# CHAPTER 2

# LITERATURE REVIEW

Nowadays, the use of herbs as a source of medical substances is increasingly developed. Various species of herbs have been cultivated in order to extract substances for medical usage. Considered as a familiar local herb, Bua Bok has been used in herbal medicine for at least 200 years. It has been found that certain substances in Bua Bok have substantial benefits in the treatment of diseases which can be utilized in the medical industry. This paper aims to study the botanical characteristics of Bua Bok, cultivation methods for high productivity, natural chemical content, harvesting and production safety

## 2.1 Bua Bok

### 2.1.1 Botanical Characteristics of Bua Bok

In the journal Flora of Thailand by Hedge and Lamond (1992), the scientific name of Bua Bok is *C. asiatica* (L.) Urban. It belongs to the family Umbelliferea, genus *Centella*. In Thailand, there is only *asiatica* species. It is an annual prostrate-creeping herb with simple leaves, often rooting at nodes and glabrous stems. The circular-reniform leaves are 2 to 6 cm long and 1.5 to 5 cm wide, with crenate margin and five to nine ribs. The petioles are 3 to 30 cm long. The pedicles are 1.2 to 4 cm. long. The sepals of the epicalyxes are oval to circular with a membranous border. They are about 2.5 to 3 mm long and 1.5 to 2.5 mm wide. The umbels have two or three sessile or short pedicle florets. The petals are white to purple or pink (Fig. 2.1 and 2.2). The calyx is not generally dentate. The fruit is oval to globose and has a diameter of 2 to 5 mm. The mericarps are clearly flattened at the sides and usually have seven to nine ribs and are raised rugose. There are many vernacular names of *C. asiatica* in Thailand such as Bua Bok (Central), Pa-na-e-kha-do (Karen-Mae Hong Son), Phak waen (Peninsular) and Phak nok (Northern) Forest Herbrium, 2001).

In the family Umbelliferea in Thailand, there are many genera, but the genus that is close to genus Centella is genus Hydrocotyle. Both genera Hydrocotyle and Centella are prostrate-creeping or ascending herbs with simple leaves, often rooting at nodes. The botanical difference between Centella and Hydrocotyle is that Centella has seven to nine ribbes of mericarp and stipules are absent, but Hydrocotyle has five ribbes of mericarp, leaves stipules, glabrous to densely tomentose.

In Thailand, genus Hydrocotyle has four species, which are (also know as "Phak Nok"), *H. javanica* (also know as "Bua Bok Khao") *H. chiangdoaensis* (also know as "Phak Nok Chiang Doa") and *H. sibthropioides* (also know as "Ya Klet Hoi). Among members of Hydrocotyl, *H. javanica* is most similar to *C. asiatica. H. javanica* can be found in evergreen forests, damp places, and edges of rice fields at a height ranging from 200-2,000 above sea level; the vernacular name is "Bua Bok Khao" (Fig. 2.3). It is considered as an edible plant especially in Chumporn Province, Southern Thailand.

Furthermore *H. umbellate* L., also know as Waen kaeo (The Forest Herbalium, 2001) is an exotic herb in Thailand. It is also similar to *C. asiatica*. The botanical characteristic of *H. umbellate* is that the leaf is round, peltate, succulent dark green leaves, and 1.25 to 5 cm inches long with creeping stems (Fig. 2.4), while Bua Bok's leaf shape is kidney with crenate margin (Fig. 2.2).

Furthermore, *Marsilea crenata* presl is similar to Bua Bok. It grows in shallow fresh water and in dry mud. The plant is rooted in the mud and the leaflets float on the water surface or the erect petioles extend above the surface of the water and hold the leaflets well above the surface (Fig. 2.5).

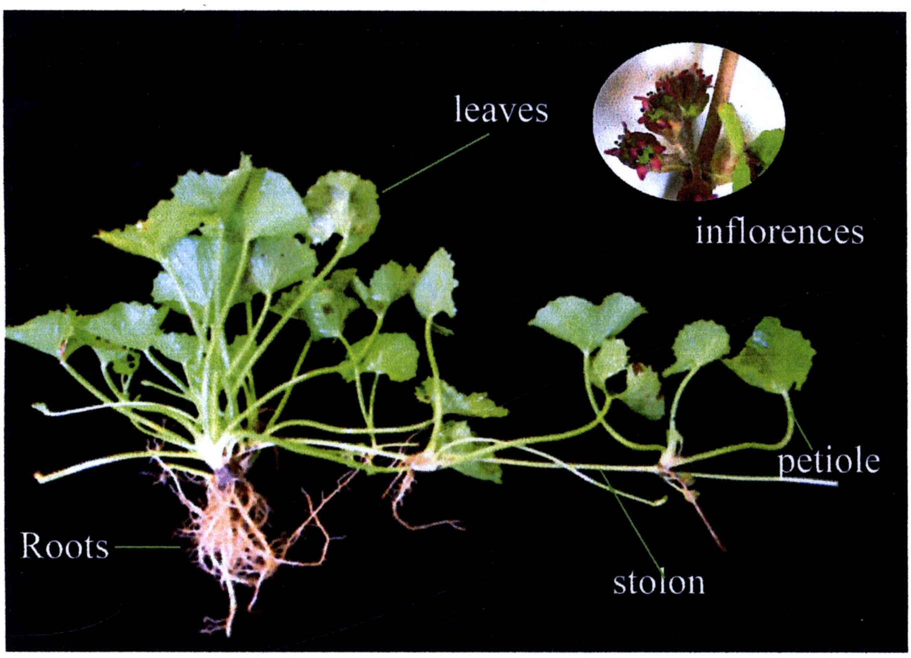



Fig. 2.1 Parts of Bua Bok

Fig. 2.2 The leaves of Bua Bok

Fig. 2.3 The leaves of *Hydrocotyle javanica*, also know as "Bua Bok Khao"

Fig. 2.4 The leaves of *Hydrocotyle umbellate* L., also know as Waen Kaeo (The Forest Herbalium, 2001), which is an exotic herb in Thailand

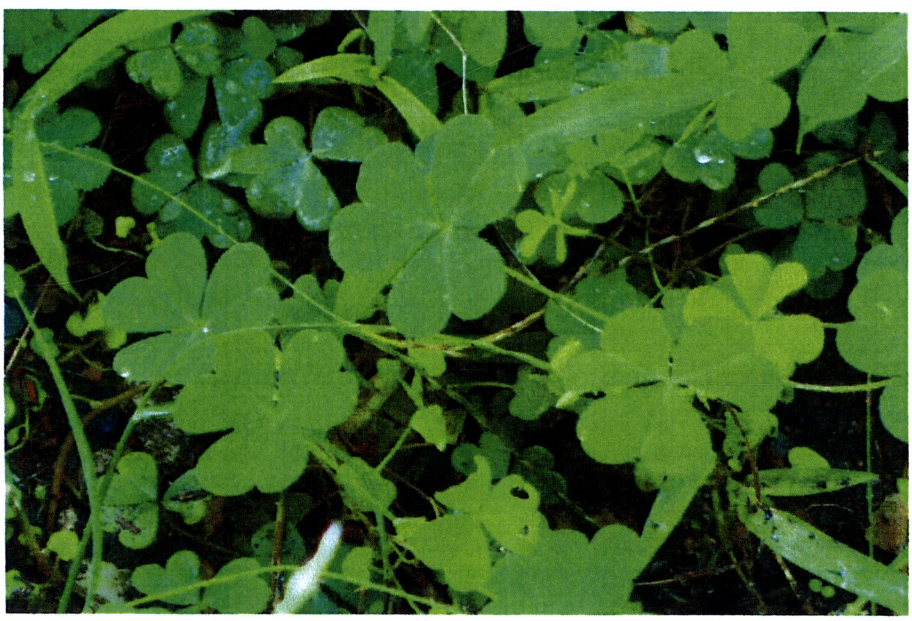


Fig. 2.5 The leaves of *Marsilea crenata* presl., which is in family Marsileaceae

### 2.1.2 Distribution and propagation of Bua Bok

Bua Bok is distributed throughout tropical and subtropical areas (Fig. 2.6). It is found in Southeast Asia countries; India, Sri Lanka, certain parts of China, western South Sea Islands, Madagascar, South Africa, southeast U.S., Mexico, Venezuela, Columbia, and eastern South America. It can grow in humidity, direct sunlight, dim light, or shade as well as from low plains to high plains up to 3,480 meters above the sea level (Hedge and Lamond, 1992).

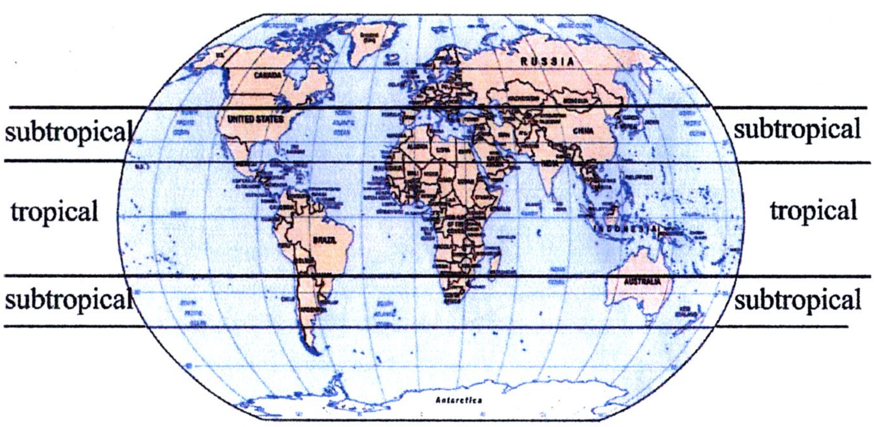



Fig. 2.6 Tropical and sub-tropical zone of the world

Bua Bok can also be propagated by tissue culture technique. . The parts used are immature stalks and leaves. After that, the tissues will be fed with culture medium in order to induce callus before growing into plantlets. Culturing immature stalks in culture medium; Murashige and Skoog (MS) mixed with 1.0 mg/l 2, 4-D (2,4-Dichlorophenoxyacetic acid) + 1.0 mg/l kinetin lead to make embryonic celli. Culturing with mixed 5.0 mg/l NAA (α-naphthaleneacetic acid) + 1.0 mg/l kinetin will result in development of hypocotyls and cotyledon. On the other hand, culturing buds in MS with BA (6-benzylaminopurine), IAA (indole-3-acetic acid) and NAA, results in shoots when mixed with 22.2 μM BA + 2.68 μM NAA, resulting in long shoots when mixed with 6.7 μM BA + 2.88 μM IAA, resulting in long roots, when mixed with 2.46 μM IBA. After transferring to growing area, they continue growing well (Kavinda et al., 2000). In addition, culturing buds in MS mixed with sucrose 30 g/l and casein hydrolysis 500 g/l and subculture every 14 days in flask rotation under 24°C and 16 hours light, so it will get cell suspension which can be found thiocolchicine in seven days of cell suspension (Solet et al., 1993).

Fig. 2.7 Seedling from seed of Bua Bok

Fig. 2.8 Cultivation by cutting stolon of Bua Bok, 1 month after cutting

### 2.1.3 The importance and advantages of Bua Bok

#### 2.1.3.1 Vegetable and nutritional

Bua Bok is an annual crop that many Thai people like to consume as a side dish with chili sauce or spicy minced meat salad; or take as a medicine for an apthous ulcer or other diseases. Today more annual crops are utilized in pharmaceuticals and adapted into different health products, such as Bua Bok juice Bua Bok pellets, Bua Bok tea, Bua Bok tea powder juice, Bua Bok shampoo, etc. Bua Bok use is popular especially in Europe. Bua Bok has high vitamin B1 content essential for nervous system, cardiovascular and muscular function (Shukla et al., 1999). Thai traditional medicine uses Bua Bok to cure many diseases especially keloids and bruises.

Reports on the nutritional value of Bua Bok from overseas and in Thailand vary. According to the information on herbs in Southeast Asia (de Padua et al., 1999), 100 g of fresh edible Bua Bok consists of water 88 g, protein 2 g, fat 0.2 g, carbohydrate 7 g, fiber 1.6 g calcium 170 ml, potassium 32 ml, iron 6 ml, pro-vitamin A 4.5 ml., and vitamin C 49 ml. Duke (1981) analyzed nutrients in 100 g of Bua Bok's leaves, which consisted of energy 34 calories, water 89.3 g, protein 1.6 g, fat 0.6 g, carbohydrate 6.9 g, fiber 2 g, ash 1.6 g, calcium 170 ml, phosphorus 30 ml, iron 3.1 ml, potassium 414. ml, beta-carotene 6.58 ml, thiamin 0.15 ml, riboflavin 0.14 ml, niacin 1.2 ml and vitamin C 4 ml. In Thailand, it is reported that the nutrition value of Bua Bok 100 g consists of energy 44 calories, water 86 g, protein 1.8 g, fat 0.9 g, carbohydrate 7.1 g, fiber 2.6 g, ash 1.7 g, calcium 146 ml, phosphorus 30 ml, iron 3.9 ml, total vitamin A 10962 IU, thiamin 0.24 ml, riboflavin 0.09 ml, niacin 0.8 ml and vitamin C 4 ml (Division of Nutrition, 1992).

**2.1.3.2 Herbal value of Bua Bok**

Bua Bok has been used as a traditional herbal medicine in Asian countries for more than 200 years. In the 1800s, it was indicated in Indian medicinal practice to be used for the treatment of many skin conditions including leprosy, varicose ulcers, and eczema, as well as fever, digestive disorders, and the absence of menses. In China, Bua Bok was popular as an agent for longevity. It had also been used to treat syphilis, hepatitis, stomach ulcers, mental fatigue, epilepsy, and asthma. At present, American and European herbalists use Bua Bok for disorders that cause connective tissue swelling, such as scleroderma, psoriatic arthritis, anklylosing spondylitis (arthritis of spine), and rheumatoid arthritis. In traditional Thai medicine, the fresh herb of Bua Bok was reputed as a diuretic. It also is used as a tonic and to treat dysentery (National Identity Bord, 1991). In Thailand the popularity of Bua Bok as a vegetable and soft drink is certainly related to its medicinal properties. Recent studies confirm some of the traditional uses and suggest possible new applications for Bua Bok. This are: lowering high blood pressure, treating venous insufficiency, boosting memory and intelligence, easing anxiety and speeding wound healing. Bua Bok has been found to be a good herbal remedy with antimicrobial, anti-inflammatory and sedative properties. The wound healing and anti-inflammatory properties of Bua Bok led to research to see if it has any protective effect on gastric ulcer formation. There were several reports supporting the antiulcer effect of Bua Bok. Oral administration of Bua Bok extracted (0.05 g/kg, 0.25 g/kg and 0.50 g/kg) before ethanol administration in male Sparague-Dawley rats significantly inhibited gastric lesion formation and decreased mucosal myeloperoxidase (MPO) activity (Cheng and Koo, 2000).

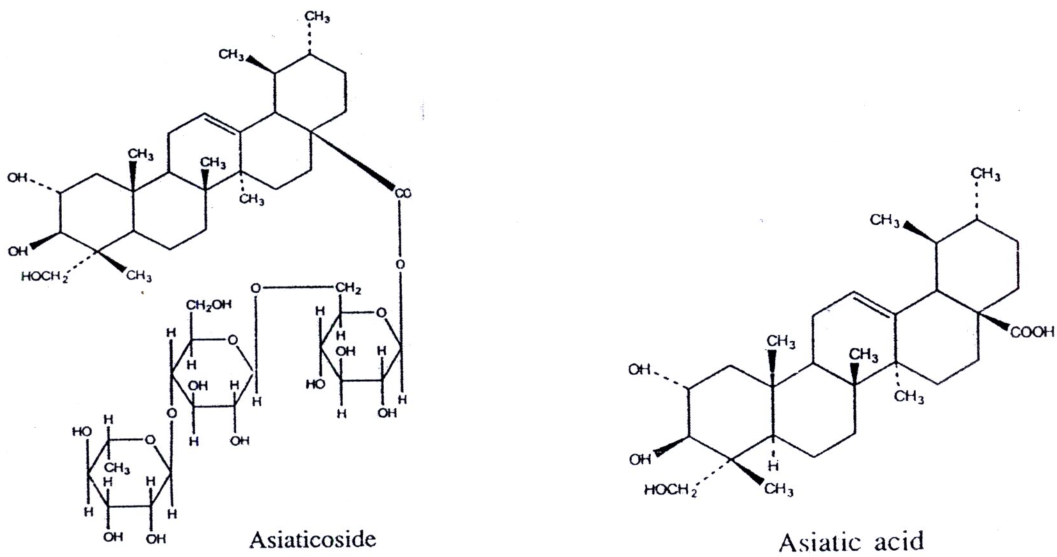
## 2.2 Chemical Compounds in Bua Bok

The major principles in Bua Bok are the triterpenes asiatic acid and madecassic acid, and their derived triterpene ester glycosides, asiaticoside, and madecassoside (World Health Organization, 1999: Collins et al., 1992; Sung et al., 1992). The amount of substances is found between 1-8% depending on the source of raw materials. In addition, the aerial parts of Bua Bok contain 0.1% of essential oil which composed of 80% sesquiterpenoids such as β-caryophyllene, α-humulene and germacrene-D, elemene and bicycloelemene, trans-farnesene (Wong and Tan, 1994). In Addition there are Kaempferol-3-glucoside and quercentin-3-glucoside (Newall et. al., 1996), stigmasterol and sitosterol (Ramaswamy et al., 1970) and amino acid. Free amino acids found in the leaf and stem are glutamate, serine, alanine. Amino acid mostly found in the root are aspartate, glutamate, serine, theonine, alanine, lysine, histidine and amino butylate (Pramongkit, 1995; Newall et. al., 1996).

## 2.3 Asiaticoside

### 2.3.1 Structure and properties

The chemical name of asiaticoside is (2α,3β,4 α)-2,3,23-Trihydroxyurs-12-en-28-oic acid o-6-deoxy-a-L-mannopyranosyl-(1->4)-o-β-D-glucopyranosyl-(1->6)-o-β-D-glucopyranosyl ester (Pramongkit, 1995). Molecular formula; $C_{48}H_{78}O_{18}$ has formula weight equal with 959.12 and solubility in propylene glycol or ethanol. The structure of asiaticoside is composed of 2 parts; aglycone, which is sapogenin named asiatic acid and glycone, which has 2 glucose molecules and 1 rhamnose molecule (Fig. 2.9). Besides, the synthesis process of asiaticoside in Bua Bik has not been clearly identified yet. On the other hand, it is clearly that asiaticoside is a group of saponin glycoside, specifically triterpenoid and its main structure, aglycone is asiatic acid, so the synthesis process of asiaticoside is relevant to the synthesis process of triterpenoid saponin in the plant.




(A) (B)

Fig. 2.9 Structure of asiaticoside (A) and asiatic acid (B)

**2.3.2 Advantages of Asiaticoside**

There were a great number of studies on pharmacological actions of asiaticoside with experiments on both *in vitro* and *in vivo*. Specifically, the action is wound healing. It was found that asiaticoside from Bua Bok can heal wounds more quickly by using an asiaticoside solution at 0.2% and 0.4% with wounds on guinea pigs and streptozin diabetic rats respectively. This causes tensile stress and increases collagen; the skin regrows quickly. It is also found that giving 1 mg/kg asiaticoside orally to the guinea pigs quickly healed the wounds. Cheng et al. (2004) studied the action of Bua Bok and asiaticoside liquor for preventing gastric ulcers that are caused by acetic acid (kissing ulcer) in rats. They found that administering Bua Bok and asiaticoside liquor orally could decrease the size of wounds. Asiaticoside is also a neuroprotective. According to the study on asiaticoside influence on learning and memory of rats riding bicycles, which were given an injection of β-amyloid protein into the space of their brains, the protein disabled the rats' learning and memory. However, orally administering 5, 10 and 25 mg/kg of asiaticoside per day to rats for a week before β-amyloid protein injection could prevent the decay of short-time memory in Y maze and long-time memory in water maze, which caused from β-amyloid protein. It did not change the rats' pattern of behavior in movement and surveying. Moreover, asiaticoside can decrease oxidative stress in the rat's brain (Salout, 2003) and it is antioxidant, immonomudulatory, antimicrobial, and antiviral

**2.3.4 Variation in asiaticoside content in Bua Bok**

Natural products are an unsurpassed source of bioactive compounds and constitute a relevant economic resource for the pharmaceutical, cosmetic and food industry. Differences between varieties in medicinal plants of the same species (chemotypes) are common and variations in secondary metabolites have been observed with identical phenotypes and growth conditions, depending on plant origin (Aziz at al., 2007).

Gupta et al. (1999) reported variable asiaticoside content in five lines of Bua Bok from India. Similarly, Rouillard-Guellec et al. (1997) investigated the secondary metabolites in India and Madagascar, and reported that plants from the latter contained the highest level of asiaticoside. The distribution of asiaticoside and madecassoside throughout the plant was organ specific with leaves of both lines containing a higher content of these compounds. In a study of Bua Bok from Madagascar, asiaticoside content of between 2.6 and 6.42% dry weight was reported (Randriamampionona et al., 2007). Aziz et al. (2007) reported two phenotypes of *C. asiatica* exhibiting differences in terpenoid content that were tissue specific and varied between glasshouse grown and tissue derived material. Triterpenoid saponin content was highest in leaves (asiaticoside and madecassoside concentrations of 0.7-0.9 and 1.1-1.6% dry weight were, respectively, reported) and roots contained the lowest content of asiaticoside.

In particular, the asiaticoside content in plants showed different responses to the variations in light intensity. In addition to the light effect on the efficiency of a medicinal plant to produce secondary metabolites the influence of the plant ontogenetic stage is also very important

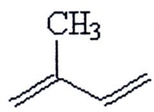
## 2.4 Nutritional in Leafy Vegetable

Leafy green vegetables are popular around the world and especially in Asia. They are quick growing crops that are harvested within four to six weeks. The vegetables are valuable sources of vitamins A and C, iron, calcium, folic acid, and dietary fiber. The content of nutrients in plants is dependent on growing conditions (Karmas and Harris, 1988)

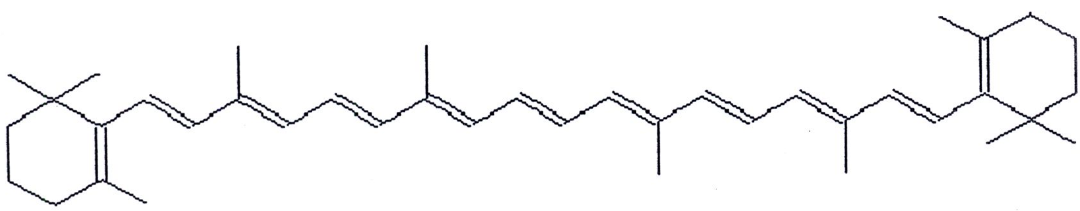
### 2.4.1 Beta-carotene

Beta-carotene is an organic compound abundant in fruit and plant. It is a carotenoid substance naturally found in plants that serves as an accessory to photosynthesis. Beta carotene is primarily responsible for the pigment in orange colored fruits and vegetables, but also contributes to the pigment in red, yellow, and green colored fruits and vegetables. Though some food sources are rich in beta carotene, including cantaloupe, broccoli, spinach, and palm oil, carrots are the major supplier of beta carotene in most people's diets (Beam, 2009).

The structure of beta-carotene is made up of eight isoprene units, which are cyclic at each end (Fig 2.10). Beta-carotene absorb light most strongly wave length between 400-500 nm. It is green/blue part of the spectrum. So beta-carotene appears orange, because the red/yellow colors are reflected back to us (Evan, 2007).




Isopene



Beta-carotene

Fig. 2.10 Structure of isoprene and beta-carotene

**Beta-carotene biosynthesis**

Beta-carotene is a member of caroteniods, their biosynthesis involve carotenoid biosynthesis pathway. Carotenoids carry out several important functions, such as the stabilization of lipid membrane (Havaux, 1998) and high collection for photosynthesis, as well as protecting the photo system from photo-oxidation by reactive oxygen species produced by the excited triplet state of chlorophyll during photosynthesis (Frank and Cogdell, 1996; Ledford and Niyogi, 2550). The protective function of carotenoid within the photosynthesis induced by a mutation such as the tomato Ghost mutation (Josses at al., 200), or by herbicides such as norflurazon (Simkin et al., 2000), result in serve photobleaching (Sandman and Böer, 1989). The carotenoids biosynthesis pathway has been intensely studied since the early 1960s (Sandman et al., 2006). While carotenoids are synthesized within the plastid compartment of the cell, the corresponding genes are located in the nucleus and their protein products are imported into the chloroplast. Chlorophyll accumulates beta-carotene and xanthophylls, which are primarily located in the photosynthetic membranes where they have been found to be associated with the light-harvesting complexes and reaction centre (Thayer and Björkman, 1992; Ruban et al., 1994 Ruban et al., 1999 ; Telfel, 2002).

The carotenoid biosynthetic pathway is presented in figure 2.11. Beta-carotene is synthesized from isopentenyl diphosphate (produced in plastids from pyruvate and glyceraldehyde-3-phosphate). Isopentenyl diphosphate is converted to gernayl gernayl diphophate (GGPP). With the condensation of two GGPP, the cell is committed to produce beta-carotene (as well as the retinal carotenoids lutein and zeaxanthin). Phytoene synthase desaturates the GGPP to form the phytoene. Phytoene desaturase adds additional double bonds in the formation of lycopene (Ye et.al., 2000). When lycopene is formed there is a branch in the pathway that leads either to the lutein or to the beta-carotene (Schalch et.al., 1999).

Beta-carotene can then be converted to zeaxanthin by two successive hydroxylation steps, while alpha-carotene is converted in two steps to lutein (Sandman, 1994; Tian et al., 2004). Zeaxanthin is epoxidized by the enzyme zeaxanthin epoxidase (ZEP) in two steps to give antheraxanthin and violaxanthin (Bouvier at al., 1996; Marin et al., 1996). Finally, the last step in carotenoid synthesis in higher plants is the formation of neoxanthin from violaxanthin by a reaction catalysed by neoxanthin synthesis (NAXS; Al-Babili at al., 2000; Bouvier at al., 2000). In high light, violaxanthin can be converted back into antheraxanthin and zeaxanthin by the activity of violaxanthin de-epoxidase (VDE)

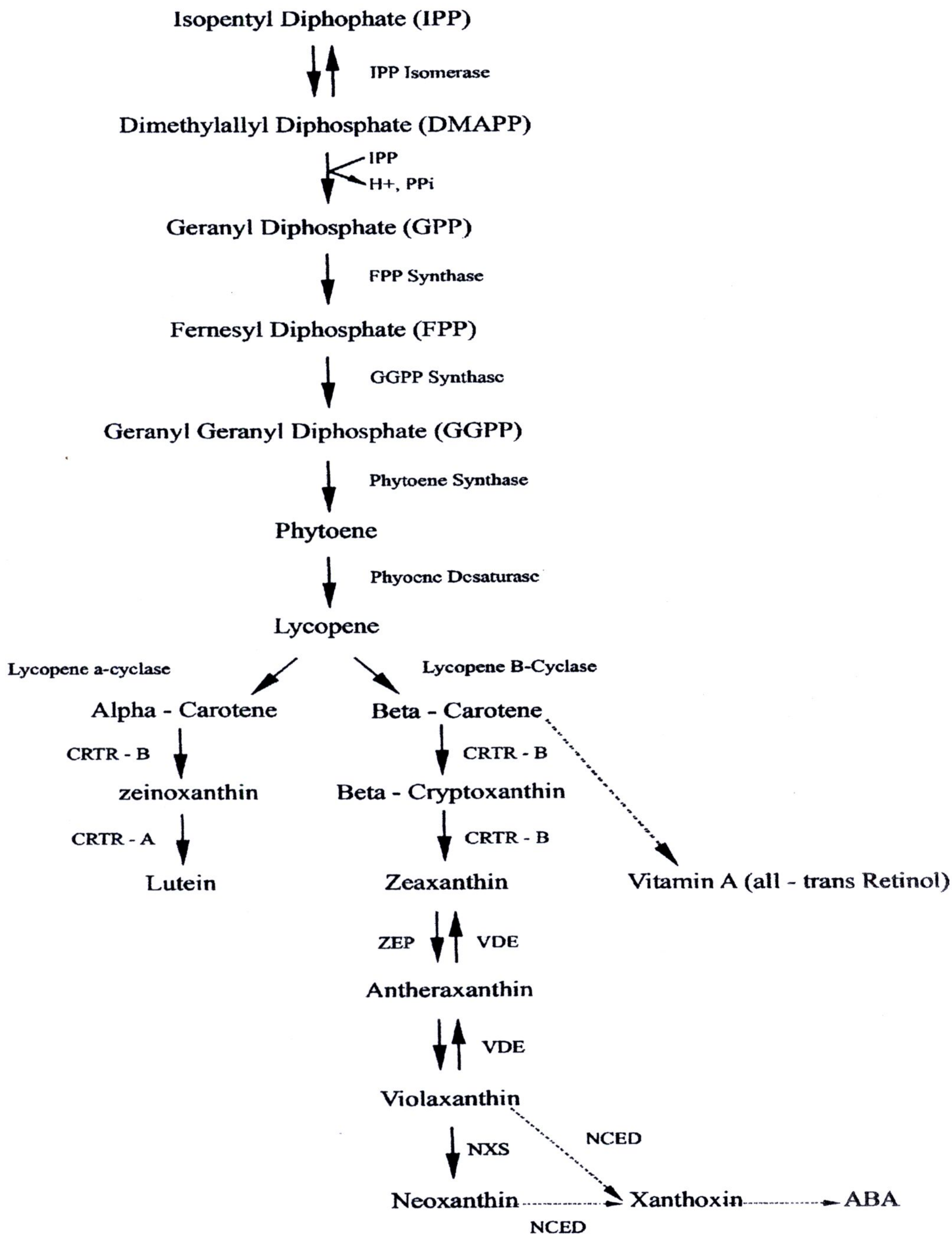



Fig 2.11 Diagram of the biosynthesis of isopenoid. IPP Isomerase, Isopentyl diphosphate isomerase; FPP synthase, Fernesyl diphosphate synthase; GGPP, Geranyl geranyl diphosphate synthase; CRTR-B, Beta-carotene hydroxylase; CRTR-E, ε-carotene hydroxylase; ZEP, Zeaxanthin epoxidase; Violaxanthin de-epoxidase; NXS, Neoxanthin synthase; NCED, 9-cis epoxycarotenoid dioxygenase. (Resource: Modified from Simkin et al., 2007; Qin et al., 2007).

**Useful of beta-carotene**

Beta-carotene can be into vitamin A in the body. It is very effective in neutralizing a highly reactive for of oxygen called single oxygen. Low level of beta-carotene in their blood has a higher incidence of heart disease and cancer, particularly lung cancer. The national Cancer Institute endorsed a study which found that beta-carotene lower change of getting cancer, including breath cancer (Abl Biotechnologies Limited, 2009). Natural beta-carotene have been used not only as a source of vitamin A but also for reducing cholesterol and lipid level, protect against strokes and cataracts, enhancing the immure system, preventing certain photo sensitivity disorders, improving antioxidant action and intra cellular communication, quenching singlet oxygen and dramatically reducing stress (Abl Biotechnologies Limited, 2009).

**Factor affecting on beta-carotene accumulation in plants**

Oyama et al. (1999) studied effects of temperature and light intensity on beta-carotene concentration in spinach and lettuce. In spinach, control and low (day/night) temperatures were 20/15°C and 15/8°C, respectively, where as for lettuce, they were 21/16°C and 16/10°C, respectively, and grown under three different light intensities 100%, 70% and 50% of transmittance of light. The beta-carotene increased in high temperature and high light intensity, but they were not clear because there were differences in growth among the treatments. On fresh weight basis, beta-carotene concentration increased in plants exposed to low air temperature and/or high light intensity. Thus low air temperature and/or high light intensity are valuable factors for increasing beta-carotene concentration in leaf vegetables because they are nutrition and are sold fresh. Liu et al. (2009) found that the beta-carotene content in Sweet potato (*Ipomoea batatas* (L.) Lam.) in Taiwan depend on crops harvested at various times.

### 2.4.2 Calcium

Calcium is the important nutrient both plant and human. In Human, calcium is important for bone health as well as many other bodily functions like muscle contraction and brain function. In plant, calcium is an essential plant nutrient. It is taken up by the root system and translocation to the aerial part of plants, like leaf, petiole and fruit, by symplastic and apoplastic transport via the xylem system (White, 2001). Calcium is almost completely immobile in the phloem and, since it cannot be retranslocated from leaves following deposition (Biddulph, 1953; Swanson and Whitney, 1953), will accumulate over time at the end of the transpiration stream. Calcium is required in large amounts by the plant and accumulation can exceed 0.1–1% dry matter (White, 2001; White and Broadley, 2003). It is a major component of the cell wall, binding pectin molecules together. In the cytosol, calcium is central to intracellular signalling, whereas, in the vacuole, the accumulation of calcium salts can represent a significant contribution to osmotic homeostasis (Fricke et al., 1995). In grasses, a dominant transpirational flow along the leaf can lead to high calcium accumulation at the leaf tip and solutes within the transpiration stream accumulate at the epidermis, specifically around the stomata (Fricke, 2004). In fleshy fruits, calcium deficiency can case physiological disorders such as bitter-pit in apple, blossom –end rot in tomato, tip-burn in strawberry or melon watercourse (Shear, 1975; Wills et al., 1998).

The different geometries of dicotyledonous leaves result in different patterns of xylem water flow across the leaf and potentially different patterns of ion accumulation.

### 2.4.3 Fiber

The fiber content and composition, however, varies depending on the fruit or vegetable maturity, type, growing environment, etc. Vegetables harvested at younger growth stage are likely to contain higher pectin and hemicellulose (soluble fibers) and less cellulose and lignin (insoluble fibers) than when harvested at later growth stages. Generally, the concentration of the insoluble fiber components increases with maturity of the vegetable or fruit, where as the reverse is true to the soluble fiber components.

### 2.4.3 Protein

In green plants, in order to create proteins it will pull nitrogen compound and other minerals from soil by roots and pulling Carbon dioxide from the air by their leaves. The plants can create amino acids that having all 20 types of amino acids. Amino acids that animals cannot create are called essential amino acid. Therefore, animals must have these amino acids by eating plants directly or indirectly by eating animals that eat plants. Proteins in plants (plant protein) can categorize into 2 types which are protein that is a component in plant leaves (leaf protein) and protein components in the seed (seed protein). The majority will consist of true protein, and the non-protein nitrogen in the form of peptides, free amino acids, amides, amines, nucleotides, ureides and inorganic in different proportions. It was found that 75% true protein of the leaf protein is found in all parts of the chloroplast (Mangan, 1982), Protein in plant leaves about 50% is soluble. The other 50% cannot be solvable (insoluble). For protein in grain will feature true protein of approximately 75-85% of total crude protein, similar to the plant leaves. Therefore, in both leaf and seed plants consists of non-protein nitrogen by approximately 15-25%, depending on age, type, species, level of fertilizers, density of sunlight, wavelength of light, and the amount of water (Hegart and Peterson, 1973).

## 2.5 Factors Effecting of Nutritional and Secondary Metabolite on Leafy Vegetable

Secondary metabolites are natural products that often have an ecological role in regulating the interactions between plants and their environment. They can be defensive substances, such as phytoalexins and phytoanticipins, anti-feedants, attractants and pheromones (Hanson, 2003). The importance of plant secondary metabolites in medicine, agriculture and industry has led to numerous studies on the synthesis, biosynthesis and biological activity of these substances. It has been estimated that over 40% of medicines have their origins in these active natural products (Gershenzon and Kreis, 1999). A prominent group of natural products are the terpenes and derivitized terpenoids. Among the environmental factors, the effect of light is very important as a consequence of its significant and complex mechanism of action on the metabolism, and influencing the accumulation and quality of the main ingredients (Bernath and Tetényi, 1978).

Each plant has certain environmental requirements. To attain the highest potential yields a crop must be grown in an environment that meets these requirements. A crop can be grown with minimal adjustments if it is well matched with its climate or growing conditions. Unfavorable environmental conditions can produce stress on plants resulting in lower yields. In such cases the environment can be artificially modified, such as in greenhouses, to meet crop requirements. The environmental conditions involved in plant growth are temperature, water content, humidity, light etc.

All light is made up of energy. Light to plants is the wavelengths of the electromagnetic spectrum including the wavelengths that humans can see (visible light) and some wavelengths that humans can't see (such as microwaves and infrared light). Light for the plant is not only used as an informational medium, but also for producing food through the process of photosynthesis. The characteristics of direction and spectral composition of light in the plant's environment is transferred to the plant through the interception and activation of pigment systems within the leaf. This information affects the morphological development (size/proportion of root and shoots) of the plant, hopefully imparting to the plant some type of ecological or physiological advantage for survival. Plants also use light for sensing and detecting competitors and keeping track of time (Decoteau, 1988)

Three principal characteristics of light affect plant growth: quantity, quality, and duration. Light quantity refers to the intensity, or concentration, of sunlight. It varies with the seasons. Up to a point, the more sunlight a plant receives the greater its capacity for producing food via photosynthesis. Light intensity is a major factor governing the rate of photosynthesis. The quantity or amount of light received by plants in a particular region is affected by the intensity of the incident (incoming) light and the length of the day; the intensity of light changes with elevation and latitude. The amount of sunlight also varies with the season of the year and time of day as well as other factors, such as clouds, dust, smoke or fog. Plants have varying preferences for light intensity. The light saturation point of the plant determines the relative light requirement. The light saturation point is the point above which an increase in light intensity does not result in an increase in photosynthetic rate. Crops such as corn, cucurbits, legumes, potato, and sweet potato require a relatively high level of light for proper plant growth, while onions, asparagus, carrot, celery, cole crops, lettuce and spinach can grow satisfactorily with lower light intensity (Decoteau, 1988).

One of the main roles of light in the life of plants is to serve as an energy source through the process of photosynthesis. Using water and carbon dioxide through photosynthesis, plants produce the foodstuffs (photosynthates) necessary for growth and survival. Subsequently, carbohydrates (starches and sugar) and stored chemical energy are produced during this biochemical process in plants. Plants capture the energy in light using a green pigment called chlorophyll. A very precise number of photons at specific wavelengths (near 680 nm) are required to split a water molecule ($H_2O$) within the green leaf, which releases oxygen ($O_2$), and provides chemical energy to continue the long biochemical process to produce more complex molecules such as carbohydrates. Carbon dioxide from the air and water from within the leaf combine to produce oxygen and photosynthates. Photomorphogenesis is defined as the ability of light to regulate plant growth and development, independent of photosynthesis. Plant processes that appear to be photomorphogenic include internode elongation, chlorophyll development, flowering, abscission, lateral bud outgrowth, and root and shoot growth. Photomorphogenesis differs from photosynthesis in several major ways. The plant pigment responsible for light-regulated growth responses is phytochrome. Phytochrome is a colorless pigment that exists in plants in very small amounts. Only the red (600 to 660 nm) and far red (700 to 740 nm) wavelengths of the electromagnetic spectrum appear to be important in the light-regulated growth of plants. The wavelengths involved in generating photosynthesis are generally broader (400 to 700 nm) and less specific. Photomorphogenesis is considered a low energy response - meaning that it requires very little light energy to get a growth-regulating response. Plants generally require greater amount of energy for photosynthesis to occur (Decoteau, 1988).

Low light intensity stress usually inhibits plant growth and productivity by affecting gas exchange (Wei et al., 2005; Gregoriou et al., 2007). Leaf area and chlorophyll content increased (Wei et al., 2005; Gregoriou et al., 2007), but leaf thickness and stomatal size decreased (Wilson and Cooper, 1969; Huang et al., 2004) in response to low light intensity environments. For example, biomass accumulation, non-structural carbohydrate content, and $CO_2$ assimilation rates were highly reduced at low radiation levels in *Grindelia chiloensis* (Zavala and Ravetta, 2001). Gregoriou et al. (2007) found that shaded leaves had increased leaf area and chlorophyll content, but decreased leaf thickness and stomatal density in *Olea europaea*. However, the mechanisms involved in physiological responses and growth acclimation of Bua Bok, in an environment of light intensity have not been clarified.

Light also influences the biosynthesis of secondary metabolites (Zavala and Ravetta, 2001; Coelho et al., 2007). In general, the resource availability hypothesis (Bryant et al., 1983), predicted a decline in allocation to secondary metabolites (Bryant et al., 1985; Bryant, 1987) when carbon gain was limited relative to nutrient availability, as during periods of low irradiance. This prediction was based on the effects of environmental conditions on carbon-nutrient balance (Zavala and Ravetta, 2001). Secondary metabolites might increase (Chauser-Volfson and Gutterman, 1998; Ralphs et al., 1998; Coelho et al., 2007) or decrease (Gershenzon, 1994; Zavala and Ravetta, 2001) under low light intensity conditions in some plants. For example, low light intensity increased methylxanthine-content in leaves of Ilex paraguariensis (Coelho et al., 2007), but decreased resin content in leaves of *Grindelia chiloensis* (Zavala and Ravetta, 2001). The major bioactive components of *G. uralensis*, saponins (mainly glycyrrhizic acid) and flavonoids (mainly liquiritin), were generally accumulated in the tissues of roots and rhizomes (Afreen et al., 2005; Zhao et al., 2006; Shen et al., 2007).

Information regarding the physiological traits of Bua Bok, such as growth properties and secondary metabolite responses, to environmental conditions such as light intensity is very limited.

Correct harvesting is also important to accumulation of nutrition and secondary metabolite. If vegetables are not harvested at the proper stage of maturity, physiological processes occur that permanently change their taste, appearance and quality. The texture, fiber and consistency of all vegetables are greatly affected by the stage of maturity at harvest, by post-harvest handling and by the time interval between harvesting and serving. While harvesting early may result in only a reduction in yield, harvesting too late can result in poor quality due to development of objectionable fiber and the conversion of sugars into starches. A late harvest can also cause plants to terminate, or stop producing as they complete their reproduction process (Westerfield, 2008).

Bua Bok is present in many commercial phytotherapeutic preparations but little is known about its agronomic management. So the present study aimed to determine the effects of leaf maturity, light intensity on nutrition and asiaticoside in Bua Bok. Our results will be used to determine whether suitable harvest time and light control might increase nutrition and asiaticoside accumulation of Bua Bok in organic farming systems.