# CHAPTER 1
# INTRODUCTION

Medicinal plants have widely been used as therapeutic drugs or herbal medicine. Some of them are useful to mankind as pharmaceuticals, fragrance, flavors, colors, stimulants and cosmetics. In the tropical zones, there is a variety of plants many of which have various kinds of interesting compounds. Therefore, medicinal plants still serve as a source for new lead to be developed into new and more active compounds.

In recent years, it has been regarded that reactive oxygen species (ROS) play a critical role in many diseases such as brain disorders, atherosclerosis, cardiovascular and colon cancer. To prevent these diseases, the intake of antioxidants has been greatly emphasized several cancer chemopreventive agents exhibit antioxidant activity through their ability to scavenge oxygen radicals, inclusive of singlet oxygen, peroxy radicals, superoxide anion, and hydroxyl radicals. Antioxidant defenses normally protect against DNA damage caused by reactive oxygen species from endogenous and exogenous sources, and reduced levels of antioxidant are associated with increased cancer risk.[1]

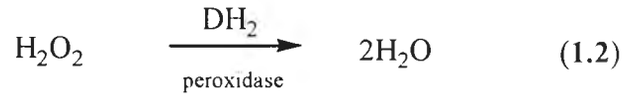
The majority of living organisms depend on molecular oxygen to sustain life, yet the oxygen molecule is a biradical and, interaction of organic substrates with oxygen can lead to a host of complex oxidation products. Not surprisingly then, there is now wide recognition that, whilst some oxidising radicals serve a useful physiological function in the healthy organism, in other circumstances they can be associated with molecular damage and hence with disease. They are also thought to contribute to the processes of aging. Under normal circumstances there exists a fine balance between the essential biochemistry of these oxidising species and the range of defense mechanisms which have been evolved to inhibit oxidation damage, not least the presence of the antioxidant vitamin C and E of which more later.

Two rather obvious processes are available whereby molecular damage may be brought about by the intervention of oxygen-centered radicals. The first involves radical-chain autoxidation, to which polyunsaturated lipids containing 1,4-diene units are particularly susceptible. To some extent, however, this is an important positive

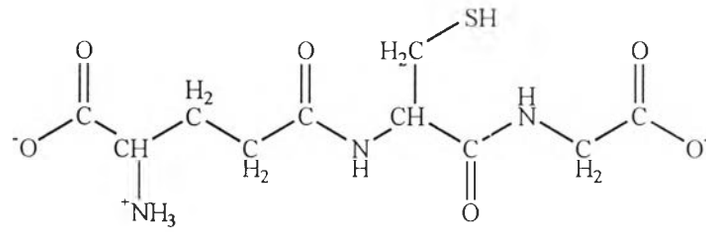
process, for, mediated by iron-dependent oxygenase enzymes, such chemistry leads to the important prostanoid and leukotriene hormones.

The second process depends on the formation of hydroxyl radicals. These are so reactive and unselective that they will attack almost any organic molecule at, or very close to, the diffusion-limited rate. This implies the possibility of damage to proteins, DNA, lipids or carbohydrates with little scope for significant prevention by so-called hydroxyl-radical scavengers. Nature has an alternative approach wherein it strives to circumvent hydroxyl radical production. One-electron reduction of oxygen gives the superoxide radical anion, $O_2^{-\bullet}$, and in the course of normal biological processes this does happen to a modest extent. For example, in red blood cells the familiar role of hemoglobin is reversible complexation with $O_2$, but occasionally the complex dissociates, giving $O_2^{-\bullet}$ together with the iron (III) species methaemoglobin which is no longer capable of binding molecular oxygen. Likewise, normal oxidative metabolism occurs with transfer of four electrons to $O_2$ and production of two molecule of water, but again the process occasionally diverts with production of superoxide. Superoxide has a $pK_a$ of 4.8, so that at physiological pH a small fraction is protonated to form $HOO^{\bullet}$. The reaction between this and superoxide is extremely rapid ($k = 10^8\ M^{-1}sec^{-1}$) giving oxygen and hydrogen peroxide [**Equation (1.1)**]. The corresponding reaction between two superoxide molecules is much slower. If this source of hydrogen peroxide seems alarming, the presence of one of the superoxide dismutase enzymes (SODs) which catalyse the reaction might engender even greater concern. However, nature has devised ways of dealing both with hydrogen peroxide, and with any iron which may catalyse chemistry. Two enzyme types are available to destroy hydrogen peroxide. Catalase enzymes promote the transformation of two hydrogen peroxide molecules into two molecules of water and one of oxygen. Peroxidases behave rather differently, depending on a hydrogen donor [$DH_2$ in **Equation (1.2)**] to deliver hydrogen. In mammalian cells the hydrogen donor is the tripeptide glutathione (**1**), two molecules of which are oxidised to the corresponding disulphide. This reaction is coupled to a further enzyme-catalysed process which regenerates the glutathione . The redox active sites in (many) SODs, catalases, and glutathione peroxidase depend respectively on copper and zinc, an iron porphyrin, and selenium.[2]

$$HOO\cdot + O_2^{-\cdot} + H^+ \longrightarrow O_2 + H_2O_2 \quad (1.1)$$



$$H_2O_2 \xrightarrow[\text{peroxidase}]{DH_2} 2H_2O \quad (1.2)$$



Tripeptide glutathione (**1**)

Reaction oxygen species such as hydroxyl (OH·) and peroxyl radicals (ROO·) and the superoxide anion ($O_2^{-\cdot}$) are constantly produced as a result of metabolic reactions in living systems. Living systems are protected from oxidative damage by these reactive species by enzymes such as superoxide dismutase and glutathione peroxidase and by antioxidant compounds such as ascorbic acid, tocopherols, and carotenoids. However, when free-radical production exceeds the antioxidant capacity of the organism, these radical species attack lipids, proteins, and DNA, thus damaging structural integrity and function of cell membranes, enzymes, and genetic material.[3]

Our recent screening tests of forty-one medicinal plants for antioxidant activity based on 2,2-diphenyl-1-picrylhydrazyl radical. *Cudrania cochinchinensis* showed the highest activity. This plant was extracted with hexane, dichloromethane, ethyl acetate and methanol, respectively. This crude extracts were also tested for antioxidant activity with DPPH. There are many models for antioxidant tests such as linoleic acid, β-carotene, xanthine oxidase, DPPH, and so on. In this study, DPPH is selected in activity-directed fractionation of free radicals scavenging activity. This model is rapid, convenient, reliable, inexpensive, sensitive, require little material. The pure compounds were also tested with xanthine oxidase.

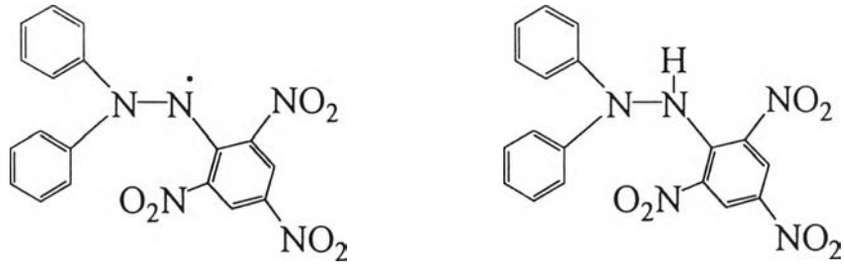
DPPH is obtained as a stable solid dye free radical, so it is easy to control the quantity of radicals. This is a kind of nitrogen-centered radical, but the reactivity is not so large as an oxygen-centered radical such as RO• and ROO• because of the widely-spreading resonant system. When this radical reacts with polyphenols, dehydrogenation occurs on polyphenol molecules and DPPH changes into DPPHn,

the structure of which is showed in **Figure 1.1**. DPPH is a deeply colored substance, but DPPHn is colorless.[4]

Xanthine oxidase is a key enzyme that catalyzes the oxidation of hypoxanthine to xanthine, in the presence of molecular oxygen, to yield uric acid and superoxide anions (**Figure 1.2**). Therefore, inhibition of xanthine oxidase is an effective therapeutic approach for treating hyperuricemia that causes gout, kidney stones, and myocardial ischemia.

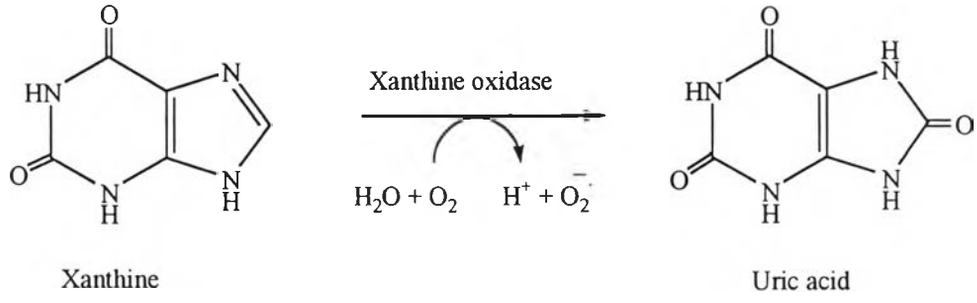
A number of compounds have been isolated from *C. cochinchinensis*, the most characteristic of which are the flavonoid and xanthone derivatives. Many studies have suggested that flavoniods exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory and vasodilating action. However, most interest has been devoted to the antioxidant of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals.

Flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase and protein kinase C. Flavonoids have been also shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, and NADH oxidase, all involved in reactive oxygen species generation.




DPPH (2,2-diphenyl-1-picrylhydrazyl) DPPHn (2,2-diphenyl-1-picrylhydrazine)

**Figure 1.1** Structure of DPPH and DPPHn




Xanthine Uric acid

**Figure 1.2** Xanthine oxidized to uric acid

A number of flavonoids efficiently chelate trace metals, which play an important role in oxygen metabolism. Free iron and copper are potential enhancers of reactive oxygen species formation, as exemplified by the reduction of hydrogen peroxide with generation of the highly aggressive hydroxyl radical, or by the copper-

$$H_2O_2 + Fe^{2+} (Cu^{+}) \rightarrow {}^{\cdot}OH + OH^{-} + Fe^{3+} (Cu^{2+})$$

mediated LDL (low-density lipoprotein) oxidation, where LH represents LDL.

$$LH \rightarrow L^{\cdot} \rightarrow LOO^{\cdot}$$

The proposed binding sites for trace metals to flavonoids are the catechol moiety in ring B, The 3-hydroxyl, 4-oxo groups in the heterocyclic ring, and the 4-oxo, 5-hydroxyl groups between the heterocyclic and the A rings (**Figure 1.3**). However, the major contribution to metal chelation is due to the catechol moiety, as exemplified by the more pronounced bathochromic shift produced by chelation of copper to quercetin compared to that of kaempferol (similar in to quercetin except that it lacks the catechol group in the B ring).

Flavonoids (Fl-OH) are thermodynamically able to reduce highly oxidizing free radicals with redox potentials in the range 2.13 - 1.0V, due to their lower redox potentials ($0.23 < E_7 < 0.75$ V, $E_7$ = One-electron), by hydrogen atom donation where $R^{\cdot}$ represents superoxide anion, peroxyl, alkoxyl, and hydroxyl radicals. The aroxyl radical ($Fl\text{-}O^{\cdot}$) may react with a second radical, acquiring a stable quinone structure (**Figure 1.4**).

$$Fl\text{-}OH + R^{\cdot} \rightarrow Fl\text{-}O^{\cdot} + RH$$

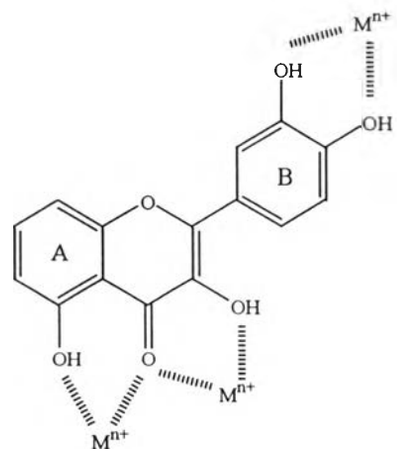



**Figure 1.3** Binding sites for trace metals.

The aroxyl radicals could interact with oxygen, generating quinones and superoxide anion, rather than terminating chain reactions. The last reaction may take place in the presence of high levels of transient metal ions and is responsible for the undesired pro-oxidant effect of flavonoids. Thus, the overall capacity of flavonoids to

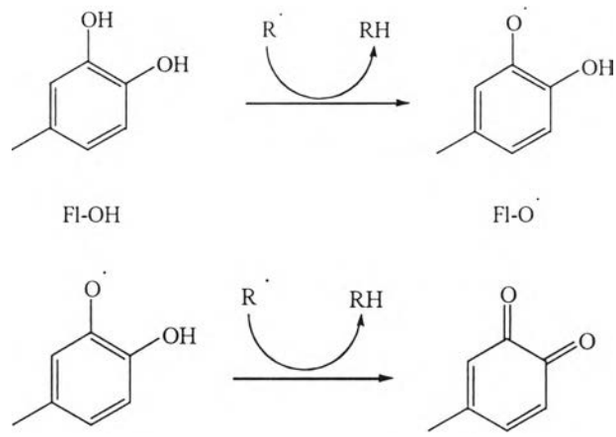
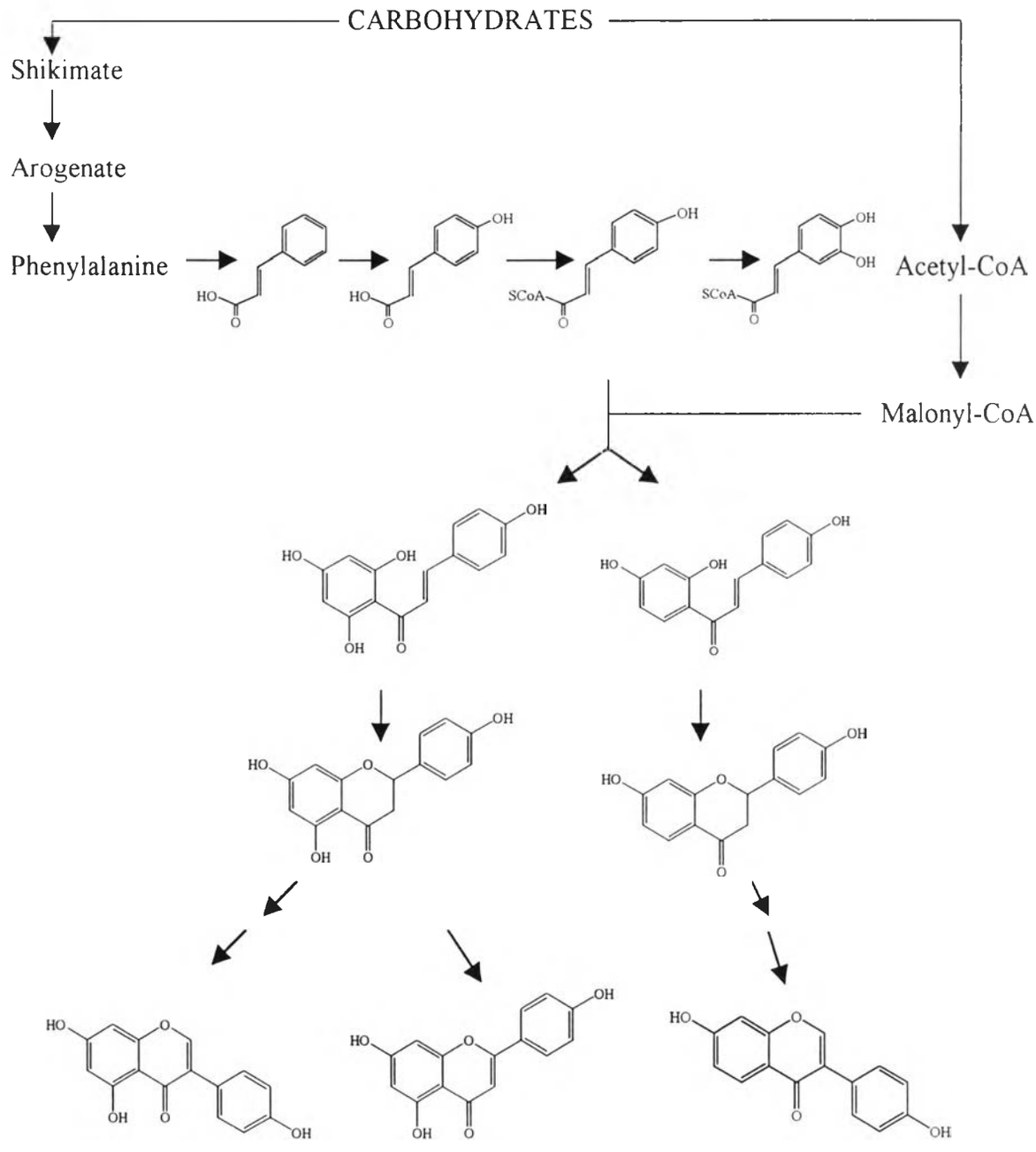



**Figure 1.4** Scavenging of ROS (R•) by flavonoids.

act as antioxidants depends not only on the redox potential of the couple Fl-O•/Fl-OH but also on possible side reactions of the aroxyl radical. Scavenging of superoxide is particularly important, because this radical is ubiquitous in aerobic cells and, despite its mild reactivity, is a potential precursor of the aggressive hydroxyl radical in the Fenton and Haber-Weiss reactions. Besides scavenging, flavonoids may stabilize free radicals involved in oxidative processes by complexing with them.[5]

Flavonoids derived biosynthetically from phenylalanine, are pigments found widespread in plants. Three moles of malonyl-coenzyme A (CoA) from glucose metabolism condense to form ring A, catalyzed by chalcone synthetase. Ring B and C also come from glucose metabolism, but via the shikimate pathway through phenylalanine, which is converted to cinnamic acid and then to coumaric acid. Coumaric acid CoA and three malonyl CoAs are condensed in a single enzymatic step to form naringenin chalcone. The ring C closes and becomes hydrated to form flavonoids which are found in nearly every plant.[6] (**Figure 1.5**)

In this study, the isolation of bioactive compounds from *C. cochinchinensis* was conducted for possible new antibiotics by bioassaying all compounds against *Escherichia coli, Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus*, Flat-Sour, and *Salmonella* spp.




**Scheme 1.1** illustrating the pathways to phenylalanine and acetyl-CoA, and the following reaction steps leading to the some flavonoid classes.[7]

*E. coli* O157:H7,the first confirmed isolation in the United States was in 1975 from a California woman with bloody diarrhea. The bacterium was first identified as a human pathogen in 1982, when it was associated with two foodborne outbreaks of hemorrhagic colitis. Unlike most foodborne pathogen, *E. coli* O157:H7 is uniquely tolerant to acidic environments. Outbreaks of *E. coli* O157:H7 infection have been

directly associated with consumption of contaminated dry salami and apple cider. The mechanism of acid tolerance has not been fully elucidated but appears to be associated with a protein (s) that can be induced by pre-exposing the bacteria to acid conditions.[8]

The most important types of listeriosis are infection of pregnant women, newborn infants, and immunosuppressed people causing meningitis, multiple adenitis, and septicemia. *L. monocytogenes* has been isolated from many foods, such as meat and meat products, soft cheeses, ice cream, vegetables, and seafood. The munber of samples found positive may vary from a few to 50 %.

Food poisoning caused by *B. cereus* may occur when foods are prepared and held without adequate refrigeration for several hours before serving. *B. cereus* is an aerobic sporeforming bacterium that is commonly found in soil, on vegetables, and in many raw and processed foods. Consumption of foods that contain $> 10^6$ *B. cereus*/g may result in food poisoning. Foods incriminated in past outbreaks include cooked meat and vegetable, boiled and fried rice, vanilla sauce, custards, soups and raw vegetable sprouts.[9]

*S. aureus* is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents. Thus, the presence of this bacterium or its enterotoxins in processed foods or on food processing equipment is generally an indication of poor sanitation. *S. aureus* can cause severe food poisoning. It has been identified as the causative agent in many food poisoning outbreaks.[9]

Flat-sour in practice, the inherent spore contamination of foods and food ingredients and of the cannery environment contributes to spoilage problems. Dry ingredients such as sugar, starches, flours and spices often contain high levels of sporeformers. Spore population can also accumulated in a food plant, such as thermophilic spores on heated equipment and saccharolytic clostridia in plants processing sugar-rich foods such as fruits.[8]

The importance of *Salmonella* spp. as the leading cause of foodborne bacterial diseases in humans, in whom reported incidents of foodborne salmonellosis tend to dwarf those associated with other foodborne pathogen.[8]

In our continuing search for potential bioactive compounds from natural products, the methanolic extracts of forty-one Thai medicinal plants were investigated.

Since *C. cochinchinensis* demonstrated the highest level of free radicals scavenging activity on DPPH. This plant was selected for further examination.

From literature, *C. cochinchinensis* has been tested for many activities such as anti-lipid peroxidation, anti-inflammatory, hepatoprotective effect and so on. The result showed in table 1.2 and 1.3. The isolation in root and stembark of this plant contained some xanthones, flavoniods and triterpenoid (**Table 1.1**).

In the present study, we report chemical constituents of the stems of this plant which has not been studied previously. The objective of this research can be summarized as follows. The investigation was undertaken to elucidate the chemical structures of the stem extracts of *C. cochinchinensis* that exhibit interesting activities. These crude extracts will be separated by means of chromatography techniques and then purified by proper methods to afford chemical structures. Structural elucidation on all of the compounds will be carried out by spectroscopy techniques. Biological activities of the pure compounds will be also investigated.

**Botanical Aspect and Distribution**

*Cudrania cochinchinensis* Lour. or *Cudrania javanensis* Trecul. in Thailand has been known as " Kae-lae", "Sakkhi", "Kae-kong (Phrae)", "Rae (Southern)", "Chang-nga-tok (Lampang)".[10] *C. cochinchinensis* Lour. is much branched or climbing shrubs or small trees often armed with axillary straight or recurved spines[11], 5-10 metres high. Leaf simple, alternate, elliptic, 1-3.5 centimetres wide, 2-9 centimetres long. Inflorescence in axillary head, unisexual, dioecious; flowers light green. Fruit syncarp; drupelets red. Wood: antidiarrheal, tonic: treatment of chronic fever with skin manifestations.[12] The plants in the Moraceae family are widely distributed over the earth and especially are abundant in tropical and sub-tropical parts of Asia, Australia, Polynesia. In Thailand, it is distributed generally in every region.

**Figure 1.5** Flowers, leaf, woods and tree of *C. cochinchinensis*

**Chemical constituents of *Cudrania cochinchinensis* Lour.**

From liturature search of *C. cochinchinensis* Lour. xanthones and flavonoids are found. They are phenolic compounds and most of them isolated from root and bark of this plants. Many types of them are reported in **Table 1.1.** Most of the chemical structures are shown in **Figure 1.6 .**

**Table 1.1** Chemical constituents of *C. cochinchinensis*[14-19]

| Parts of plant | Name of compounds | ref |
|---|---|---|
| bark | osajaxanthone<br>(-)-(s)-stachydrine<br>vanillic acid<br>p-hydroxybenzoic acid | 14 |
| periderm | Cudraniaxanthone<br>Butyrospermone<br>Kaempferol<br>Aromadendrin<br>Populnin<br>Quercetin<br>Taxifolin | 15 |
| Twig and leaf | Cudraisoflavone-A<br>3'-O-methylorobol<br>sitosterol | 16 |
| Root bark | Gerontoxanthone G<br>Gerontoxanthone E<br>Gerontoxanthone H<br>Gerontoxanthone I | 17 |
| Root bark | Gerontoxanthone C<br>Gerontoxanthone I<br>Gerontoxanthone D<br>Cudraniaxanthone | 18 |

| Parts of plant | Name of compounds | Ref |
|---|---|---|
| Root bark | Gerontoxanthone G<br>Cudraxanthone I<br>Gerontoxanthone A<br>Cudraxanthone A | 18 |
| Fresh root wood | 7,4'-dihydroxy-5,3'-dimethoxyisoflavone<br>or gerontoisoflavone A<br>Gerontoxanthone J<br>Wighteone<br>Genistein<br>Genistein-5-methyl ether<br>Orobol<br>6-C-prenylorobol<br>6-C-prenylapigenin<br>8-C-prenylapigenin<br>Naringenin<br>5,7,2',4'-tetrahydroxyflavanone<br>Artocarpesin<br>Kaempferol<br>Aromadendrin<br>Kaempferol-7-glucoside<br>Kaempferol-3,7-glucoside | 19 |

**Ethnomedical Informations and Biological Activities**

*C. cochinchinensis* Lour. has been used as a folk medicine for treatment in Asia. Ethnomedical informations are shown in **Table 1.2** and Biological activities are shown in **Table 1.3**.

**Table 1.2** Ethnomedical Information[13]

| Country | Parts of Plant | Used |
|---|---|---|
| China | Root | Amenorrhea |
| Nepal | Bark and Juice | Peptic Ulcer |
| | Latex(unspect part) | Treat Boils |
| Indonesia | Dried Root | Cure Malaria |
| | | Cure Colds |
| Taiwan | Dried Rhizome | Liver Disease |
| | Dried Root and Stem | Treat Hepatitis |
| | Dried Rootbark | Treat Rheumatism |
| | | Treat Hepatitis |
| | | Treat Neuralgia |
| | Fresh Rootwood | Hepatitis |
| | | Contused Wounds |
| | | An Antireumatic |
| | | Neuralgia |
| | | Antipyretic |
| | | Rheumatism |
| Thailand | Dried Heartwood | An Antipyretic |
| | Dried Stemwood | A Permanent Dye |
| | | An Antineoplastic |
| | Dried Wood | Blood Purification |
| | | A Cardiotonic |
| | | A CNS Stimulant |
| | | Fainting |
| | | Cancer |

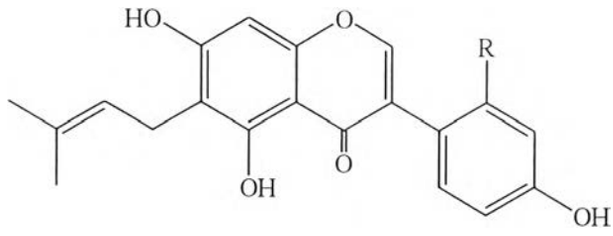
**Table 1.3** Biological Activities[13]

| Country | Parts of Plant | Activities |
|---|---|---|
| China | Root | Menstruation Induction Effect |
| Taiwan | Root + Stem | *Antibacterail Activity |
| | | **Antimycobacterial Activity |
| | Dried Rhizome | Glutamate-Pyruvate-Transaminase Inhibition |
| | | Induced Hepatotoxicity |
| | | Induced Pedal Edema |
| | Dried Rootbark | Antiviral Activity |
| | | Lipid Peroxide Formation Information |
| India | Dried Aerial Parts | Toxicity Assessment |
| | | Antitumor Activity |
| | | Cytotoxic Activity |
| Indonesia | Dried Root | Antimalarial Activity |
| | | Cell Proliferation Inhibition |
| | | $Na^+/H^+$ Exchange Inhibition |
| Japan | Dried Root | Antifungal Activity |
| Hongkong | Dried Leaf+Twig | Cytotoxic Activity |
| Thailand | Dried Part Not Specified | Cytotoxic Activity |
| | Dried Heartwood | Hypotensive Activity |
| | | Antispasmodic Activity |
| | | Antihistamine Activity |
| | Dried Stemwood | Antitumor Activity |
| | | Anticoagulant Activity |
| | | Cardiotonic Activity |
| | | CNS Stimulant Activity |
| | | Antihypotensive Activity |

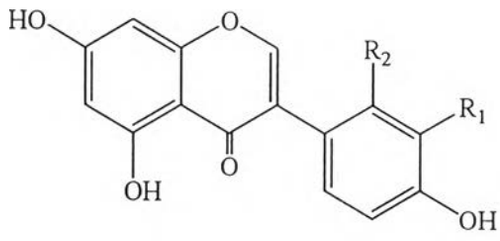
* *Bacillus subtilis*

*Staphylococcus aureus*

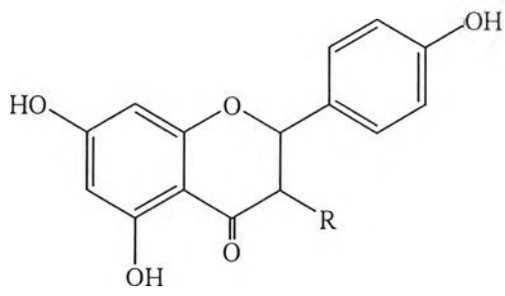
** *Mycobacterium smegmatis*



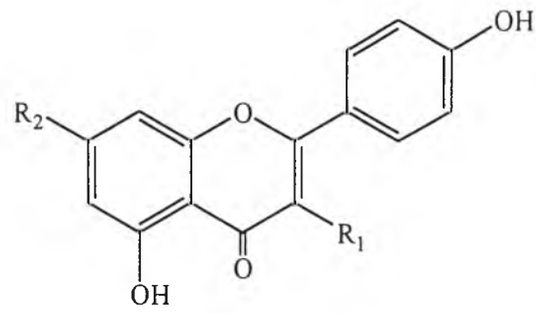

| | R |
|---|---|
| **Weighteone** | H |
| **Artocarpesin** | OH |



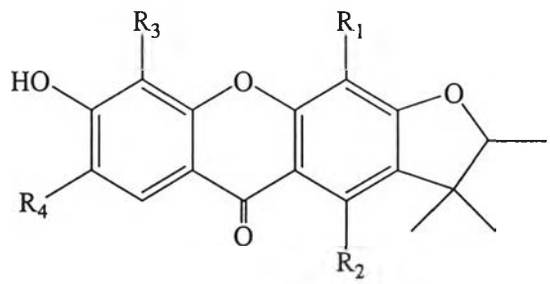

| | $R_1$ | $R_2$ |
|---|---|---|
| **Genistein** | H | H |
| **Orobol** | OH | H |
| **5,7,2'4'-tetrahydroxyflavanone** | H | OH |



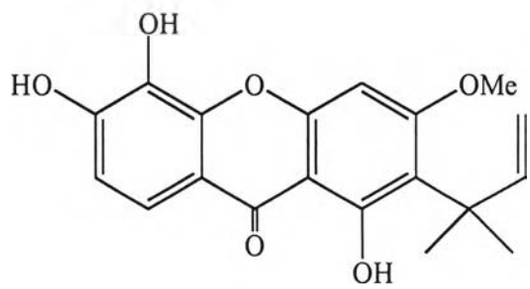

| | R |
|---|---|
| **Naringenin** | H |
| **Aromadendrin** | OH |



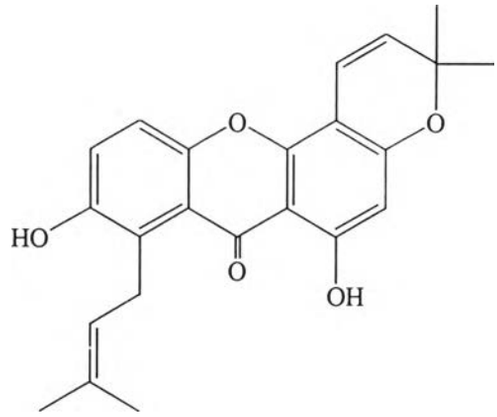

| | R1 | R2 |
|---|---|---|
| **Kaempferol** | OH | OH |
| **Kaempferol-7-glucoside** | OH | glucoside |
| **Kaempferol-3,7-diglucoside** | glucoside | glucoside |



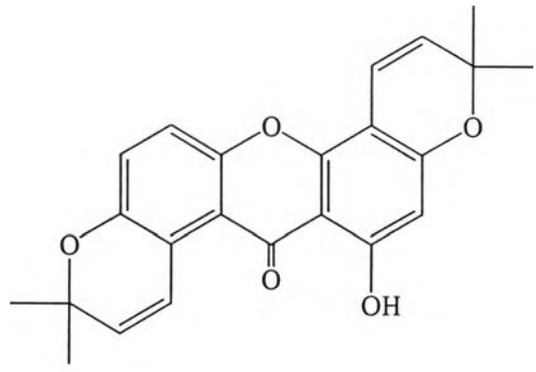

| | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| **Gerontoxanthone C** | OH |  | OH | H |
| **Gerontoxanthone D** | OH | OMe | OH | H |
| **Gerontoxanthone E** | OH | H | OMe |  |
| **Gerontoxanthone G** | OH | H | OH |  |
| **Gerontoxanthone J** | OMe | H | OH | H |



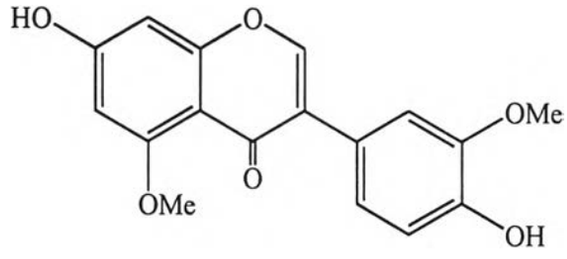

**Cudraniaxanthone**




**Cudraxanthone I**




**Cudraxanthone A**




**Gerontoisoflavone A**

**Figure 1.6** Structures of flavonoids and xanthones from *C. cochinchinensis*[14-19]