ and hypochlorous acid (Valko *et al.*, 2007).

2) The superoxide free radical anion ($O_2^{\bullet}$)

Super oxide radical anion ($O_2^{\bullet}$) is generally a poor reactive radical. These radical can be generated by adding one electron to oxygen molecules. Many molecules in the body might react with oxygen such as catecholamine and some constituents of mitochondria electron transport chains (Tandon *et al.,* 2005). $O_2^{\bullet}$ radical is also produced during the respiratory burst of phagocytic cell, forming part of body's defense system for the destruction of invading organisms. In humans, $O_2^{\bullet-}$ is the most commonly produced free radical. Phagocytic cells such as macrophages and neutrophils are prominent sources of $O_2^{\bullet-}$. In an inflammatory response, these cells generate free radicals that attack invading pathogens such as bacteria. Production of $O_2^{\bullet-}$ by activated phagocytic cells in response to inflammation is one of the most studied free radical producing systems (Gutteridge and Mitchell, 1999; Scheibmeir *et al.,* 2005).

3) Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) is another ROS found in biological system by enzymatic reactions, including SOD, d-amino acid oxidase, amine oxidase, glycolate oxidase and urate oxidase catalysis reaction. H_2O_2 appears to play role not only in phagocytic defense by immune system but also in methabolic pathways such as the production of thyroxine in the thyroid gland.

2.10.2 Reactive nitrogen species (RNS)

1) Nitric oxide ($NO^{\bullet}$)

$NO^{\bullet}$ is an abundant reactive radical that acts as an important oxidative biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation. $NO^{\bullet}$ is generated in biological tissues by specific nitric oxide synthases (NOSs), which metabolises arginine to citrulline with the formation of $NO^{\bullet}$ via a five electron oxidative reaction (Archer, 1993; Alderton *et al.,* 2001; Bergendi *et al.,* 1999; Forstermann *et al.,* 1998). NO• has greater stability in an environment with a low concentration and acts as an important oxidative biological signaling molecule in a large variety of physiological process. In immune system, both superoxide anion and nitric oxide are produced during the oxidative burst trigger

inflammatory process. They may react together with amounts of an oxidative active molecule which is a potent oxidizing agent and can cause DNA fragmentation and lipid peroxidation (Carr *et al.,* 2000).

2) Peroxynitrite ($ONO_2^{\bullet-}$)

Peroxynitrite is from the reaction between NO• and $O2^{\bullet-}$. Peroxynitrite can attack many biological important compounds including the oxidation of nonprotein sulfhydyls, sulfides, lipid, deoxyribose and ascorbate.

3) Nitrocabonate anion ($O_2NOCO_2^-$)

Nitrocabonate anion rapidly reacts with carbon dioxide to from the unstable nitrosocarboxycarbonate anion ($ONOOCO_2^{\bullet-}$). Nitrocabonate anion may serve two important biological functions. It reacts as a scavenger of $ONO_2^{\bullet-}$ and may be superior microbiocide to H_2O_2.

2.11 Reaction of free radicals with biomolecules

Free radicals and active oxygen species can attack biomolecules such as lipids, sugar, proteins and DNA that may result in oxidative damage. This may lead to cell death, cellular component destruction, DNA breaks, protein inactivation and mitochondria damage and membrane disruption.

2.11.1 Cellular components

Cellular components are involved with poly unsaturated fatty acid (PUFA) residues of phospholipids that highly sensitive to oxidation. When PUFA is attacked by free radical, it can generate fatty acids radical ($L^{\bullet}$) that rapidly adds oxygen to form fatty acid peroxyl radical ($LOOH^{\bullet}$). The fatty acid peroxyl radical can elongate the chain by reacting with other lipid molecules to produce fatty acid radical and lipid hydroperoxide (LOOH). This mechanism is called “lipid peroxidation” that causes the increasing of membrane permeability and could damage other structure of cell such as RNA, DNA, protein and enzyme (Dröge, 2002; Bryan, 2006).

$LH + R^{\bullet} \rightarrow L^{\bullet} + RH$

$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$ (Fatty acid peroxy free radical)

$LOO^{\bullet}$ + L'H (New PUFA) → LOOH (Lipid hydroperoxide) + L

LOOH → $LOO^{\bullet}$(Lipid peroxyl), $LO^{\bullet}$ (Lipid alkoxyl, Aldehydes)

2.11.2 Nucleic acid oxidation

Nucleic acids are also sensitive to the oxidation. DNA can be attacked with oxygen radicals especially hydroxyl radical ($OH^{\bullet}$). The hydroxyl radical could react with DNA resulting in DNA base transversions during DNA replication and double stranded DNA break. Moreover, free radicals also lead to single stranded DNA breaks, structure modification, loss of base and DNA-DNA or DNA-protein cross linkages (Cheeseman and Slater, 1993).

2.11.3 Carbohydrates

Free radicals such as hydroxyl radical ($OH^{\bullet}$) react with carbohydrates by randomly abstracting a hydrogen atom from one of the carbon atoms and producing a carbon-centered radical. This leads to chain breaks in important molecules like hyaluronic acid. In the synovial fluid surrounding joints, an accumulation and activation of neutrophils during inflammation produces significant amounts of oxyradicals that is also implicated in rheumatoid arthritis (Devasagayam *et al.,* 2004).

2.11.4 Protein oxidation

Proteins are also target of free radical. However, the oxidations of proteins are less sensitive than lipids. Many amino acids which have sulphur and unsaturated bond could be attacked by free radicals of both ROS and RNS. The primary radical in most oxygenated biological systems is the superoxide ion (O^{-2}) and the hydroperoxyl radical ($HO^{\bullet}$). The major sources of these radicals are leakages from the electron transport chains of mitochondria, chloroplasts and endoplasmic reticulum. Although O^{-2} is relatively unreactive in comparison with other radicals, biological systems can convert it into more reactive species such as peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$) and hydroxyl radical ($OH^{\bullet}$). These radicals may lead to protein structure modification such as

fragmentation, polymerization, conformational change, loss of activity and loss of amino acids. The effect of protein oxidation may result in a deleterious loss of protein function and loss of enzymatic activity or protein signaling. There are numerous disease caused by protein oxidative modification as shown in Table 2.4 (Sivanandham, 2011).

Table 2.4 Protein oxidative modification and disease (Shacter, 2000)

Modification	**Disease/tissue**
Carbonyls	
Glutamine synthetase	Aging, Alzheimer, ischemia/reperfusion
IgG	Rheumatoid arthritis
Kidney proteins	Chronic estrogen exposure
Lung proteins	Hyperoxia
Brain proteins	Ischemia/reperfusion
Eye lens proteins	Cataracts
Muscle proteins	Muscular dystrophy
Unidentified proteins	Aging, Parkinson's disease, Neonatal lung fluids
Methionine sulfoxide	
α-1 - proteinase inhibitor	Bronchitis
Lipid peroxidation adducts	
LDL	Athersclerosis
Glutathiolation (SH oxidation)	
Carbonic anhydrase III	Aging
Muscle proteins	Muscular dystrophy
Unidentified proteins	Activated monocytes
3-Chlorotyrosine, dityrosine	
LDL	Atherosclerosis
Hydro (pero) xyleucine	
Unidentified proteins	Cataracts
Nitrotyrosine	
Unidentified proteins	Acute inflammatory lung tissue, Atherosclerosis, Rheumatoid arthritis
Aggregates	
IgG	Rheumatoid arthritis

2.12 Antioxidants

An antioxidant is substance that present at low concentration compared with those of an oxidizable substrate. It significantly delays or prevents oxidation of that substrate.

2.12.1 Type of antioxidant

Antioxidants are divided into 2 types

1) Synthetic antioxidants

Mostly the synthetic antioxidants that are phenolic compounds for example butylates hydroxyanisol (BHA), butylates hydroxyl toluene (BHT), tertiary butyhydroquinone (TBHQ) and gallic acid derivatives.

2) Natural antioxidants

Natural antioxidants are found in many sources such as plants, fungi, microorganism and even animal tissue. Phenolic compounds are also the majority group of natural antioxidants and the three important groups of antioxidant are tocopherol, flavonoids and phenolic acid. Natural antioxidants have been widely used as alternative antioxidants but the synthetic antioxidant has new toxicology data that imposed caution in their use. Some natural antioxidants are more potent of efficiency and safer than synthetic antioxidants (Pokorny *et al.*, 2001).

Moreover, antioxidant is divided into two classes based on mechanism of action.

1) Chain-breaking antioxidants

Antioxidant such as vitamin E and β- carotene can break the chain of free radical formation by donating an electron to stabilize an existing free radical. Chain-breaking antioxidants are found in the blood and the fluid of the extracellular space, where preventive antioxidant enzymes are absent or present in very small quantities (McDermott, 2000). These small molecule of antioxidants include both water and lipid soluble varieties. The lipid soluble antioxidants are located in the cellular membranes and lipoproteins, whereas the water soluble antioxidants are present in the aqueous environments, such as fluid inside cells and in the blood (Clark, 2002).

2) Preventive antioxidants

Preventive antioxidants are enzymes that scavenge initiating radicals before they start an oxidation chain. The antioxidant enzymes inside cells are an important defense against free radicals. The main enzymatic scavengers responsible for the prevention of ROS formation and oxidation are superoxide dismutase (SOD), catalase and glutathione.

SOD is found in virtually every oxygen based organism, and its major function is to catalyze the dismutation of superoxide to hydrogenperoxide. This reaction is generally considered to be the primary antioxidant defense in the body because it prevents further generation of free radicals. In humans, the highest levels of SOD are found in the liver, adrenal gland, kidney, and spleen (Halliwell, 1996).

Catalase and glutathione peroxidase work to detoxify oxygen reactive radicals by catalyzing the formation of H_2O_2 derived from superoxide. The liver, kidney, and red blood cells possess high levels of catalase which helps to detoxify chemicals in the body.

Glutathione also plays an important role in a variety of detoxification processes. Glutathione readily interacts with free radicals, especially the hydroxyl radical, by donating a hydrogen atom. This reaction provides protection by neutralizing reactive hydroxyl radicals that are thought to be a major source of free radical pathology, including cancer (Clark, 2002). The mechanism of action of some antioxidants is shown in Table 2.5.

Table 2.5 Mechanism of action of various antioxidants against disease (Shacter, 2000)

Compounds	**Pathology**	**Mechanism of action**
Catalase (CAT)	Cancer, diabetic retinopathy	Destroys hydrogen peroxide in high concentration by catalyzing its two-electron dismutation into oxygen and water.
Glutathione peroxidase (GPx)	Neurodegenerative diseases	Catalyze the reduction of hydroperoxides at the expense of GSH. In this process, hydrogen peroxide is reduced to water whereas organic hydroperoxides are reduced to alcohols.
Superoxide dismutase (SOD)	Neurodegenerative diseases	Catalyze the one-electron dismutation of superoxide into hydrogen peroxide and oxygen.
Alkaloids	Cancer, Neurodegenerative diseases, chronic inflammation	Shown a variety of biological activities such as inhibition of topoisomerase I and II; cytotoxicity against different tumor cell lines.
Catechins	Neurodegenerative diseases	Enhance activity of SOD and catalase.
Carotenoids	Cancer, diabetic retinopathy, chronic inflammation	Mainly act as physical quenchers of reactive oxygen.
α-tocopherol	Cancer, neurodegenerative diseases, chronic inflammation	Scavenges lipid peroxyl radicals (LOO) through hydrogen atom transfer.

Table 2.5 (continued)

Compounds	**Pathology**	**Mechanism of action**
Glutathione	Cancer	Glutathione in the nucleus maintains the redox state of critical protein sulphydryls that are necessary for DNArepair and expression
Proanthocyanidin (GSPE)	Cardiovascular disorders	Inhibitory effect on pro-apoptotic and cardio regulatory genes. Modulating apoptotic regulatory *bcl-XL*, *p53* and *c-myc* genes
Phenolics	Cancer, diabetic retinopathy, chronic inflammation	Inhibit the oxidation of lipids, fats, and proteins (RH) by donation of a phenolic hydrogen atom to the free radical
Quercetin, Kaempferol, genistein, resveratrol	Colon cancer	Suppresses COX-2 expression by inhibiting tyrosine kinases important for induction of COX-2 gene expression
Tannins	Cardiovascular disorders	Tannins are known to enhance synthesis of nitric oxide and relax vascular segments precontracted with norepinephrine

2.13 Phenolic compounds

Phenolic compounds are a class of chemical compounds consisting of a hydroxyl-group (-OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol, which is also called carbolic acid C_6H_5OH (Figure 2.6). In plants, there are a variety of phenolic compounds such as flavonoid, α-tocopherol and (-)-epigallocathechin gallate (EGCG). Plants polyphenols cover wide range of compound consisting of hydrolysable tannin, condensed tannin, caffeates, lignans, flavonoids and compounds that having polyphenolic structure in their molecules such as caffeic acid and derivatives (Table 2.6, Figure 2.7) (Lester *et al.,* 1999).

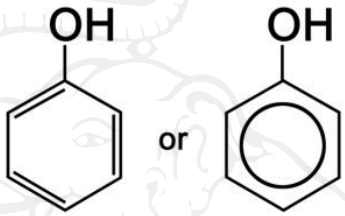

Figure 2.6 Basic structure of phenolic

Flavonoids are the most important group of polyphenol in food. This group consists of anthocyandins, proanthocyanidins, cathechins, flavones, flavonol and their glycoside derivatives. Recently, flavonoids have been proposed to act as antioxidants because of their radical scavenging abilities. Some flavonoids are strong oxygen radical scavengers and metal chealators, which are effective in preventing lipid peroxidation.

Alkoaloids are a chemically heterogeneous group of basic nitrogen containing substance that found predominantly in higher plants. Alkaloids usually contain one or more nitrogen atom. In plants, alkaloids occur largely as salts of organic acids like acetic, oxalic, citric acid etc. A few alkaloids are present as glycosides of common sugars such as glucose, rhammose, galactose or esters of organic acids.

Table 2.6 The important classes of phenolic compounds in plants

Number of C-atoms	Basic skeleton	Class
6	C_6	Simple phenolics, Benzoquinones
7	C_6-C_1	Phenolic acids
8	C_6-C_2	Acetophenone, Phenylacetic acid
9	C_6-C_3	Hydroxycinnamic acid, Coumarin,
10	C_6-C_4	Naphthoquinone
13	C_6-C_1-C_6	Xanthone
14	C_6-C_2-C_6	Anthrachinone
15	C_6-C_3-C_6	Flavonoids, Isoflavonoids
18	$(C_6$-$C_3)_2$	Lignans, Neolignans
30	$(C_6$-C_3-$C_6)_2$	Biflavonoids
n	$(C_6$-$C_3)_n$	Lignins
	$(C_6)_n$	Catecholmelanine
	$(C_6$-C_3-$C_6)_n$	(condensed tannins)

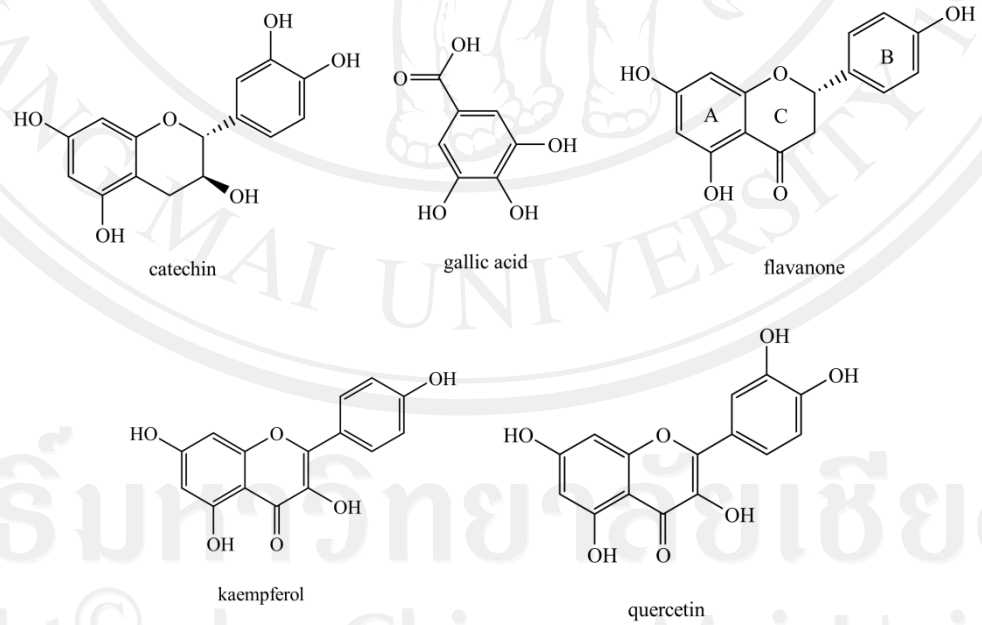

Figure 2.7 Plant phenolic compounds

2.14 Measurement of Total antioxidant activity

The antioxidant activity assay can be classified into two types based on the reaction involved including hydrogen atom transfer (HAT) reaction and single electron transfer (SET) reaction.

2.14.1 Hydrogen atom transfer (HAT) reaction base assay

HAT methods measure the ability of an antioxidant to quench free radicals by hydrogen donation. HAT-based assay, represented by the oxygen radical absorbance capacity (ORAC) assay, involves peroxyl radicals as the oxidant and will provide useful information on radical chain breaking capacity. Antioxidant reactivity and capacity measurements are based on competition kinetics. HAT reactions are solvent and pH independent and are usually quite rapid, typically completed in seconds to minutes. The presence of reducing agents, including metals, is a complication in HAT assays and can lead to erroneously reactivity. In food system, antioxidants normally refer to substance that can inhibit fatty acid auto-oxidation. The major antioxidants are metal chelators e.g., EDTA, preventive) and chain-breaking antioxidants (e.g., BHT, sacrificial) acting as hydrogen atom donors (Huang *et al.*, 2005; Prior *et al.*, 2005).

2.14.2 Single electron transfer (SET) reaction base assay

SET-based methods detect the ability of potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls and radicals. Overall, there are a multitude of electron transfer-based assays for measuring the reducing capacity of antioxidants. The assays are carried out at acidic (Ferric reducing antioxidant power, FRAP), neutral (Trolox equivalent antioxidant capacity, TEAC) or basic (total phenols assay by Folin- Ciocalteau) conditions. Relative reactivity in SET methods is based primarily on deprotonation and ionization potential of reactive functional group and the pH values have an important effect on the reducing capacity of antioxidants, so SET reactions are pH dependent. SET reactions are usually slow and can require long times to reach completion. Therefore, the antioxidant calculation is based on the decrease in product rather than kinetics. Moreover, SET methods are very sensitive to ascorbic acid and uric acid, which are important in maintaining plasma redox tone, and reducing polyphenols are also detected. Importantly, trace components

and contaminants particularly metals interfere with SET methods and can account for high variability and poor reducibility of results (Prior *et al.*, 2005; Wright *et al.*, 2001).

2.15 Method for antioxidant activity testing

2.15.1 DPPH method

DPPH• (2, 2-diphenyl-1-picrylhydrazyl) is a stable nitrogen free radical. The structure of DPPH is shown in Figure 2.8. It is commercially available and does not have to generate before assay. The decolorization on the DPPH• molecule determines the occurrence of a purple color, with an absorption band at a maximum around 520 nm. When DPPH• reacts with a hydrogen donor, the reduced form (DPPH) is generated and accompanied by the disappearance of the violet color. Therefore, the absorbance diminution depends linearly on the antioxidant concentration. The DPPH assay is considered to be mainly based on an electron transfer reaction and hydrogen atom abstraction in a marginal reaction pathway (Prior *et al.*, 2005).

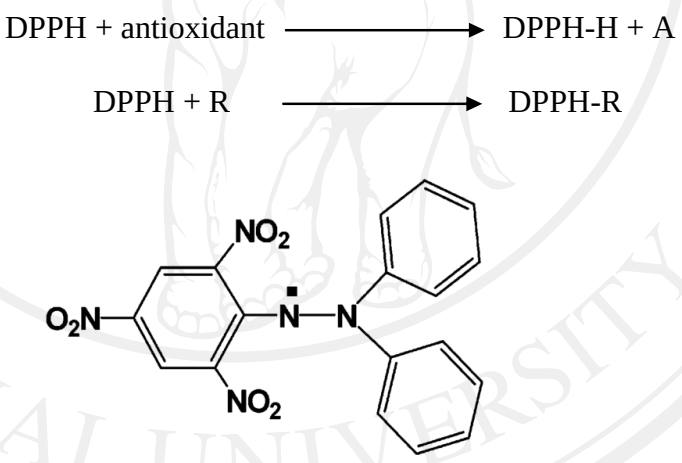

Figure 2.8 DPPH structure (Huang *et al.*, 2005)

Advantages/Disadvantages of DPPH method: The test is simple rapid and needs only a UV-VS spectrophotometer to perform. Thus, it is widespread use in antioxidant screening. However, interpretation is complicated when the test compounds have spectra that overlap DPPH at 515 nm. Moreover, DPPH have many drawbacks since it is not a competitive reaction because DPPH is both radical probe and oxidant so its color can be lost via radical reaction (HAT) or reduction (SET) as well as unrelated reactions and steric accessibility is a major limit of the reaction.

2.15.2 ABTS method

The ABTS cation radical ($ABTS^{\bullet+}$) absorbs at 743 nm giving a bluish-green color, which is formed by the loss of an electron by the nitrogen atom of ABTS (2,2'- azino- bis (3-ethylbenzthiazoline-6-sulphonic acid)) (Figure 2.9). In the presence of trolox or another hydrogen donating antioxidant, the nitrogen atom quenches the hydrogen atom, yielding the solution decolorization. ABTS can be oxidized by potassium persulfate or manganese dioxide giving rise to the ABTS cation radical ($ABTS^{\bullet+}$) and absorbance diminution at 743 nm was monitored in the presence of trolox that is chosen as standard antioxidant.

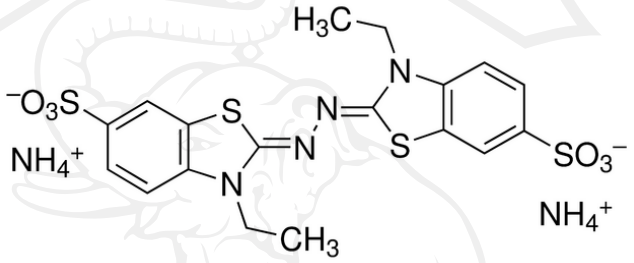

Figure 2.9 Structure of 2, 2′- azinobis (3-ethylbenzothiazoline-6-sulfonic acid) cation (ABTS •+) (Zulueta *et al.,* 2009)

Advantages/Disadvantages of ABTS method: ABTS method is simple operation and has been used in many researches for studying antioxidant capacity. ABTS•+ reacts quickly with antioxidants, normally within 30 minutes (Figure 2.10). It can be used over a wide pH range and can be used to study effects of pH on antioxidant mechanisms. TEAC reactions can be automated and adapted to microplates, flow injection and stopped flow. However, in thermodynamically, a compound can reduce ABTS•+ if it has redox potential lower that of ABTS (0.68 V). Many phenolic compounds have lower redox potentials and can thus react with ABTS•+. In addition, the TEAC reaction may not be the same for slow reactions and it may take a long time to reach endpoint. Thus, using short duration endpoint (4 or 6 min), may interpret before the reaction is finished and result in lower TEAC values (Prior *et al.,* 2005).

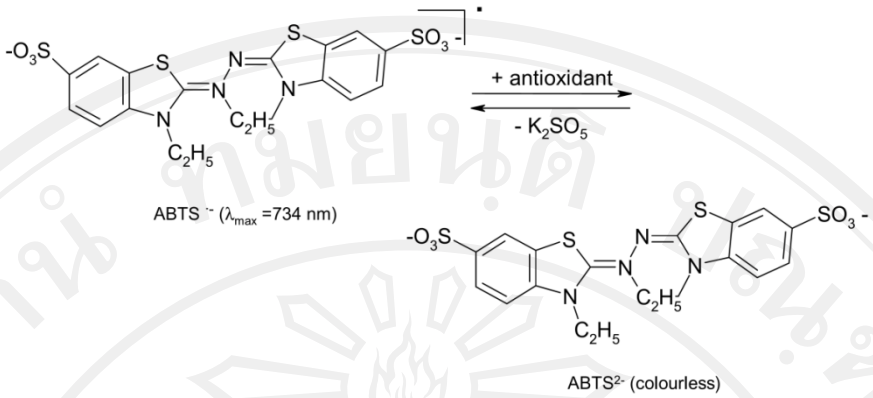

Figure 2.10 Reaction of the ABTS radical in the presence of the antioxidant compound during the ABTS assay (Zulueta *et al.*, 2009)

2.15.3 FRAP assay

Frap assay is based on the ability of phenolics to reduce yellow ferric tripyridyltriazine (Fe (III) - TPTZ) to blue ferrous complex (Fe (II) - TPTZ) by the action of electron donating antioxidants (Benzie and Strain, 1996). However, FRAP cannot detect compounds that act by radical quenching (H transfer), especially thiol and proteins (Figure 2.11).

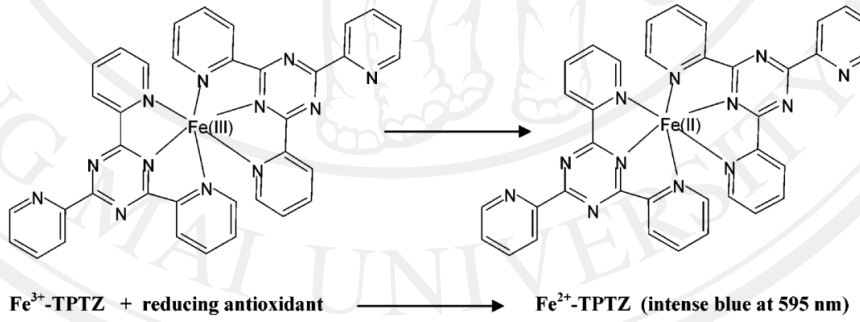

Figure 2.11 Reaction for FRAP assay (Prior *et al.*, 2005)

Advantage/Disadvantage of FRAP assay: The FRAP assay is simple, speedy, inexpensive and required less specialized equipment. It can also perform using automated, semi-automic or manual methods. FRAP is restricted to measure thiol antioxidants, such as glutathione. The limitation of FRAP is, it actually measures only the reducing capability based upon the ferric ion, which is not relevant to antioxidant mechanistically and physiologically (Prior *et al.*, 2005).

2.15.4 Total phenolic content by Folin-Ciocalteau method

The Folin-Ciocalteau method is simple and can be useful in characterizing and standardizing in plant sample. However, this method is interfered from a number of substances particularly sugars, aromatic amines, sulfur dioxide, ascorbic acid, organic acids, Fe (II), enediols and reductones. Moreover, some non phenolic organic substances and inorganic substances may also react with Folin-Ciocalteau reagent to give elevated apparent phenolic concentrations (Prior *et al.*, 2005).

2.16 Plant extraction

Phytochemical constituents in plant extracts depend on the types of solvent used in the extraction procedure. The good solvent for plant extraction includes low toxicity, easy of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. Moreover, the solvent should be nontoxic and should not interfere with the bioassay. The solvent will also depend on the targeted compounds to be extracted (Eloff, 1998). There are various solvent to use for plant phytochemical extraction as follows (Table 2.7).

Table 2.7 Solvents used for active component extraction (Cowan, 1999)

Water	**Ethanol**	**Methanol**	**Chlorofor**	**Ether**	**Acetone**
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenols
Starches	Polyphenols	Terpenoids	Flavones	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonols	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxyllines			
Polypeptides	Sterols	Quassinoids			
Lectins	Alkaloids	Lactones			
		Flavones			
		Phenones			
		Polyphenols			

2.16.1 Water

Water is universal solvent and used to extract plant products with antimicrobial activity. However, plant extracts from organic solvents have been found to give more consistent antimicrobial activity than water extract. Previous research found that water soluble flavonoids mostly anthocyanins did not have significant antimicrobial activity and water soluble phenolics were important as antioxidant compound (Das *et al.*, 2010).

2.16.2 Acetone

Acetone can dissolve many hydrophilic and lipophilic components from the plants. Therefore, it is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported that acetone used to extract tannins and phenolics better than alcohols (Eloff, 1998; Das *et al.*, 2010). In addition, both acetone and methanol were found to extract saponins, which have antimicrobial activity (Ncube *et al.*, 2008).

2.16.3 Alcohol

The higher activity of the ethanolic extracts when compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols than aqueous extracts. Furthermore, the aqueous extract usually has low activity because of enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium for the occurrence of the microorganisms as compared to ethanol (Lapornik *et al.*, 2005). Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material (Wang, 2010). However, methanol is more polarity than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of substances as it may lead to incorrect results.

2.16.4 Chloroform

Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents (Cowan, 1999).

2.17 Medicinal plants used in this study

2.17.1 *Andrographis paniculata* Nees

Thai name: Fah Thalai Chon

Family: Acanthaceae

Characteristic: This plant is an annual herb with a high of 30-110 cm in moist shady places. The lance-shaped leaves are glabrous, up to 8 cm long and 2.5 cm broad. The flowers are small, white with rose-purple spots on the petals. The stem is slender, dark green and glabrous. It is extremely bitter in taste in every part of plant body (Deng *et al.*, 2011) (Figure 2.12).

Traditional used: It is used in traditional medicine for treatment of fever, common cold, laryngitis, diabetes, pharyngitis, tonsillitis, pneumonia, respiratory tract infection, hepatitis, dysentery, enteritis, peptic ulcer, non infectious diarrhea, hypertension, snake bite and some skin infection.

Bioactive constituents: Andrographolide, flavonoids, steroids, alkaloids, polyphenol are found (Koteswara *et al.*, 2004; Zhou *et al.*, 2008; Akbar, 2011).

Figure 2.12 *Andrographis paniculata* Nees

2.17.2 *Cissus quadrangularis* L.

Family: Vitaceae

Thai name: Phet Sangkhat

Characteristic: This plant is climbing shrubs and has quadrangular sectioned branches. Stem is thick, glabrous, and freshly with a constrictions at its node. Simple leaves are also thick and ovate with 8 cm long and 6 cm broad. Flowers are small and greenish or reddish white (Chen *et al.*, 2007) (Figure 2.13).

Traditional used: It is used for treatment of gastritis, bone fractures, skin infections, constipations, eye diseases, piles, anemia, asthma, irregular menstruation, burns and wounds.

Bioactive constituents: Quadrangularin A, flavanoids, triterpenoids, resveratrol, piceatannol, pallidol perthenocissin, phytosterols, ascorbic acid, triterpene, β-sitosterol, ketosteroid are found (Enechi and Odonwodo, 2003; Jainu and Devi, 2003; Mishra *et al.*, 2010).

Figure 2.13 *Cissus quadrangularis* L.

2.17.3 *Coscinium fenestratum* (Gaertn.) Colebr.

Family: Menispermaceae

Thai name: Hamm

Characteristic: This plant is creeping and climbing vine with 25-30 cm high and has yellow wood. Leaves are single, alternate and lower blade white green with 9-15 cm long and 6-8 cm wide. Flowers are green yellow, inflorescence flat, and rounded top rising from node (Rai *et al.*, 2012) (Figure 2.14).

Traditional used: The stems are used for treatment of wound, ulcers, fever, jaundice, snakebite, acne, inflammatory, diabetes and hepatotoxic. Roots are used for treatment of bitter tonic, dressing wounds, ulcers, stomachache, antiseptic and dysentery.

Bioactive constituents: Berberine, steroids, benzylisoquinoline alkaloids, palmatine, tetrahydroberberine, berberrubine, protoberberine alkaloids, aporphine alkaloids, quaternary protoberberine alkaloids, tetrahydroproto-berberine alkaloids are found (Siva, 2007; Eswani *et al.* 2010).

Figure 2.14 *Coscinium fenestratum* (Gaertn.) Colebr. (Tushar *et al.*, 2008)

2.17.4 *Derris scandens* (Roxb.) Benth.

Family: Fabaceae

Thai name: Thao Wan Priang

Characteristic: This plant is woody vine. Leaves are compounds, alternate simple pinnate with 7-13 opposite leaflets. Flower are in short branches, bisexual and whitish and pinkish color. Fruit are narrowly oblong with a broad wing along one side. Inflorescence is a raceme of pea-like flowers, twice as long as the leaves (Chen and Padley, 2010) (Figure 2.15).

Traditional used: It is used for treatment of mucous congestion, internal infections, severe colds, dysentery, gastrointestinal tract infection and detoxifying purgative.

Bioactive constituents: Benzyls and isoflavones including genistein, coumarins, scandinone, scandenin, prenylated isoflavones, isoflavone glycosides are found (Rukachaisirikul *et al.,* 2002; Salguero, 2003; Rao *et al.,* 2007).

Figure 2.15 *Derris scandens* (Roxb.) Benth.

2.17.5 *Eclipta prostrata* (L.) L.

Family: Asteraceae

Thai name: Kra Meng

Characteristic: This plant is annual herb, erect or prostrate, much branched, roughly hairy, rooting at the nodes. Stems are reddish-purple with short, flat and up-turned hairs. Leaves are 2.5-7.5 cm long, opposite, sessile and lanceolate. Solitary flower heads are 6 -8 mm in diameter with white floret (Tzonev, 2007) (Figure 2.16).

Traditional used: It is used for treatment of asthma, bronchitis, cirrhosis, hepatitis, anemia, skin disease, microbial infection and enteritis.

Bioactive constituents: Leaves have coumestans such as desmethyl-wedelolactone and wedelolactone. Roots have hentriacontanol, heptacosanol, stigmasterol, ecliptal and eclalbatin. Wedelolactone, sterols and ecliptalbine are found in stem (Wagner *et al.*, 1986; Jadhav *et al.,* 2009).

Figure 2.16 *Eclipta prostrata* (L.) L.

2.17.6 *Glycyrrhiza glabra* L.

Family: Fabaceae

Thai name: Cha Em Tet

Characteristics: This plant is perennial herb with underground stem (rhizome). Leaves are compound, pinnate, alternate, and divided into 9-17 leaflets. The leaflets are 2-4 cm long and bear dotted glands on the surface. Flowers are light blue to violet and resembling sweet pea flowers in shape. Fruits are reddish-brown, 1-3 cm long and 4-5 mm wide. Each pod contains 2-5 brown to blackish seeds (Bao and Larsen, 2010) (Figure 2.17).

Traditional used: It is used for treatment of gastric, duodenal ulcer, peptic ulcers, cold, flu, fever, cough, stomach pain, sore throat, laryngitis, lung disease and bronchial infections.

Bioactive constituents: Glycyrrhizin (triterpenoid saponins), liquiritigenin, liquiritin (flavonones). isoliquiritigenin, isoliquiritin (chalcones), genistein, glicoricone, glisoflavone, isoangustone A (isoflavones) are found (Salguero, 2003; Saxena, 2005).

Figure 2.17 *Glycyrrhiza glabra* L. (Saxena, 2005)

2.17.7 *Gynostemma pentaphyllum* (Thunb.) Makino

Family: Cucurbitaceae

Thai name: Jeaw Ku Lan

Characteristics: This plant is perennial climbing vine. Stem and branches are slender**,** augular-sulcate and glabrous. Leaves are sedately 3-9 foliolate, usually 5-7 foliolate, membranous and glabrous. Leaflets are ovate-oblong or lanceolate, serrate and has small lateral leaflets. Fruits are indehiscent, black when mature and globose (Chen and Jeffrey, 2011) (Figure 2.18).

Traditional used: It is used for reduction of blood pressure, chloresterol reduction, diabetes and cardiovascular diseases treatment.

Bioactive constituents: Triterpene, saponins, gypenosides, ergostane, cholestane, stigmastane skeletons are found (Cour *et al.*, 1995; Hoa *et al.*, 2009; Mishra and Joshi, 2011).

Figure 2.18 *Gynostemma pentaphyllum* (Thunb.) Makino

2.17.8 *Hiptage* cf. *benghalensis* ssp. *benghalensis*

Family: Malphigiaceae

Characteristics: This plant has height of 4.5 m shrub plant. Stem are squarish or terete, lenticelled and glabrous. Branches are pilose and leaves are simple, opposite, oblong ovate, green above and shining pale below. Fruits are 3-wingged (Figure 2.19).

Traditional used: It is used to provide energy, relieve back pain, relieve flank pain, help in case of exhaustion and rheumatism (Wiart, 2006).

Figure 2.19 *Hiptage* cf. *benghalensis* ssp. *benghalensis*

2.17.9 *Houttuynia cordata* Thunb.

Family: Saururaceae

Thai name: Khao Thong

Characteristics: This plant is herbaceous perennial plant with 30-60 cm height. The proximal part of the stem is trailing and produces adventitious roots, while the distal part of the stem grows vertically. Leaves are alternate, broadly heart-shaped with purple underneath, 4-9 cm long and 3-8 cm broad. Flowers are small, greenish-yellow, densely clusters on a terminal spike 2-3 cm long with 4-6 large white basal bracts (Xia and Brach, 1999) (Figure 2.20).

Traditional used: It is used for treatment of dieresis, detoxification, relieve abnormal lung symptoms, infectious disease, refractory hemoptysis and malignant pleural effusion.

Bioactive constituents: Houttuynoside A, houttuynamide A, epharanone B, phytol and stigmast-4-ene-3,6-dione are found (Bauer *et al.*, 1996; Chou *et al.*, 2009).

Figure 2.20 *Houttuynia cordata* Thunb.

2.17.10 *Momordica charantia* L.

Family: Cucurbitaceae

Thai name: Mara Kee Nok

Characteristics: This plant is herbaceous climbing plant. Stems are angled and grooved. Alternate leaves have 3-7 deeply separated lobes. Flowers are yellow, monoecious. The fruit has a distinct warty exterior and an oblong shape. Seed is oblong, sub-bidentate at base and apex, sculptured on sides, cream or grey color (Lu and Jeffrey, 2011) (Figure 2.21).

Traditional used: It is used for treatment of tonic, stomachic, stimulant, emetic, anti-bilious, laxative and alterative, rheumatism and sub acute cases of the spleen and liver diseases.

Bioactive constituents: Seeds have triterpene glycosides namely momordicosides A, B, C, D and E. Leaves and vine have tetracyclic triterpenes-momordicines I, II and III (bitter principles). Fruits have cucurbitacin glycosides (Kumar *et al.*, 2010; Sharma *et al.*, 2011).

Figure 2.21 *Momordica charantia* L.

2.17.11 *Phyllanthus amarus* Schumach.

Family: Euphorbiaceae

Thai name: Luk Tai Bai

Characteristics: This plant is erect annual herb with 30-60 cm height. Stems are simple or branched, smooth and light green. Leaves are pale green, 3-4.0 mm wide and 5-9 long, elliptic to oblong. Flowers have proximal 2-3 axis with unisexual cymules. Fruits are tiny and smooth capsules containing seeds (Li and Gilbert, 2008) (Figure 2.22).

Traditional used: It is used for treatment of gonorrhea, frequent menstruation, diabetes, skin ulcers, sores, swelling, itchiness, jaundice, diarrhea, dysentery, intermittent fevers, diseases of urinal-genital system, scabies ulcers and wounds.

Bioactive constituents: Lignans such as hypophyllanthine and phyllanthine, flavonaoids such as quercertin, astralgin, quercertrin, isoquercitrin and rutin, hydrolysable tannins like phyllanthusiin D and alkaloids like sobubbialine, epibubbialine, phyllnirurin are found (Foo, 1993; Houghton *et al.*, 1996; Sharma *et al.*, 1993; Joseph and Raj, 2011).

Figure 2.22 *Phyllanthus amarus* Schumach.

2.17.12 *Pluchea indica* (L.) Less.

Family: Asteraceae

Thai name: Khlu

Characteristics: This plant is branched shrub, up to 2 m tall. Leaves are papery, oval shape and often coating with hair. Flowers are pink-purple color and grow in dense cluster in the leaf axils or in the branches tip. Fruits are brown about 1 mm with white corners (Salguero, 2003) (Figure 2.23).

Traditional used: It use for treatment of diuretic action, hemorrhoids, diabetes, hypoglycemia and ulcers.

Bioactive constituents: β-sitosterol, stigmasterol βH-silphiperfol-5-ene and β-selinene (Salguero, 2003; Gomes *et al.*, 2007).

Figure 2.23 *Pluchea indica* (L.) Less.

2.17.13 *Pseuderanthemum palatiferum* (Nees) Radlk. ex Lindau

Family: Acanthaceae

Thai name: Hoan Ngoc

Characteristics: This plant has small shrub stems, 1-2 m high. Bark is smooth and green. Leaves are green, 3-5 cm wide and 10-20 cm long, semi-oval, lanceolate, basal unequal sided, apex obuse to acute. Flowers are purple-white (Figure 2.24).

Traditional used: It is used to treat diarrhea, diabetes, cancer, hemorrhoids, stomach inflammation, skin disease (itchy skin & allergy), high blood pressure, uterus pain , rheumatoid , liver and kidney inflammation.

Bioactive constituents: Stigmasterol, β-Sitosterol, flavonoid are found (Tran and Ziegler, 2001).

Figure 2.24 *Pseuderanthemum palatiferum* (Nees) Radlk. ex Lindau

2.17.14 *Rhinacanthus nasutus* Kuntze

Family: Acanthaceae

Thai name: Thong Phan Chang

Characteristics: This plant is erect shrub and 1-1.5 cm height. Leaves are oblong, 8-12 cm long and 4-8 cm wide, elliptic and acute at both ends. Flowers are white and zygomorphic shape (Hu and Daniel, 2011) (Figure 2.25).

Traditional used: It is used to treat of eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension and several skin diseases.

Bioactive constituents: Flavonoids, benzenoids, coumarin, sterols, anthraquinone, quinone, glycosides, carbohydrate, triterpenes, terpenoids, lignans, rhinacanthins, rhinacanthone, dehydro α-lapachone are found (Rao *et al.*, 2010; Bukke *et al.*, 2011).

Figure 2.25 *Rhinacanthus nasutus* Kuntze

2.17.15 *Schefflera leucantha* R.Vig.

Family: Araliaceae

Thai name: Hanuman Prasan Kai

Characteristics: This plant is shrub and highly branched, 1-2 m height. The finger-like leaf composed of 5-6 compound leaves, oblanceolate-subulate and glabrous. Flowers are white, small and inflorescence. Fruits are yellow and dense aggregate (Xiang and Lowry, 2007) (Figure 2.26).

Traditional used: It is used to treat cold, respiratory tract infection, cough, asthma, difficulty breathing, a hemostatic to contusions, cuts, and bleeding wounds.

Bioactive constituents: Oleic acids, butulinic acid, D-glucose, D-xylose, L- rhamnose are found (Salguero, 2003).

Figure 2.26 *Schefflera leucantha* R.Vig.

2.17.16 *Senna alata* (L.) Roxb.

Family: Fabaceae

Thai name: Chumhet Thet

Characteristics: This plant is shrub, 3-4 m height. Leaves are paripinnate, 30 cm wide and 60 cm long. Leaflet is oblong or elliptic oblong glabrous, 3-7 × 1.5–2.5 cm, yellow flowers are densely in axillary racemes. Fruit shape likes a straight pod, thick, flattened, wing and glabrous (Figure 2.27).

Traditional used: It is used for treatment of constipation, flatulence, diarrhea caused by intestinal parasites, and blood or mucous in the stools, antiseptic and antiparasitic for treatment of ringworm, fungal and bacterial skin infections, and wound.

Bioactive constituents: Anthraquinone glycosides, rhein, emodin and chrysophanol are found (Salguero, 2003).

Figure 2.27 *Senna alata* (L.) Roxb. (The Botanical Organization, 2014)

2.17.17 *Stemona* sp.

Family: Stemonaceae

Thai name: Non Taai Yaak

Characteristics: This plant is perennial herb climbing as high 4-10 m. Stem is often cylindrical, branched and woody. Leaves are shallow heart- shaped base, 8-30 cm long and 2.5-10 cm wide, opposite or whorled, rarely alternate. Inflorescence is auxiliary and raceme-like, 1-3 flowered (Ji and Duyfjes, 2000) (Figure 2.28).

Traditional used: It is used for treatment of respiratory disorders such as bronchitis, pertussis, tuberculosis and cough.

Bioactive constituents: Alkaloids, tuberostemonine, isotuberostemonine, stilbenoides, tocopherols, stemoninestemonidine, sinostemonine and bibenzyls are found (Zhao *et al.*, 1995; Li e*t al.*, 2007; Schinner *et al.*, 2007).

Figure 2.28 *Stemona* sp.

2.17.18 *Stephania venosa* (Blume) Spreng.

Family: Menispermaceae

Thai name: Sabuu Lueat

Characteristics: This plant is herbaceous perennial vines around 4 m tall. Stems are woody with bitter taste and red latex. Leaves are arranged spirally on the stem, simple, entire, peltate, petiole usually geniculate at base. Inflorescences are axillary or cauliflorous. Flowers are orange and yellow and unisexual (Hu *et al.*, 2008) (Figure 2.29).

Traditional used: It is used for treatment of nerve tonic, aphrodisiac, appetizer, asthma, microbial infection, hyperglycemia and cancer.

Bioactive constituents: Protoberberine, stephanine, oxostephanosine, cyclanoline, kamaline, vitexin, lignin, terpenoids, tannins, saponins, alkaloid, phenolics, steroids and flavonoids are found (Pharadai *et al.*, 1985; Charles *et al.*, 1987; Banerji *et al.*, 1994).

Figure 2.29 *Stephania venosa* (Blume) Spreng.

2.17.19 *Thunbergia laurifolia* Lindl.

Family: Acanthaceae

Thai name: Rang Chuet

Characteristics: This plant is vigorous perennial climbing vine. Oval-shaped leaves are mostly 7-18 cm long and 2.5-6 cm wide, grow in opposite pairs along the stem. The trumpet-shaped flower begins as a short broad tube, white outside and yellowish inside. The coreela is pale lavender-blue petals and opens out into five rounded, one larger than the others. Seed capsule are brown in cone shaped pods (Hu and Daniel, 2011) (Figure 2.30).

Traditional used: It is used for treatment of blood detoxifying, snake or insect bites, digestion, flatulence, diarrhea, mucous or blood in the stool, and intestinal parasites, a remedy for fever, allergies, asthma, diabetes and hypoglycemia**.**

Bioactive constituents: Iridoid glucosides (such as 8-epi-grandiforic and 3'-*O*-β-glucopyranosyl-stibericoside), phenolic compound; delphinidin, apigenin, and chlorogenic acid) are found (Kanchanapoom *et al.,* 2002; Thongsaard and Marsden, 2002; Salguero, 2003).

Figure 2.30 *Thunbergia laurifolia* Lindl.

2.17.20 *Tinospora crispa* (L.) Hook.f. & Thomson

Family: Menispermaceae

Thai name: Bora Phet

Characteristics: This plant is deciduous vine herb. Old stem is freshly, with very prominent blunt tubercles. Younger stems are slightly freshly, brownish and glabrous. Leaves are lenticels large, broadly ovate to orbicular, size 6-13 x 6-13, slightly freshly and lobes rounded. Inflorescences racemes, unbranched or occasionally shortly branched, appearing before leaves, flowers 2- or 3-fascicled (Hu *et al.*, 2008) (Figure 2.31).

Traditional used: It is used for treatment of fever, headache, cough, sinusitis, arthritis, malarial infection, cholera, diabetes, rheumatism and snake-bites.

Bioactive constituents: Picroretine, berberine, tinosporine ,tinosporidine (alkaloid), bergenin, β-carotene, triterpine (cycloeucalenol and cycloeucalenone), flavone oglycosides (apigenin), picroretoside, palmatine, picroretine, and resin are found (Kongkathip *et al*, 2002; Salguero, 2003; Wiart, 2006).

Figure 2.31 *Tinospora crispa* (L.) Hook.f. & Thomson

2.17.21 *Vernonia cinerea* (L.) Less.

Family: Asteraceae

Thai name: Ya Dok Khao

Characteristics: This plant is annual or perennial erect herb, up to 100 cm tall. Stems are rounded, hairy and brown. Leaves are alternate spiral, elliptic, ovate and acute in both ends, 3-6.5 × 1.5-3 cm. Flowers are tubular, dense in terminal head, sessile, white or purple color (Figure 2.32).

Traditional used: It is used for treatment of diabetes, control blood sugar, prevent sore and skin ulcer, cold, respiratory disorder such as cough, asthma, arthritis, urinary tract infection and back pain.

Bioactive constituents: Lupeol, 12-oleanen-3-ol-3ß-acetate, Stigmasterol, ß-sitosterol, (+)-lirioresinol B, stigmasterol-3-O-beta-D-glucoside, 4-sulfo-benzocyclobutene are found (Salguero, 2003; Haque *et al.*, 2012).

Figure 2.32 *Vernonia cinerea* (Linn.) Less.

2.17.22 *Zingiber montanum* Link ex A. Dietr.

Family: Zingiberaceae

Thai name: Phali

Characteristics: This plant is herbaceous plant, 0.7-1.5 m height. Rhizome branched with yellowish inside, thickened, fleshy, and strongly aromatic. The pseudo stems are made up of sheathes. The leaflets are bifarious, sessile on their sheath, linear-lanceolate, deep green above with 3.5-5.5 cm wide and 18-35 cm long. Inflorescences arise from rhizome, oblong in shape, bracts pale greenish brown. Creamy white petals and purple fruit color (Figure 2.33).

Traditional used: It is used for treatment of indigestion, dysentery, diarrhea, intestine inflammation, internal organs injury, asthma, joints inflammation, ligaments, wounds, cuts, and skin infections.

Bioactive constituents: Phenylbutenoids, saponins, flavonoids, phenolics, triterpine, diterpene, curcuminoid namely cassumunarin types A, B, and C, sabinene, γ-tepinene, α-tepinene and terpinen are found (Ozaki *et al.*, 1991; Jitoe, 1994; Jeenapongsa *et al.*, 2003; Salguero, 2003).

Figure 2.33 *Zingiber montanum* Link ex A. Dietr.

2.18 Antimicrobial and antioxidant properties of medicinal plants

The traditional medicine based on natural products has played a significant role in the treatment of a wide range of medical conditions, including infectious diseases. Some naturally occurring chemical compounds serve as models for a large percentage clinically proven drugs, and many are now being reassessed as antimicrobial agents. The primary reason for this situation is the fact that infectious disease remains a significant cause of morbidity and mortality worldwide. Another reason is the prevalence of drug resistant microorganisms and previously unknown disease causing by microbes poses an enormous threat to global public health (Singh and Pandeya, 2011; Saleem *et al.,* 2010).

Medicinal plants have therapeutic properties due to the presence of complex mixture of phytochemical acting individually or synergistically. Numerous studies have been investigated the new compounds from medicinal plants. The antimicrobial action of 2′,4′-dihydroxychalcone, a flavones compound, exhibited the most significant activity against MSSA and MRSA (Sufian *et al.,* 2013). Similarly, Shan *et al.* (2008) reported that stilbenes and hydroxyanthraquinones were found as major bioactive compounds in *Polygonum cuspidatum* roots and also had greatly contributed to the antibacterial properties against *Bacillus cereus, Listeria monocytogenes, S. aureus, E. coli* ATCC25922, and *Salmonella anatum.* In another study, ethyl acetate extract of *Terminalia muelleri* Benth. leaves, was found to inhibit *S. aureus* and MRSA growth. Moreover, the extract of *T. muelleri* also altered cell morphology of *S. aureus* (Anam *et al.,* 2010). The previous report found that hydrophobic components of the essential oils could interrupt lipids of bacterial cell membrane resulting in cell component disruption and permeable. Moreover, chemical compounds from essential oils also act on cytoplasmic membrane proteins (Sikkema *et al.,* 1994). There are numerous compounds that affect bacterial cell such as carvacrol, thymol, eugenol, carvone and p-cymene. Cavacrol and thimol are members of a class of plant chemicals known as monoterpene phenols. They have potent natural antimicrobial properties and are major components of the essential oils of thyme and oregano. Both substances seem to make the membrane permeable (Lambert *et al.,* 2001). Moreover, eugenol, phenylpropene, inhibited the production of protease and amylase of *B. cereus* and they also degraded

and lysed cell membrane (Thoroski *et al.,* 1989). Carvone, is a member of a family of chemicals called terpenoids. It could disturb the general metabolize in cells and also dissipates gradient pH and cell membrane potential (Oosterhaven *et al.,* 1995). Furthermore, p-cymene, a hydrophobic compound was a precursor of carvacrol. It could make cytoplasmic membrane swelling more than carvacrol (Ultee *et al.,* 2002). Tannins are water-soluble polyphenols that are commonly found in higher herbaceous and woody plants. Several tannins such as tannic acid, gallic acid, ellagic acid, (–)-epicatechin and (–)-epicatechin gallate had been reported to be antimicrobial agents against *S. aureus* (Akiyama *et al.,* 2001). Moreover, previous report showed that capsaicin, the main constituents in pepper had the antimicrobial effect against *Helicobacter pyroli* (Zeyrek and Oguz, 2005). The sites or structures of the bacterial cell were considered targets for action by the components of natural products that are illustrated in Table 2.8.

Table 2.8 Main groups of plant compounds with antimicrobial activity (Cowan, 1999; Silva and Fernandes, 2010)

Class	**Subclass**	**Examples**	**Mechanism**
Phenolics	simple phenols	catechol	Substrate deprivation
		epicatechin	Membrane disruption
	phenolic acids	cinnamic acid	
	quinones	hypericin	Bind to adhesions, complex with cell wall, inactivate enzyme,
	flavonoids	chrysin	Bind to adhesions, complex with cell wall
		abyssinone	inactivate enzyme
	tannins	ellagitannin	Bind to protein, adhesion, Enzyme inhibition, substrate deprivation, complex with cell wall, membrane
Terpenoids, essential oils		capsaicin	Membrane disruption
Alkaloids		berberine	Intercalate into cell wall and/or DNA
		piperine	

Recently, anti-virulence approach has gained the increase of interest as an alternative strategy for the treatment of bacterial infections. Many researchers investigated the effect of medicinal plants on the expression of some virulence factors. It has been reported that the production of some virulence factors could be affected by some natural compounds. The sub inhibitory concentration of costus oil and perilla oil decreased the expression of α -toxin, enterotoxins A and B and toxic shock syndrome toxin 1 (TSST-1) in MSSA and MRSA strains (Qiu *et al.*, 2010a; Qiu *et al.*, 2011). Moreover, licochalcone A which is one of the many flavonoids present in Chinese liquorice root also inhibited the enterotoxin (SEA and SEB) in *S. aureus* strains (Qiu *et al.*, 2010b). Moreover, allicin is one of the active compounds in garlic. It could inhibit α –toxin in *S. aureus* (Leng *et al.*, 2011).

Potential sources of antioxidant compounds have been searched in several types of plant materials. There are many reports about the antioxidant activity from various species of plants that harvested in different places and at different development stages of plant. Recently, phenolics have been considered as powerful antioxidant in natural sources such as phenolic acid, phenolic diterpenes, flavonoids and volatile oils. Phenolic antioxidants could inhibit free radical formation and interrupt the oxidation reaction. The antioxidant properties of phenolic compounds acted as free radical scavengers, hydrogen donators, metal chelators and singlet oxygen quenchers (Miller and Rice-Evans *et al.*, 1996). Tocopherols, for example, are important antioxidants occurring naturally in vegetable oils. Tocopherols have function both as free radical terminators and as scavengers for singlet oxygen. Flavonoids are one of phenolic compounds which, largely include anthoxanthins (flavones, flavonols, flavanones, flavanols, chalcones and isoflavones), anthocyanins, leucoxanthins and flavonoidal alkaloids. Flavonoids are naturally found in various plant species and possess antioxidant properties. Previous study showed that six flavonoids including 5-(1, 1-dimethylallyl)-3,4,4′-trihydroxy-2-methoxychalcone, licochalcone B, licochalcone A, echinatin, glycycoumarin and glyurallin B were isolated from the extracts of licorice (*Glycyrrhiza inflata* and *Glycyrrhiza uralensis*). The flavonoids had high antioxidant activity and exhibited potent inhibition of lipid peroxidation (Fu *et al.*, 2013). Several Thai plants had been reported to be high antioxidant activity including the methanolic extracts of *Cayratia trifolia*, *Pouzolzia indica* and the ethyl acetate extract of *Oroxylum*

indicum using DPPH method. Moreover, the methanolic and ethyl acetate extracts of *Oroxylum indicum* showed the most potent inhibition of lipid-peroxidation (Siriwatanametanon *et al.*, 2010). In other studies, Li *et al*. (2013) investigated the antioxidant activity in 223 medicinal plants that was commonly used in China using ferric-reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC) assays, and total phenolic contents (TPC). It was found that several plants possessed high antioxidant and total phenolic content such as *Sanguisorba officinalis, Rosa chinensis, Cimicifuga foetida, Salvia miltiorrhiza, Fraxinus rhynchophylla, Rhodiola sacra, Tussilago farfara, Sargentodoxa cuneata, Polygonum multiflorum* and *Paeonia lactiflora*. Other studies had shown that kaempferol and rhamnocitrin isolated from *Rhamnus alaternus* leaves had high effective antioxidant activity after testing with various assays including cupric reducing antioxidant capacity (CUPRAC), reducing power and FRAP assay (Bhouri *et al.*, 2012).