2.0 LITERATURE REVIEW

2.1 Secondary Metabolites

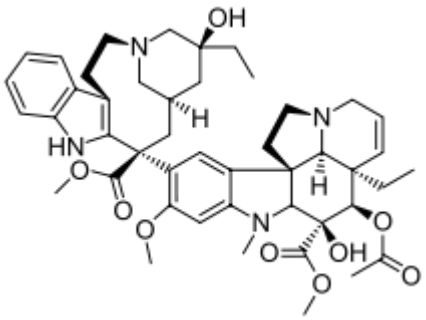
Since the early days of human existence, plants with secondary metabolites have been used by humans to treat health disorders, illness and infection (Wyk & Wink, 2005). Plant secondary metabolite is a generic term used for more than 30,000 different substances which are produced by plants. They are often created by modified primary metabolite synthase. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism but carry out protective functions in the human body. For instance, secondary metabolites can protect the body from free radicals, modulate the immune system and kill pathogenic microbes. Plant secondary metabolites also contribute to the plant defense against herbivory (Stamp & Nancy, 2003).

Secondary metabolites are heterogeneous group of natural compounds that may assist in survival and basic functions of the plants, such as symbiosis, metal transport, competition, differentiation and so on (Demain & Fang, 2000). They are also widely used for pharmaceutical, medical, or agricultural purposes (Calvo *et. al.*, 2002) including natural antibiotics which are capable of inhibiting microbial growth (Mapleston *et. al*, 1992; Sekiguchi & Gaucher, 1977; Stone & Williams, 1992). Secondary metabolites have a scientifically proven effect on health but many of these effects are still unknown and their effects are currently being intensively investigated and researched.

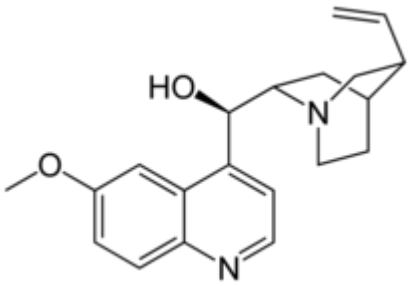
Secondary metabolites can be classified based on the chemical composition (containing nitrogen or not), chemical structure (for example, having rings, containing a sugar), the biosynthetic pathway (e.g., phenylpropanoid, which produces tannins) or their solubility in various solvents. Secondary metabolites can be divided into three large categories, namely alkaloids, terpenes and phenolics.

(a) Alkaloids

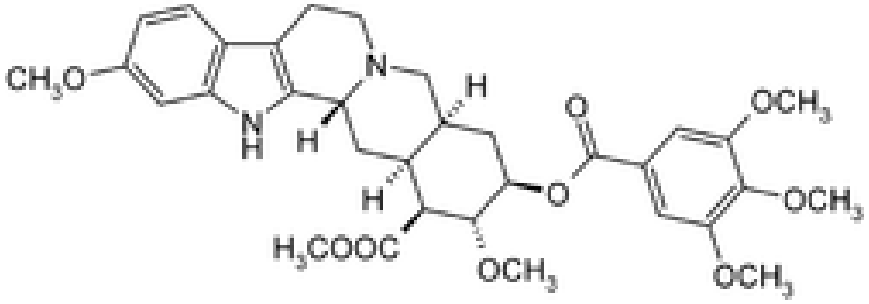
Alkaloids are derived from amino acid. Approximately, more than 10,000 different alkaloids have been discovered from more than 300 plant families (Raffauf, 1996). Alkaloids mostly contain one or more carbon rings which usually contain nitrogen. The positions of nitrogen atom in the carbon ring depend on the type of alkaloid and plant families. Medicinal use of alkaloid plants has a long history. The first alkaloids were synthesized in the 19th century and they immediately found application in clinical practice (Hesse, 2002). Many alkaloids are still being used in medicine, usually in the form of salts. Some examples include vinblastine [Figure 2.1(a)] which has antitumor properties (Jordan & Leslie, 2004); quinine [Figure 2.1(b)] which has antipyretics and antimalarial properties (Reyburn *et. al,* 2009); and reserpine [Figure 2.1(c)] which can be used to treat high blood pressure (Moser, 1987).



Vinblastine (a)



Quinine (b)



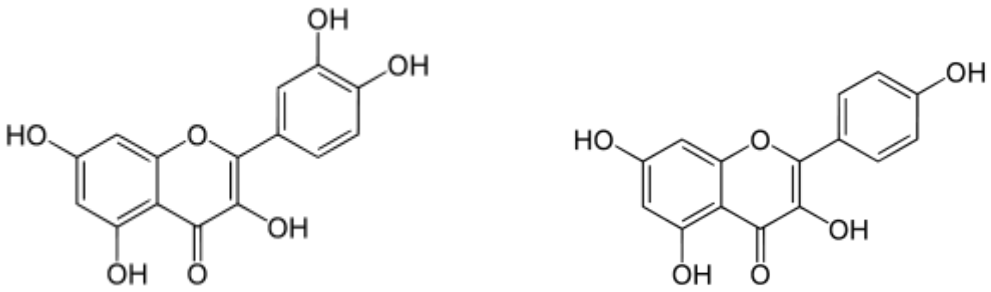
Reserpine (c)

Figure 2.1: Structures of alkaloids vinblastine (a), quinine (b) and reserpine (c)

(b) Phenolic compounds

Phenolic compounds are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. There are more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Phenolics are synthesized primarily from products of the shikimic acid pathway (Knaggs & Andrew, 2001). A common feature of phenolic compounds is the presence of at least one hydroxyl-substituted aromatic ring system. Phenolic compounds include flavonoids, phenolic acid, tannins and the less common stilbenes and lignans. Most phenolic compounds belong to the flavonoids (anthocyanin,

chalcons, flavones, quercetin, kaempferol). Structures of quercetin and kaempferol are shown in Figure 2.2. Flavonoids are most commonly known for their antioxidant activity in vitro. Reports from in vitro studies have shown that natural phenols have antimicrobial (Rauha *et al.*, 2000), antiviral (Perez, 2003), anti-inflammatory (Santos *et.al.*, 2006) and vasodilatory actions (Padilla *et. al.*, 2005).



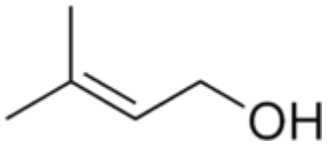
Quercetin (a) Kaempferol (b)

Figure 2.2 : Structures of flavonoids quercetin (a) and kaempferol (b)

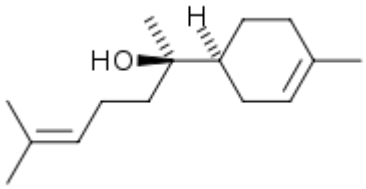
(c) Terpenes

Terpenes are hydrocarbons resulting from the combination of several five-carbon isoprene units. Terpenoids can be assumed as modified terpenes in which methyl groups have been moved or removed, or oxygen atoms added. Terpenoid can be classified according to the number of isoprene unit such as hemiterpenoid (1 isoprene unit), monoterpenoids (2 isoprene units), sesquiterpenoids (3 isoprene units), diterpenoids (4 isoprene units), sesterterpenoids (5 isoprene units), triterpenoids (6 isoprene units), tetraterpenoids (7 isoprene units) and polyterpenoid (>7 isoprene units). Terpenoids can be synthesized through the mevalonic acid pathway (Newman & Chappell, 1999) or 2-*C*-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP

pathway) (Rohmer *et. al.*, 1993). Terpenoids are commercially important fragrance and flavouring agents (Ohloff, 1994). Prenol [Figure 2.3(a)] and α-bisabolol [Figure 2.3(b)] are used in fragrance due to fruity odor and sweet floral aroma respectively. The roles of terpenoids as pharmaceutical agents with activities such as antibacterial and antineoplasia are still under investigation.



Prenol (a)



α-bisabolol (b)

Figure 2.3: Structures of terpenoids prenol (a) and α-bisabolol (b)

2.2 Free Radical

Free radical is an electron-deficient species which includes atom, molecule or ion with at least one unpaired electron in the valence cell that renders a free radical to be energetically and kinetically unstable (Pierrefiche & Laborit, 1995). This unpaired electron then contributes to the extreme reactivity of free radical. High reactivity of the free radical results in their low chemical specificity, which means they can react and cause damage to stable molecules in the surroundings including protein, lipids, nucleic acid and carbohydrates (Stephan *et al.*, 1997).

Highly reactive free radicals are present in biological system through a wide variety of sources. They are produced either from normal essential metabolic processes in the

human body or from external sources. Free radicals formation occurs continuously in the cells as by products of metabolism and deliberately as in phagocytosis inside human body. Enzymatic reactions that are involved in prostaglandin synthesis, respiratory chain and cytochrome P450 also serve as sources of free radicals (Mueller *et al.*, 2005). Meanwhile, free radicals resulting from X-rays, cigarette smoking, environmental pollutant, industrial chemicals and ozone serve as external sources.

Free radicals are naturally produced by the body as necessary intermediates in a variety of normal biochemical reactions. The immune system is the main system that utilizes free radicals to fight infection, in which the phagocytic cells generate radicals to kill invading pathogen. However, excess levels of free radicals in the body create a situation known as oxidative stress which leads to a variety of biochemical and physiological lesions resulting in metabolic impairment and cell death. These lesions in turn lead to various disease and degenerative process such as carcinogenesis (Wiseman & Halliwell, 1996), rheumatoid arthritis (Tatli Seven *et al.,* 2008), hypertension (Harrison *et al.,* 2007), Alzheimer's disease (Cai & Yan, 2007) and myocardial ischemia (Kaminski *et al,* 2002). Reducing exposure to free radicals and increasing intake of antioxidant nutrients has potential to reduce the risk of free radical related health problem.

2.2.1 Type of Free Radicals

There are numerous types of free radicals. The main types of free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS).

a) Reactive Oxygen Species (ROS)

Free radicals which contain oxygen are referred as reactive oxygen species (ROS) and include superoxide, hydroxyl radical, peroxyl radical, hydrogen peroxide, singlet oxygen and hypochlorous acid. ROS are the most important free radicals in the body. ROS are formed in mitochondria as oxygen is reduced along the electron transport chain which is as part of normal aerobic life (Turrens, 2003) and also produced by numerous biochemical processes in the body. ROS participate in signal transduction in a variety of enzyme reactions (Scherz-Shouval & Elazar, 2007) but excessive production may lead to oxidative stress, loss of cell function and eventually apoptosis or necrosis.

b) Reactive Nitrogen Species (RNS)

Reactive nitrogen species (RNS) are produced in the biological system with specific function in the cells. RNS are highly active compounds that can cause damage through oxidation and nitration of biomolecules. They play an important role in maintaining various physiological functions at basal levels and contribute to several pathological processes at high levels (McQuaid & Keenan, 1997; Chabot *et al.,* 1998). Reactive nitrogen species (RNS) include both nitric oxide and nitrogen dioxide.

2.2.2 Diseases Associated with Oxidative Stress

Overproduction of free radicals in the body creates a situation known as oxidative stress. Accumulation of oxidative damage by free radicals results in the development of

chronic degenerative diseases such as atherosclerosis, diabetes, cancer, and diseases of the central nervous system such as Alzheimer's disease, and Parkinson's disease.

(a) Atherosclerosis

Atherosclerosis is a condition in which deposition of atheromas or atherosclerotic plaques occurs in the arterial walls leading to the narrowing and hardening the arteries. Biochemical studies suggested that oxidize modification of low density lipoprotein (LDL) play a key role during early atherogenesis and there is increasing evidence for the role of oxidative modification of LDL by free radicals in the development of atherosclerosis (Singh & Jialal, 2006). Oxidized LDL is taken up by macrophages at an enhance rate via their scavenger receptor which lead to the formation of lipid laden foam cell which is the hallmark of the early atherosclerosis (Henriksen *et al.*, 1983).

(b) Diabetes

Diabetes is a multi-systemic disease cause by a defect in glucose metabolism. Excessive production of ROS by glycated proteins may be responsible for the development of complications in diabetes such as polyneuritis, retinopathy, perforating ulcers, and impaired healing (Paolisso & Giugliano, 1996).

(c) Cancer

Free radicals were found to be involved in stimulating cancer development in three stages which are initiation, promotion and progression. These highly reactive free radicals

can act as the initiators or promoters, cause DNA damage, activate procarcinogens, and then alter the cellular antioxidant defense system in carcinogenesis (Trueba *et al.,* 2004). DNA damage can result in either arrest or induction of transcription, induction of signal transduction pathways, replication errors and genomic instability. A common form of DNA damage is the formation of hydroxyl bases of DNA which is considered an important event in chemical carcinogenesis (Valko *et. al.,* 2006)

(d) Alzheimer's Disease

Alzheimer's disease is a neurodegenerative disorder which is characterized by the development of tangles of nerve fibers, senile plaques (which contain aluminium, calcium and iron) and the loss of brain cells. This disorder involves oxidative stress, which accumulates free radicals leading to excessive lipid peroxidation and neuronal degeneration in certain brain regions (Mielke & Lyketsos, 2006). Brain tissues are susceptible to free radical damage due to the highest oxygen consumption rate in the brain in comparison to any organ in the body, high concentrations of easily-oxidizable lipids, and a relative deficiency of antioxidant enzymes compared to other tissues (Cai & Yan, 2007).

(e) Parkinson's Disease

Parkinson's disease involves the progressive death of dopaminergenic cells in substantia nigra which lead to reduce availability of the dopamine to the striatum which controls movement. According to Danielson and Andersen (2008), oxidative stress involving lipid peroxidation is one of the mechanisms that have been proposed as possible

causes of the dopamine cell degeneration in Parkinson's disease. The increased production of oxidants causes damage to the substantia nigra of the brain through iron-dependent free radical reactions.

2.3 Antioxidant

Antioxidant compound in our food has a vital role as a health-protecting factor. Therefore, it is considered useful nutraceuticals on account of many health benefits (Droge, 2002; Lee *et al.*, 2004; Valko *et al.*, 2007). The most important function of antioxidant is trapping the free radical particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS) which are involved in the pathogenesis of several chronical and degenerative diseases such as cancer, inflammation, neurodegenerative diseases, cardiovascular diseases and aging-related disorders. ROS and RNS can act as secondary messenger in normal physiological functions of the organism and participate in various regulatory redox-mechanism. However, problems arise with overproduction of these species which can overwhelm protective enzymes leading to destructive and lethal cellular effects (Valko *et al.*, 2007).

Antioxidant works by preventing or slowing the oxidation of other molecules by free radical. Oxidation is a process of chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can generate and produce free radicals, which will initiate chain reactions that cause damage to the cells. These radicals will react with biological molecules for example DNA, proteins and phospholipids and eventually

damage and destroy the structure of tissues and other membranes. (Vuillaume, 1987; Imlay *et. al*, 1988; Meneghini, 1988).

Conversely, antioxidants will terminate these chain reactions by removing and eliminating free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Recently, the use of natural antioxidants has been promoted and become important because of concerns regarding the safety of synthetic ones (Shahidi, 2000). Hence, many studies have been conducted to investigate the antioxidant activity of plant extracts (Arlorio *et al.*, 2008; Madhujith & Shahidi, 2006).

2.3.1 Types of Antioxidant

Antioxidants can be classified into two major groups which are enzymatic and non-enzymatic antioxidants based on their origin and function in the cells (Karlsson, 1997).

(a) Enzymatic Antioxidants

Antioxidant enzymes are not consumed and have high affinity and rate of reaction with ROS. Glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) are the major antioxidant enzymes which are involved in antioxidant defense. These enzymes require trace metal cofactors for maximal efficiency, including selenium for glutathione peroxidase; copper; zinc, or manganese for superoxide dismutase; and iron for catalase (Halliwell, 1995).

Glutathione peroxidase (GPx) can reduce hydrogen peroxide (H_2O_2) to water (H_2O) by oxidizing glutathione. The reduction of the oxidized form of glutathione (GSSG) is then catalyzed by glutathione reductase. GPx is located in both of mitochondria and the cytosol where it serves as an important cellular protectant against free radical induced damage to membrane lipids, proteins and nucleic acid (Powers & Lennon, 1999). It has also been reported that GPx is a major class of enzyme that regulates cells homeostasis by neutralizing the effect of lipid peroxidase (Boutet *et al.,* 2007).

Superoxide dismutase (SOD) are metalloenzymes that catalyze the dismutation of superoxide radical into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) and consequently provide an important defense mechanism against superoxide radical toxicity. There are three types of SODs in humans which include cytosolic copper/zink (Cu/Zn) SOD, mitochondrial manganese (Mn) SOD and extracellular SOD. Cu/Zn-SOD is the major intracellular SOD which can be found in mostly cytoplasm and acts as a bulk scavenger of superoxide (Crapo *et al.,* 1992). Induction of Mn-SOD gene expression under oxidative stress is one of the self-defense mechanisms to alleviate oxidative damage to mitochondria.

In addition, catalase (CAT) is one of the enzymes that is involved in catalyzing the decomposition of hydrogen peroxide into harmless oxygen and water (Gutteridge, 1995). It is a heme protein that can be found in peroxisomes except in cells like erythrocytes that do not contain organelles (Loewen, 1992). CAT is the predominant enzyme that catabolizes the decomposition of exogenous hydrogen peroxide in eukaryotic cells especially in red blood cells (Scott *et al.,* 1991). Besides that, CAT can also consumed nitric oxide in the

presence of H_2O_2 to form nitrite and limit the formation of peroxynitrite by providing an alternative route to scavenge nitric oxide (Brown, 1995).

(b) Non-enzymatic Antioxidants

Carotenoids, natural flavonoids, vitamin C, and vitamin E are the non-enzymatic antioxidants (Mc Call & Frei, 1999). Non-enzymatic antioxidants are mostly derived from food intake. Human antioxidant defense system is incomplete without dietary antioxidant.

Carotenoids are natural pigments which are synthesized by plants and are responsible for the bright colors of various fruits and vegetables. There are several dozen carotenoids in the foods that we eat, and most of these carotenoids have antioxidant activity. Beta-carotene is one class of carotene which is an effective vitamin A precursor. It has been reported can protect humans against certain types of cancer (Steinmetz & Potter, 1996). It was also supported by Ziegler and colleagues (1996) that individuals who consume diets relatively large amount of vegetables and fruits which are rich in carotenoids are at lower risk of cancer at several tumour sites especially lung cancer.

Flavonoids are compounds found in fruits, vegetables and certain beverages that have diverse beneficial biochemical and antioxidant effects. The antioxidant activity of flavonoids depends on their molecular structure. The stable delocalization system in flavonoid consisting of aromatic and heterocyclic rings as well as multiple unsaturated bonds helps to delocalize the resulting free radicals (Rice-Evans *et al.*,1997). Many flavonoids such as quercetin, catechins and luteolins are better antioxidants than the

antioxidant nutrients vitamin C, vitamin E and L-carotene on a mole for mole basic (Rice-Evans *et al.,* 1995).

Meanwhile, vitamins C (ascorbic acid) and E protect the body against the destructive effects of free radicals. Vitamin E is one of the most efficient chain-breaking antioxidants available. Vitamin E is a major antioxidant that is responsible for terminating free radical chain reactions resulting from the oxidation of polyunsaturated fatty acids (PUFA) in cell membranes (Ciocoiu *et al.,* 2007). Ascorbic acid is an important water-soluble antioxidant which is reported can neutralize ROS and reduce oxidative stress (Verma *et al*., 2007). According to Dingchao *et al.* (1994), high dose vitamin C supplementation decreases lipid peroxidation and protects the myocardium from ischemia-reperfusion injury during and after open-heart surgery. Ascorbic acid has also been suggested to have the ability to regenerate vitamin E from its tocopheroxyl radical form (Benzie & Strain, 1999) and thereby restore its free radical scavenging capacity.

2.4 Antimicrobial

An antimicrobial can be defined as any substance of natural, semi-synthetic, or synthetic origin that kills or inhibits the growth of a microorganism, but causes little or no host damage. The continuous spread of multidrug-resistant pathogens has become a serious threat to public health and a major concern for infection control practitioners worldwide (Sanders & Sanders, 1992). The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization (WHO) directives culminating in several pre-clinical and clinical studies that have provided the

scientific basis for the efficacy of many plants used in folk medicine to treat infections (Vijaya & Ananthan, 1997; Dilhuydy & Patients, 2003). Medicinal plants represent a rich source of antimicrobial agents. Hundreds of plant species have been tested for antimicrobial properties but the vast majority of have not been adequately evaluated (Balandrin *et al.,* 1985).

Most of the common antibiotics used in therapeutics such as penicillin, amphotericine B and 5-fluorocytosine are produced by fermentation process. Even though they are much cheaper than antibiotics derived from plants but the clinical effectiveness of these antibiotics in the treatment of infectious diseases is limited by the rapidity of the susceptible microorganisms in developing resistance. The resistance developed during prolong treatment period using the same antibiotic (Iwata, 1986). Continuous investigation on chemical and pharmacological activities of antimicrobial plants is useful in discovering new drugs to overcome resistance against antibiotics that are already established. Antimicrobial agents from higher plants are essential to provide new or lead compounds for chemists to improve the bioactivity through structural modification which lead to the production of synthetic materials that are cheaper and independent of the plant materials (Colegate & Molyneux, 1993).

2.4.1 Major Groups of Antimicrobial Compounds from Plants

Therapeutic properties and bioactivities of plants depend on secondary metabolites found in them (Goh *et al,* 1994). Compounds with antimicrobial activities can be described and classified into major classes namely phenolics, terpenoid, essential oils, alkaloid,

lectins and polypeptides and polyacetylenes. Table 2.1 shows examples of secondary metabolites with antimicrobial activities together with their mechanisms of action.

Table 2.1: Major classes of antimicrobial compounds from plants

Class	Subclass	Example (s)	Mechanism	Reference(s)
Phenolics	Simple phenols	Catechol	Substrate deprivation	Peres *et al*, 1997
		Epicatechin	Membrane disruption	Toda *et al*, 1992
	Phenolic acids	Cinnamic acid		Fernandez *et al*, 1996
	Quinones	Hypericin	Bind to adhesions, complex with cell wall, inactivate enzymes	Duke, 1985; King &Tempesta, 1994
	Flavanoids	Chrysin	Bind to adhesions	Perrett *et al*, 1995; Rojas *et al*, 1992
	Flavones		Compex with cell wall	
		Abyssinone	Inactivate enzymes	Brinkworth *et al*, 1992; Taniguchi & Kubo, 1993
			Inhibit HIV reverse transcriptase	Ono *et al*, 1990
	Tannins	Ellagitannin	Bind to proteins	Schultz, 1988; Stern *et al*, 1996
			Bind to adhesions	Scalbert, 1991
			Enzyme inhibition	Haslam, 1996; Brownlee *et al*, 1990; Butler, 1988
			Substrate deprivation	
			Complex with cell wall	
			Membrane disruption	
			Metal Ion complexation	
	Coumarins	Warfarins	Interaction with eukaryotic DNA (antiviral activity)	Bose, 1958; Hoult & Paya, 1996; Keating & O'Kennedy, 1997; Yoshikawa *et al*, 1994
Terpenoids, essential oils		Capsaicin	Membrane disruption	Cichewicz & Thorpe, 1996
Alkaloids		Berberine	Intercalate into cell wall and/or DNA	Atta-ur-Rahman & Choudhary, 1995, Burdick, 1971, Freiburghaus *et al*, 1996; Houghton *et al*, 1994
		Piperine		
Lectins and polypeptides		Mannose-specific agglutinin	Block viral fusion or adsorption	Meyer *et al*, 1997; Zhang & Lewis, 1997
		Fabatin	Form disulfide bridges	

2.5 Sinusitis

Sinusitis is very often a sequela of an acute upper respiratory tract infection. The normal oropharyngeal flora contained aerobic and anaerobic bacteria that can cause respiratory infections including sinusitis (Socransky and Manganiello, 1971). Sinusitis can be defined as inflammation of the paranasal sinuses, which may be due to infection, allergy or autoimmune issues. Among the most potent inflammatory mediators are free radicals that can be neutralized by antioxidants. It is a common condition with over than 24 million cases occurring in the United States annually (Anon, 2010). Degree of impairment from sinusitis is substantial, and is comparable to other chronic diseases, such as angina, chronic obstructive lung disease, and back pain (Glikilich & Metson, 1995). Sinusitis can be categorized into acute, subacute and chronic. All three types of sinusitis have similar symptoms, and are therefore often difficult to distinguish and detect.

Acute sinusitis can be defined as an infection of the paranasal sinuses, with accompanying symptoms present for more than 10 days and less than 4 weeks. Acute sinusitis is usually precipitated by an earlier upper respiratory tract infection. Acute sinusitis is very common, which is self-limiting and treatable. Approximately, about ninety percent of adults had sinusitis at some point in their life (Pearlman & Conley, 2008). The most common bacteria capable of causing acute sinusitis are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* (Leung & Katial, 2008).

Other sinusitis causing bacterial pathogens include *Staphylococcus aureus* and other streptococci species, anaerobic bacteria and, less commonly, gram negative bacteria. Virus

also capable of causing sinusitis but it is difficult to distinguish between bacterial and viral sinusitis. Viral sinusitis typically lasts for 7 to 10 days (Leung & Katial, 2008) but bacterial sinusitis is more persistent (Piccirillo, 2004). In addition, acute episodes of sinusitis can also result from fungal invasion. Fungal sinusitis is common in immunocompromised or diabetic individuals (Brook, 2001).

Chronic sinusitis can be defined as sinusitis that lasts longer than three months and also can be caused by many different diseases that share chronic inflammation of the sinuses as a common symptom. However, to fully define chronic sinusitis has been difficult because of the variation in clinical expression of the disease, and the discordance between patient symptoms and objective findings. Some theories have implicated anatomic, infectious, allergic, and inflammatory disease, but none have been proven (Leung & Katial, 2008). Chronic sinusitis cases are subdivided into cases with polyps and cases without polyps. In nasal samples of patients with polyps, there were significantly more eosinophils, plasma cells, and stromal edema compared with those without polyps. When polyps are present, the condition is called chronic hyperplastic sinusitis but the causes are poorly understood (Leung & Katial, 2008) and may include environmental factors such as dust or pollution, allergy, bacterial infection, or fungus.

Although the pathogenesis of chronic sinusitis is unclear, bacteria has been isolated in patients with chronic sinusitis includes anaerobes (pigmented *Prebotella*, *Fusobacterium* and *Peptostreptococcus* spp) and aerobic bacteria (*Staphylococcus aureus*, *Moraxella catarrhalis* and *Haemophilus* spp.) (Brook, 2001).

2.6 *Ervatamia coronaria*

2.6.1 Description and Taxonomy

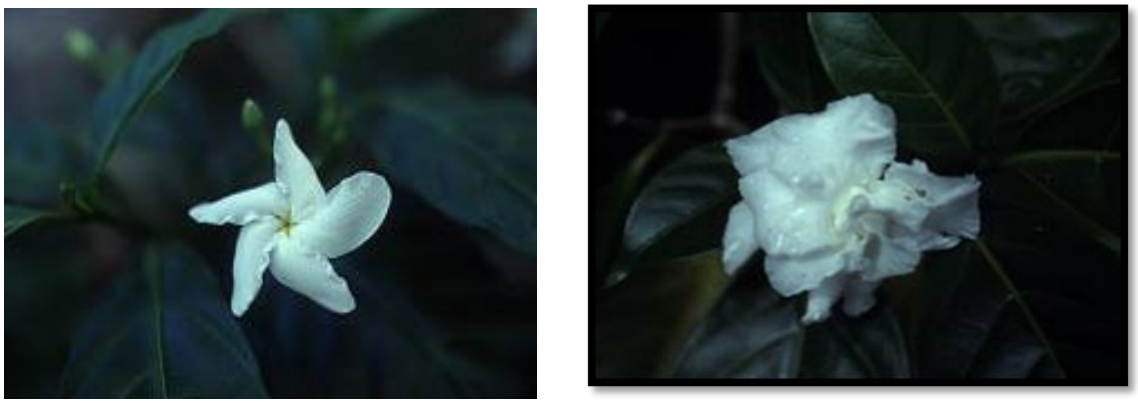


Figure 2.4: *Ervatamia coronaria* (single flower-left picture and double flower-right picture)

Ervatamia coronaria is also known as *Tabernaemontana divaricata.* The common name of *Ervatamia coronaria* is susur kelapa. It belongs to the Apocynaceae family. The basionym of *T. divaricata* is *T. siamensis* (Pitard, 1933). The plant *Ervatamia coronaria* is widely distributed in tropical countries including Brazil, Egypt, India, Sri Lanka, Vietnam, Malaysia and Thailand. It is cultivated as an ornamental plant, grows wild in hedges and shady forests (Kirthikar & Basu, 1998; National Institute of Science Communication, Council of Scientific and Industrial Research, 2000).

Kingdom……Plantae
Class……..Magnoliopsida
Subclass…….Lamiidae
Order…….Apocynales
Family……...Apocynaceae
Genus……*Ervatamia*
Species……..*coronaria*

There are two distinct varieties namely the single-flower and double-flower (Flore-Pleno) and both occur widely in Malaysia. *Ervatamia coronaria* was first described by Linnaeus in 1973 (Van Beek *et al.,* 1984). It has evergreen shrub forms shaped like symmetrical with 6 feet high and horizontal branches with almost horizontal shrub. The leaves are large, shiny, dark green leaves with 6 or more inches length and 2 inches wide. The flower is white in colour, waxy flowers with five petal pinwheels which gathered in small clusters (Leeuwenberg, 1991). These flowers have some fragrance at night and are produced most of the year. This plant can be grown in full sunlight or partial shade. It can also tolerate many different soil conditions, including some flooding and can thrive on soil of pH 4.6–6.0, organic mulch over the root zone, and constant moisture. However, it does not tolerate salinity.

2.6.2 Phytochemistry and Chemical Investigation

There are two varieties of *Ervatamia coronaria* which are the single-flower (single whirl) and double-flower (two whirls) variety. Studies by Raghuvanshi and Chatthan (1969) have shown marked morphological differences between these two. Nevertheless, not many researches were done to compare the chemical constituents between both of the variety and no mention of any differences in their chemical constituents has been published. According to research by Danieli and Palmisiano (1986), bisindole alkaloid of conophylline was discovered in both varieties.

The phytochemistry and chemical constituents from the leaves, stems, and roots have been reported previously in a number of instances which include alkaloids (Raj *et al.*,

1974; Egypkarawya & Aboutabl, 1979; Rastogi *et al.*, 1980; Pawelka & Stockigt, 1983; Atta-Ur-Rahman *et al.*, 1983; Atta-Ur-Rahman *et al.*, 1984; Atta-Ur-Rahman *et al.*, 1985; Atta-Ur-Rahman & Muzaffar, 1985; Atta-Ur-Rahman *et al.*, 1986; Van Beek *et al.*, 1985; Sharma & Cordell, 1988; Van der Heidjen *et al.*, 1988, 1990; Arambewela & Ranatunge, 1991; Henriques *et al.*, 1996; Kam et al., 1992, 1996, 2003, 2004; Kam & Anuradha, 1995; Kam & Pang, 2004; Ingkaninan *et al.*, 2006), steroids (Sharma & Cordell, 1988;, Dagino *et al*, 1991), flavonoids (Daniel & Sabnis, 1978), triterpenoids (Rastogi *et al.*, 1980, Sharma & Cordell, 1988; Van der Heidjen, 1989), phenolic acid (Henriques *et al.*, 1996) and phenyl propanoids (Daniel & Sabnis, 1978; Dagino *et al.*, 1991).

Since 1974, about 66 different alkaloids of *Ervatamia coronaria* have been extracted and identified by several methods such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography-mass spectrophotometry (GC-MS) (Pratchayasakul *et al*., 2008). For instance, five alkaloids such as coronaridine, lochnericine, tabernaemontanine, voacangine and voaphylline were identified (Raj *et al*., 1974) while vobasine and dregamine were reported from leaves, stems, barks and roots (EgyptKarawya & Aboutabl, 1979).

A re-investigation of the root bark of *Ervatamia coronaria* resulted in discovery of seven alkaloid compounds which were 5-hydroxyl-6-oxocoronaridine, 3-oxocoronaridine, 5-oxocoronaridine, 6-oxocoronaridine, ibogamine, 19-hydroxycoronaridine and coronaridine hydroxyindolenine (Rastogi *et al.*, 1980). Based on research of indole alkaloids, six monoterpenoid indole alkaloids have been isolated and identified from cell suspension cultures of *Ervatamia coronaria* grown under standard conditions such as

catharanthine, apparicine (pericalline), tubotaiwine (dihydrocondylocarpine), conoflorine, vinervine (12-hydroxyakuammicine) and coronaridine (Pawelka & Stockigt, 1983).

In addition, various alkaloid compounds have been isolated from the leaves of *Ervatamia coronaria* such as mehranine and hyderabadine (Atta-ur Rahman *et al.*, 1983), lahoricine (Atta-ur Rahman *et al.*, 1984), 5-oxo-11-hydroxyl voaphylline (Atta-ur Rahman & Muzaffar, 1985), ervatinine (Atta-ur Rahman *et al.*, 1985), 5-hydroxyvoaphylline, ervaticine and stapfinine (Atta-ur Rahman *et al*., 1986). Meanwhile, bis-indole alkaloid of pseudovobparicine heve been isolated from roots and bark of *Ervatamia coronaria* (Van Beck *et al.*, 1985).

Based on the report from Sharma and Cordell (1988), the whole plant of *Ervatamia coronaria* grown in Thailand has afforded a new indole alkaloid 19S-heyneanine hydroxyindolenine whose structure was deduced through interpretation of spectral data, 3-Oxovoacangine, heyneanine, voacangine hydroxyindolenine, voacristine and voacristine hydroxyindolenine. Since several interesting activities have been reported for alkaloids isolated from *Ervatamia coronaria*, therefore cell cultures have become important techniques to study chemical constituents of this plant. For instance, the discovery of pericyclivine, perivine, vellesamine and voaphylline hydroxyindolenine (Van der Heidjen *et al.*, 1988), o-acetylvallesamine and stemmadenine from the cell suspension culture of *Ervatamia coronaria* (Van der Heidjen *et al.*, 1990).

Four alkaloid compounds such as 11-methoxy-N-methyl dihydropericyclivine, 19-epivoacangine, isovoacangine and isovoacristine were discovered in the leaves, flowers and

roots of *Ervatamia coronaria* in Sri Lanka whereas 11-methoxy-N-methyl dihydropericyclivine was a new alkaloid compound (Arambewela & Ranatunge, 1991). A new bisindole alkaloid known as 19,20-dihydroervahanine A was isolated and discovered in the stems of this plant grown in Brazil (Henriques *et al.*, 1996).

Based on the review of several studies by Kam and colleagues, approximately 19 alkaloid compounds were isolated in leaves, stems and barks part of the plants. There are summarized in Table 2.2.

Table 2.2 : List of isolated alkaloids from *Ervatamia coronaria*

Alkaloids	Class	Plant part	References
Canophylline	Bis-indole	Leaves	Kam *et al.*, 1992
Voaharine	Plumeran	Leaves	Kam *et al.*, 1992
19-Epivoacristine	Ibogan	Leaves	Kam *et al.*, 1992
Canophyllidine	Bis-indole	Leaves	Kam *et al.*, 1992
N1-methylvoaphylline	Plumeran	Leaves	Kam and Anuradha,1995
N-methylvoafinine	Aspidospermatan	Leaves	Kam and Anuradha,1995
Pachysiphine	Plumeran	Leaves	Kam and Anuradha,1995
Voafinine	Aspidospermatan	Leaves	Kam and Anuradha,1995
Conofoline	Bis-indole	Leaves	Kam and Anuradha,1995
Voafinidine	Aspidospermatan	Leaves	Kam *et al.*, 1996
Voalenine	Aspidospermatan	Leaves	Kam *et al.*, 1996
Canophyllinine	Bis-indole	Leaves	Kam *et al.*, 2003
Taberhanine	Aspidospermatan	Leaves	Kam *et al.*, 2003
3S-Cyanocoronaridine	Ibogan	Stems, barks	Kam *et al.*, 2004
3S-Cyanoisovoacangine	Ibogan	Stems, barks	Kam *et al.*, 2004
Conodusarine	Bis-indole	Stems, barks	Kam and Pang, 2004
Conolidine	Aspidospermatan	Stems, barks	Kam *et al.*, 2004
Conolobine A	Aspidospermatan	Stems, barks	Kam *et al.*, 2004
Conolobine B	Aspidospermatan	Stems, barks	Kam *et al.*, 2004

The latest discoveries were ibogan alkaloids of conodurine, tabernaelegantine A and bis-indole alkaloid of 19,20 Dihydrotabernamine (Ingkanan *et al.,* 2006). All the alkaloids were discovered in roots of *Ervatamia coronaria* in Thailand.

Even though most of phytochemical work has been concerned with the alkaloidal constituents, some non-alkaloidal constituents such as terpenoids, enzymes, hydrocarbons and steroids have also been isolated from this plant. For instance, the enzyme anthranilate synthase was detected from *Ervatamia coronaria* cell cultures by HPLC assay (Poulsen *et al,* 1991) while five known enzymes such as isopentenyl diphosphate isomerase, prenyl transferase, squalene synthetase, qualene 2,3-oxide cycloartenol cyclase and squalene 2,3-oxide cyclase were detected for the first time in *Ervatamia coronaria* cell-suspension culture (Fulton *et al,*1994). Besides, five enzymes were also reported in cell culture including strictosidine synthase, tryptophan decarboxylase, isopentenyl pyrophosphate, strictosidine glucosidase and geratinol 10-hydroxylase (Dagino *et al.,* 1995)

Another non-alkaloidal enzyme was α-D-glucosidase (Luijendijk *et al.,* 1996) and squalene synthase (Kroon & Threlfall, 1997) which were partially purified from cell-suspension culture. In addition, enzyme farnesyl diphosphate synthase was reported by Ramas-Valdivia and colleagues (1998) from cultured cell of *Ervatamia coronaria* by western blotting assay and chromatography. Other than that, the discovery of free radical scavenging enzymes such as ascorbate peroxidase, phenolic peroxidase, superoxide dismutase and glutathione reductase from roadside plants in India indicated this plant is good scavenging system to combat air pollution (Mandal & Mukherji, 2001).

According to Sharma and Prasad (1986), the leaves and stems of India *Ervatamia coronaria* have solid char and pyrolytic oil that can be converted to petroleum and ethanol which can be used to produce gasohol fuel while Behera and colleagues (1995) demonstrated the hexane extracts from roots, flower, old leaves and stems of the plant which was rich in hydrocarbons. Besides, five phenolic acids such as vanillic, gentisic, syringic, salicyclic acid and 4-hydroxybenzoic were also isolated from the stems of *Ervatamia coronaria* grown in Brazil (Henriques *et al*, 1996). Meanwhile, eight non-alkaloid compounds were isolated from the root bark such as cycloartenol, α-amyrin lupeol, α-amyrin acetate, campesterol, benzoid acid, β-sitosterol, lupeol acetate and aurantiamide acetate (Rastogi *et al.,* 1980)

2.6.3 Medicinal Value

The pharmacological properties of *Ervatamia coronaria* have previously been investigated both *in vivo* and *in vitro*. Various parts of the plant were used in traditional medicine. The most common medicinal use of this plant was antimicrobial action against infectious diseases sush as syphilis, gonorrhea, leprosy and as well as antiparasitic action against dysentery, diarrhoea, worms and malaria (Van Beek *et al.,* 1984).

The plant material was widely used as tonic for the brain, liver and the spleen, purgative useful in paralysis (Kirtikar & Basu, 1975), treatment for cancer (Hsu, 1967) and used in treatment of wounds and inflammations (Atta-Ur-Rahman *et al*, 1985). Anti-inflammatory mechanism was thought to be due to the presence of phenolic acid (Daniel & Sabnis, 1978). Besides, *Ervatamia coronaria* was also believed to have antitumor effect.

The effect of crude methanol extract from *Ervatamia coronaria* could suppress mesangial cell proliferation of cell cancer via the reduction of IL-1, IL-6 and TNF-α expression (Kuo *et al.,* 1999)

The ethanol extract of *Ervatamia coronaria* was found to cause dose-related decreased in motor activity, ataxia, loss of righting reflex, decreased respiratory rate and loss of screen grip which indicated the depressive effects on the central nervous system (CNS) (Taesotikul *et al.,* 1989). However, Ingkaninan and colleagues (2003) reported that the roots and stems of *T. divaricata* have been used in Thai traditional medicine as components of rejuvenating and neurotonic remedies which are believed can prevent forgetfulness and improve memory as well as being a CNS stimulant. The research was supported by *in vivo* study that *Ervatamia coronaria* administration in various doses (250, 500 and 1000mg/ml) can significantly decrease neuronal AChE activity in the cerebral cortex (Chattipakorn *et al.,* 2007). Therefore, *Ervatamia coronaria* may be a new therapeutic target for Alzheimer's disease.

The plant extract was also found to possess antipyretic, analgesic, vasodilator and CNS depressant effects (Taesotikul *et. al,* 1989), uterine stimulant effect (Da Sil Va *et al*, 1984), cytotoxic activity (Yamamoto *et al,* 1997) antiplasmodic and hypotensive activity (Dhar *et. al,*1968). The latest research on methanolic extract of *Ervatamia coronaria* flower exhibited acid neutralizing, anti-secretory and ulcer preventive properties and thereby produces significant gastroprotective effect at a dose of 500 mg/kg (Mohammed, 2011).

2.7 *Tinospora crispa*

2.7.1 Description and Taxonomy

Figure 2.5: *Tinospora crispa*

This wild medicinal plant has numerous synonyms *such as Tinospora rumphii* and *Menispermatum crispum*. The botanical name, *Tinospora crispa* is also known as common local names. However these common names vary depending on the country. For instance, it is known as Patawali in Malaysia (Wan Omar Abdullah, 1998), Brotawali or Andawali in Indonesia (Burkill, 1966), Boraphet in Thailand (Burkill, 1966), Ratnawali in Brunei (Goh *et. al*, 1995) and Makabuhay in Philippines (Burkill, 1966).

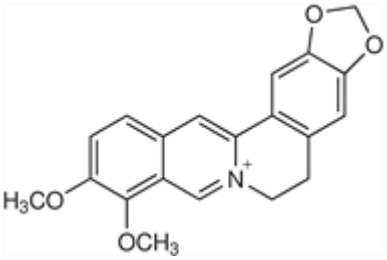
Kingdom…….Plantae
Class……....Dicoteledoneae
Subclass……..Magnoliidae
Order……...Ranunculales
Family…….....Menispermaceae
Genus……..*Tinospora*
Species…….*crispa*

Tinospora crispa belongs to the family Menispermaceae and genus *Tinospora*. It can be found in primary rainforests or mixed deciduous forests throughout a large part of Asia and Africa (Pathak *et al.*, 1995) including all parts of Thailand, Malaysia and Indonesia. *Tinospora crispa* can also be found in tropical and subtropical India and parts of the Far East.

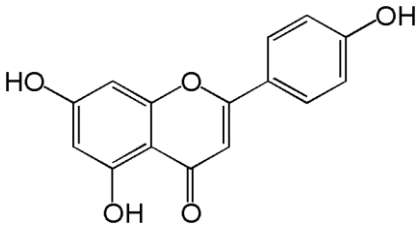
The plant is a woody climber and can be found in the length up to 15 meters long. Its leaves are thin, ovate and are about 6-12 centimeters long and 7-12 centimeters wide. It has a pointed tip with broad heart-shaped base and is smooth and shiny. The petiole can be up to 6 centimeters long. It is either racemes solitary or in pairs arising from axils of the leaves, light green and short pedicelled. The flower of this herb has 3 petals which are light green in colour. The fruit of *Tinospora crispa* is an ellipsoidal drupe with the length up to 8 mm long and they grow in long clusters with orange or pink in colour (Burkill, 1966). In addition, the stem has the diameter of approximately 1 cm and on the older stems has numerous prominent protrusions. The stems are fleshy and contain a very bitter milky sap (Burkill, 1935).

2.7.2 Phytochemistry and Chemical Investigation

A number of chemical constituents have already been isolated from the plant. The whole plant contains bitter principles which are columbine, traces of an alkaloid, a flavanoids glycoside and also contains amorphous bitter principles which are picroretine and traces of berberine (Lily, 1981; Burkill, 1935). From the root-bark a bitter principle (which is not a glycoside) and some alkaloids were isolated.



Berberine (Alkaloid)



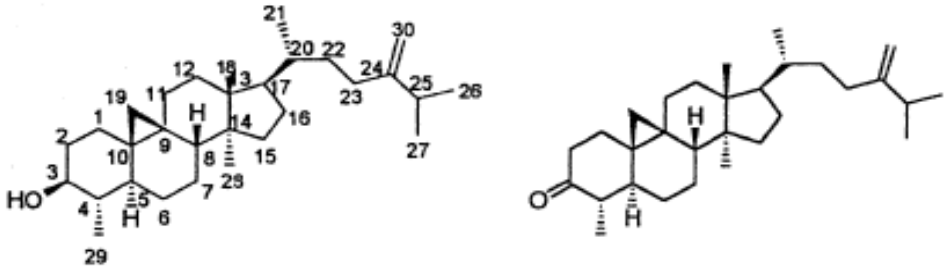
Apigenin (Flavanoid)

Figure 2.6: Structures of berberine and apigenin

The bitter, aqueous extract of the stem does not contain alkaloids but amorphous and resinous substances in Philippines. When the plant stem was re-examined it was found to contain berberine [Figure 2.6], a glucoside and a bitter principle which was glucosidal in nature (Kongkathip *et al*., 2002). The stems of *Tinospora crispa* also contain flavones O-glycosides (apigenin), picroretoside, picroretine and resin (Kongkathip *et al*., 2002). It is well known that apigenin [Figure 2.6] has an ability to act as a powerful antioxidant. There are two triterpenes, namely cycloeucalenol [Figure 2.7] and cycloeucalenone [Figure 2.7] which were first time reported in this plant by Kongsaktrakoon and colleagues (1994). Table 2.3 shows the chemical compounds isolated from the plant.

Table 2.3: Chemical compounds isolated from *Tinospora crispa*

Chemical Compounds	**References**
Palmatine	Bisset and Nwaiwu, 1984;
Berberine	Bisset and Nwaiwu, 1984; Kongkathip *et al.*, 2002
Jatrorrhizine	Bisset and Nwaiwu, 1984
Tembetarine	Bisset and Nwaiwu, 1984
Choline	Bisset and Nwaiwu, 1984
N-trans-feruloytryramine	Fukada *et. al*, 1983, Cavin *et. al*, 1998
N-cis-feruloytryramine	Fukada *et. al*, 1983, Cavin *et al*, 1998
N-formylannonaine	Pachaly *et. al*, 1992
N-acetylnornuciferine	Pachaly *et. al*, 1992; Patchak *et. al*, 1995
N-formylnornuciferine	Pathak *et. al*, 1995
Borapetoside A	Fukada, 1985; Pathak *et. al*, 1995
Borapetoside B	Murakoshi *et. al*, 1993; Pathak *et. al*, 1995
Tinocrisposide	Fukada *et. al*, 1983, Pathak *et. al*, 1995
Borapetoside H	Fukada *et. al*, 1985
Tinotuberide	Fukada *et. al*, 1983; Pathak *et. al*,1995
Borapetol A	Fukada *et. al*, 1985; Patchak *et. al*, 1995
Borapetol B	Murakoshi *et. al*, 1993; Pathak *et. al*, 1995
Siringin	Murakoshi *et. al*, 1993
Secoisolaricinesinol	Cavin *et. al*, 1998
γ- sitosterol	Pathak *et. al*, 1995
Tinotubride	Pathak *et. al*, 1995
Picrotein	Pathak *et. al*, 1995
Cycloeucalenol	Kongsaktrakoon *et. al*, 1994
Cycloeucalenone	Kongsaktrakoon *et. al*, 1994
Cis-clerodane-type furanoditerpenoids	Choudhary *et. al*, 2010
Tinotufolin A-B	Fukada *et. al*, 1985
Tinotufolin C-F	Fukada *et. al*, 1985



Cycloeucalenol Cycloeucalenone

Figure 2.7: Structures of cycloeucalenol and cycloeucalenone

2.7.3 Medicinal Value

The Filipinos and Malays consider this plant as a universal medicine. It is the most popular of local medicinal plants. The plant part that is thought to have medicinal value is the stem. It is customarily put to use either in its raw form or dried form (Burkill, 1935).

The young stems of *Tinospora crispa* have been shown to lower high blood pressure and assuage abdominal pains when eaten raw. However, the bitter taste of the sap contained in the stem hindered the consumption of the raw plant. Therefore, the decoction of the plant is produced by boiling the stem in water. The consumption of this decoction is used as a remedy for cholera and diabetes (Perry, 1980; Burkill, 1966; Umi Kalsom *et al*, 1999), washing sore eyes and syphilitic sores (Burkill, 1966). An infusion of *Tinospora crispa* stems is also consumed to treat fever, jaundice, cholera, malaria and against worms in children (Umi Kalsom *et al,* 1999). A preparation with coconut oil is an effective cure for rheumatism and also for flatulence in children (Quisumbingm, 1978).

Scientifically it is reported that malaria can be countered with the extract of *Tinospora crispa* (Hasimah *et al.,* 1991) because its stem and root comprise quarternary alkaloids that also include berberine (Bisset & Nwaiwu, 1984). These alkaloids are presumed to deter the synthesis of protein in *Plasmodium falciparum* (Elford, 1986). Meanwhile, the leaves part of the plant can also be crushed to a fine pulp for external application on wounds. Poultices made from the leaves of *Tinospora crispa* are also used for relieving itches (Burkill, 1966; Quisumingm, 1978).

Tinospora crispa have also been used to treat rheumatism and internal inflammation (Burkill, 1996) which are associated with oxidative stress. Besides, *Tinospora crispa* is also used as a health tonic in the treatment and prevention of diabetes mellitus and hypertension (Shahimi & Mohsien, 1979). Its use in the control of hypertension may be justified as its extract was found to reduce tachycardia induced by noradrenaline and isoprenaline (Shahimi & Mohsien, 1997). The analgesic qualities of *Tinospora crispa* also help to relieve pain (Wan Omar Abdullah, 1998; Muhammad & Mustafa, 1994). *Tinospora crispa* was also considered to be an effective remedy for snake bites (Perry, 1980; Selvanayagam *et al*, 1994).

In another finding, it was suggested that the relatively polar compounds of the chloroform extract of *Tinospora crispa* possessed negative ionotropic and vasodilating activities. Both of these activities could reduce blood pressure that may further justify its anti-hypertensive medicinal properties (Nor Aziyah *et al*, 2001). Furthermore, *Tinospora crispa* extract has shown to be effective in reducing the glucose level in plasma by

increasing the plasma insulin level (Noor & Ashcroft, 1989). In addition, *Tinospora.crispa* has been demonstrated to possess antibacterial property (Sulaiman *et. al,* 2008).