CHAPTER II

Literature review

2.1 Plant prenylated aromatic compounds

Prenylated aromatic compounds are a group of secondary metabolites that have been found ubiquitously in plant kingdom, particularly the family of Leguminosae, Moraceae, Umbelliferae, and Rutaceae (Botta, et al., 2009; Botta, et al., 2005; Epifano, et al., 2007). Diversity of compound structures is due to various precursors and prenyl side chain in prenylated aromatic biosynthesis. . According to the structure, these compounds can be classified into three types which are prenylated flavonoids, prenylated xanthone, and prenylated quinone.

2.1.1 Prenylated flavonoids

Prenylated flavonoids (Figure 1) are a group of secondary metabolites found in plants and bacteria and their structures contain prenyl moieties on the flavonoid nucleus. Generally, these compounds have been found in many prenylated derivative forms after binding to flavonoids such as flavones, flavonols, flavanones, flavanonols, isoflavones, isoflavanones, isoflavans, and chalcones. An example of prenylated flavanone, Sophoraflavanone G **(1),** was isolated from root of *Sophora flavescen* and showed antibacterial activity and inhibitory activity against cycloxigenase 1 (COX1), 5-lipoxigenase (5-LOX), and tyrosinase (Cha, et al., 2007; Kim, et al., 2002; Son, et al., 2003; Tashiro, et al., 2001). Papyriflavonol A **(2)** is a prenylated flavonol isolated from 6 *Broussonetia papyrifera* root that exhibited anti-tyrosinase and anti-5-lipoxigenase (5-LOX) activities (Lee, et al., 2004; Son, et al., 2001; Zheng, et al., 2008). Artelastin **(3)** was extracted from wood bark of *Artocarpus elasticus* and showed broad range inhibitory activities of reactive oxygen species

(ROS) and nitric oxide (NO) production, lymphocyte proliferation, and DNA replication in MCF-7 human breast cancer cell line (Cerqueira, et al., 2008; Cerqueira, et al., 2003; Pedro, et al., 2005). Kuwanon G **(4)** is a prenylated derivative of isoflavone has been isolated from root bark of *Morus alba*. This compound has biological activities as antibacterial agent against oral pathogens and nitric oxide production inhibitor (Cheon, et al., 2000; Park, et al., 2003). Ganconin Q **(5)**, 6-prenylapigenin **(6)**, and 8-prenylapigenin **(7)** are prenylated flavones have been extracted from genus *Dorstenia* and it has been reported that they showed cytotoxic activity and inhibited cancer cell proliferations (Kuete, et al., 2011; Wang, et al., 2006).

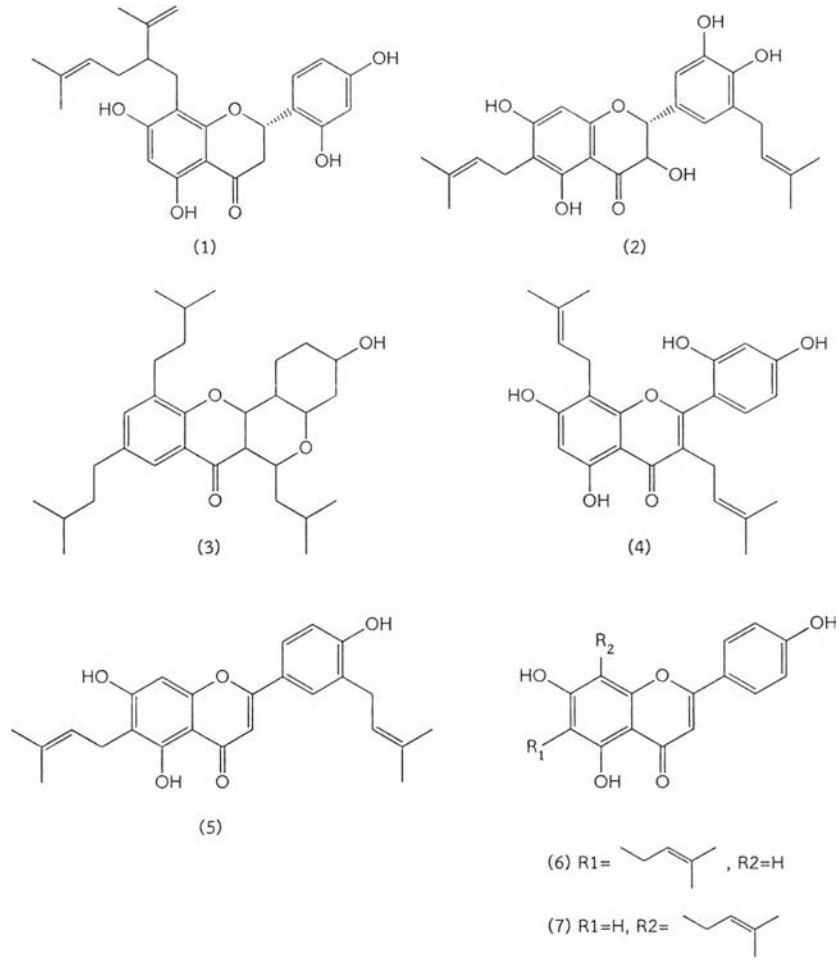

Figure 1 The example of plant prenylated flavonoids.

2.1.2 Prenylated xanthones

Prenylated xanthones are a group of xanthones that having prenyl moieties attached to different positions of xanthone structure. The prenylated xanthones were found in ethanol extract from leaves of *Garcinia griffithii* pericarb consisting of garcinones C **(8)**, garcinones D **(9)**, garcinones E **(10)**, gartanin **(11)**, xanthone I **(12)**, and γ- mangostin **(13)**, and also Rubraxanthone **(14)** (Figure 2). They showed cytotoxicity 8 against human cancer cells (Chen, 2002; Xu, et al., 2014) and inhibitory effects on platelet-activating factor (PAF) (Alkadi K. A. A, et al., 2013; Jantan, et al., 2002).

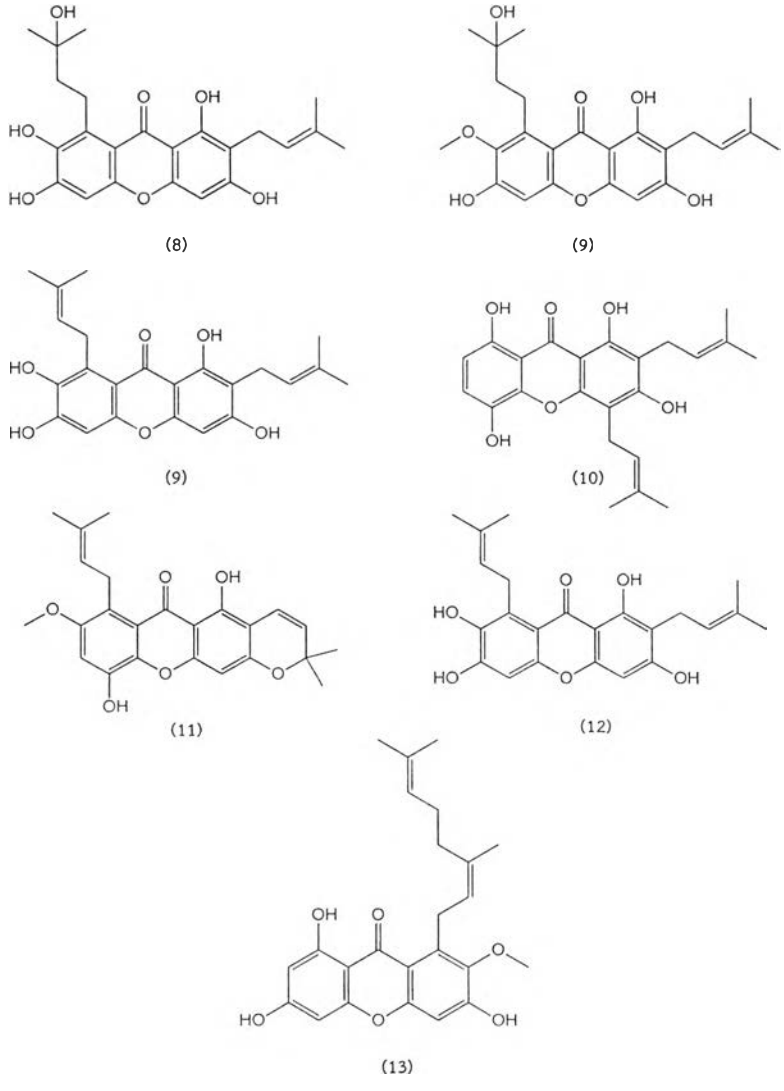

Figure 2 The example of plant prenylated xanthones.

2.1.3 Prenylated quinones

In higher plants, prenylated quinones (Figure 3) are a group of aromatic compounds consisting of prenyl moieties attached to an aromatic ring e.g. 4-hydroxybenzoate (4-HB) and homogentisate (HGA). The phylloquinone (vitamin K1) **(14)**, plastoquinone **(15)**, ubiquinone **(16)** and tocochromanol (tocopherol **(17)** and tocotrienol **(18)**) belong to lipoquinones that are important for electron transport system in photosynthetic organelle (Biggins and Mathis, 1988; Nowicka and Kruk,

2010; Pshybytko, et al., 2008). The tocopherols (α-, γ-, β- and δ- form) have inhibitory activity against cancer cells by upregulating the mRNA and protein expressions of cleaved-caspase 3, peroxisome proliferator activated receptor γ (PPAR-γ), and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and also decreasing the gene expression of interleukin 8 (IL-8) (Smolarek, et al., 2013; Soo, et al., 2004; Stone, et al., 2004; Zingg, et al., 2013).

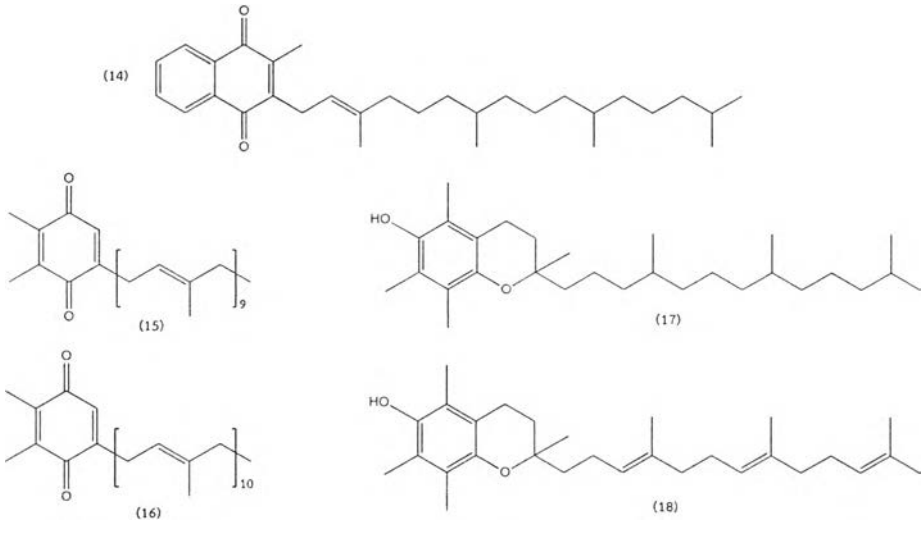

Figure 3 The example of plant prenylated quinones.

2.2 Plant aromatic prenyltransferase

The aromatic prenytransferases (PTases) are groups of catalytic enzymes involved in prenylation reaction by transferring an isoprenoid molecule in form of allylic isoprenyl diphosphate such as dimethylallyl pyrophosphate (DMAPP), isopentyl pyrophosphate (IPP), geranylgeranyl pyrophosphate (GGPP) or phytyl pyrophosphate (PDP) to an aromatic molecule that contributed significantly to structure diversity of prenylated aromatic compounds. In plant kingdom, the aromatic PTase usually belongs to UbiA superfamily of membrane bound enzymes that accept various aromatic compounds as a substrate leading to two groups of

compounds including prenylated flavonoids and prenylated quinones. Recently, several studies of aromatic PTase in terms of molecular biology and enzyme activity were successfully investigated.

2.2.1 Aromatic prenyltransferase in prenylated flavonoid biosynthesis

In prenylated flavonoid biosynthesis, flavonoid and prenyl groups are precursors of flavonoid PTases. The flavonoid core structure arises mainly from shikimate pathway via cinnamoyl-CoA and chain extension using three molecules of malonyl-CoA to produce initially polyketide and change to chalcone scaffold forming a flavonoid core structure, naringenin, by chalcone synthase. The prenyl units can come from two pathways: mevalonate (MVA) and methylerythritol phosphate (MEP) pathway. These pathways occur in different organelles in plant. The MVA pathway has been found in cytolsol or mitochondria while the MEP pathway occurs in chloroplast. All identified flavonoid PTases from plants utilized DMAPP rather than IPP to connect with flavonoid core structure and Mg^{2+} or other cation are required in catalytic reaction. Despite numerous prenylated flavonoids were found in plant, six flavonoid PTases of UbiA superfamily have currently been identified and characterized. The naringenin 8-dimethylallyltransferase (SfN8DT) has been isolated from *Sophora flavescen* suspension cells and it transferred DMAPP to C-8 position of narigenin to produce 8-dimethylallyl naringenin (Sasaki, et al., 2009). Hence, isoflavone PTase (SfG6DT) and chalcone PTase (SfiLDT) have been isolated from S. *flavescen* which corresponding to prenylation of the genistein at 6 position and isoliquiritigenin to produce dimethylalyl genistein and dimethylalyl isoliquiritigenin, respectively (Sasaki, et al., 2011). LaPT1 was identified from white lupin (*Lupinus albus*). This enzyme prenylated the genistin and 2'-hydroxygenistin with DMAPP at C-3' position to produce isowighteone and licoisoflavone, respectively (Shen, et al., 2012). The biosynthetic pathway of these prenylated flavonoids was shown in figure

4. The glycinol-4-dimethylallytransferase (G4DT) has been isolated from soybean (*Glycine max*) and its function was to produce 4-dimethylallyglycinol which is an intermediate in glyciolin I biosynthesis (Akashi, et al., 2009a). MaIDT and CtDT are flavonoid PTase identified from *Morus alba* and *Cudrania tricuspidata* (Wang, et al., 2014). These enzymes can accommodate a broad range of substrates, for example, isoliquiritigenin, dihydroxychalcone, butein, genistein, and hydroxygenistein for prenylation reaction to produce 3'-dimethylalylisoliquiritigenin, 3'-dimethylalyl-2,4-dihydroxychalcone, 3'-dimethylalyl-2,4,2',4'-tetrahydroxychalcone, 3'-dimethylalylbutein, 6'-dimethylalylgenistein, 6'-dimethylalyl-2'-hydroxygenistein, respectively. The Figure 4 showed the plant flavonoid PTases activity. Recently, PT1 which is an enzyme involved in bitter acid biosynthetic pathway was characterized in hop (*Humulus lupulus*). This enzyme catalyzed prenylation by transferring DMAPP to naringenin chalcone to obtain desmethylxanthohumol (Li, et al., 2015).

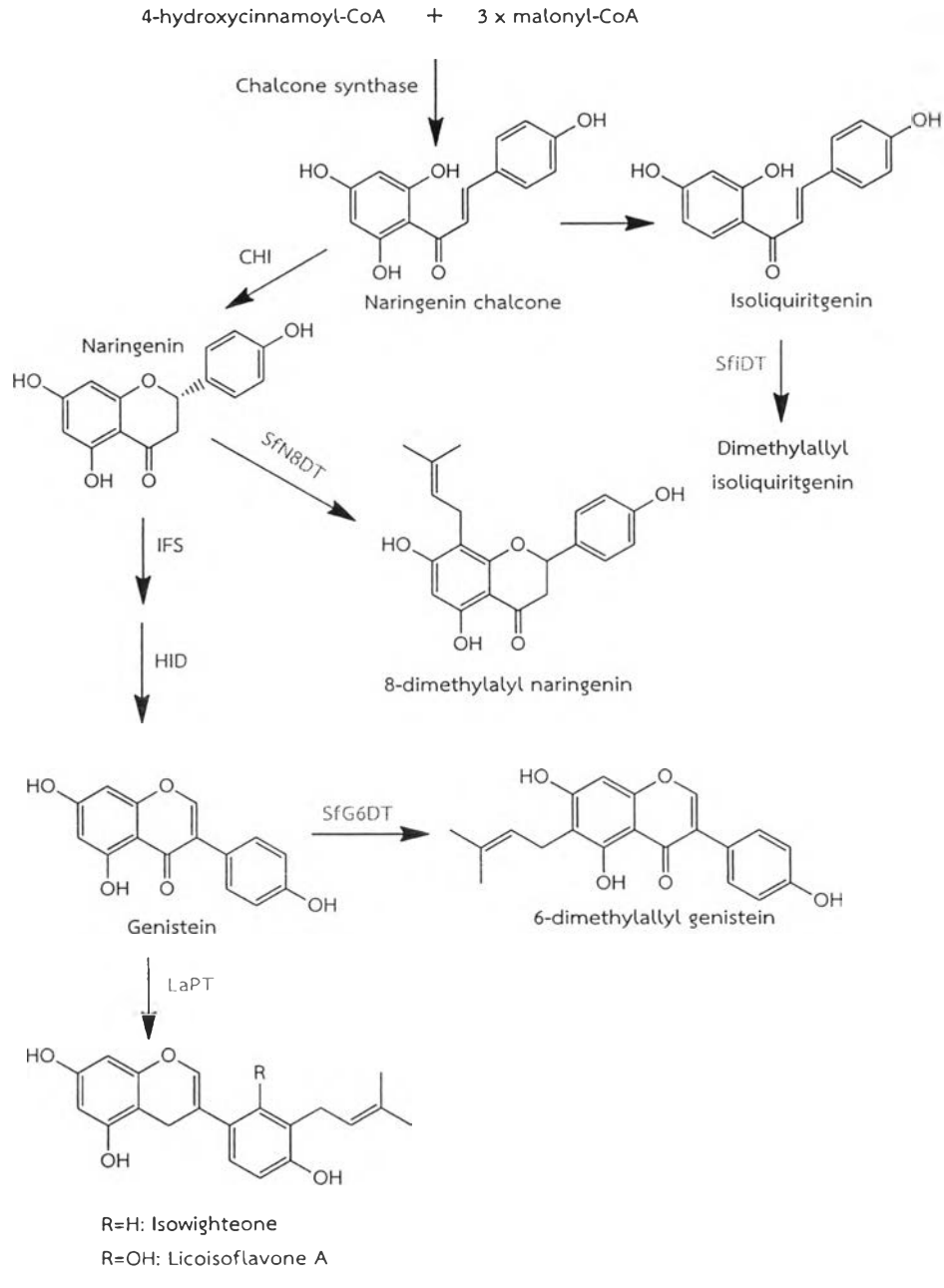

Figure 4 The example of plant flavonoid prenylated prenyltransferases activity. CHI: chalcone isomerase; IFS: 2-hydroxyisoflavanone synthase and HID: 2-hydroxyisoflavanone dehydratase. SfN8DT: naringenin 8-dimethylallyltransferase, SfG6DT: genistein 6-dimethylallyltransferase, SfiLDT: isoliquiritigenin dimethylalyltransferase.

2.2.2 Aromatic prenyltransferase in prenylated quinone biosynthesis

In plants, the prenyl, quinone, or lipoquinone serve as electron transporters in photosystem I and II (PSI and PSII) and protect against lipid oxidation. The biosynthesis of these compounds is started from hybridization of aromatic head group and prenyl side chain by aromatic PTase. The aromatic head group and prenyl side chain are derived from shikimate pathway and MVA or MEP pathway, respectively. The p-hydroxybenzoate polyprenyltransferase (PPT) catalyzes prenylation reaction in critical step of ubiquinone biosynthesis by connecting p-hydroxybenzoate (PHB) with polyprenyl side chain to form C-C bond to generate polyprenyl PHB (Ohara, et al., 2006). In addition, the PPT is involved in napthoquinone and shikonin biosynthesis. The p-hydroxybenzoate geranyltransferase (PGT) showed substrate specificity with geranyl diphosphate (GPP) to produce geranyl PHB as an intermediate in the pathway as shown in Figure 5 (Ohara, et al., 2009; Yazaki, et al., 2002).

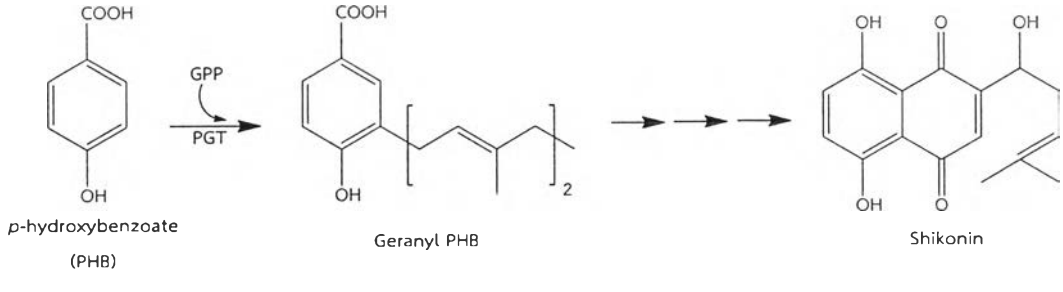

Figure 5 The activity of p-hydroxybenzoate geranyltransferase (PGT) in shikonin biosynthesis.

Homogentisate phytyltransferase (HPT/VTE2) is another group of aromatic quinone PTase involved in the rate limiting step of tocopherol biosynthesis in plant.

Biosynthesis of tocopherol, tocotrienol and plastoquinone in plants was illustrated in Figure 7. It is first started from homogentisic acid (HGA) that is synthesized from p-hydroxyprenyl pyruvate by 4-hydroxyphenylpyruvate diogenase (HPPD), followed by the addition of phytyl pyrophosphate (PDP) or geranylgeranyl pyrophosphate (GGPP) or solanezyl pyrophosphate to HGA by HPT/VTE2 or homogentisate geranylgeranyltransferase (HGGT) or homogentisate solanesyl transferase (HST) for the production of key intermediates in the biosynthesis of tocopherol, tocotrienol and plastoquinone, respectively. To produce α-tocopherol, HPT/VTE2 prenylates HGA with PDP to produce the first intermediate of 2-methyl-6-phytyl-1,4-benzoquinone (MPBQ), then methylation reaction of MPBQ catalyzes by MPBQ methyltransferase (MPBQ MT/VTE3) to yield 2,3-dimethyl-5-phytyl-1,4-benzoquinone (DMPBQ). The DMPBQ is cyclized by tocopherol cyclase (TC/VTE1) to produce γ-tocopherol and subsequently γ-tocopherol is methylated by γ-tocopherol methyltransferase (γ-TMT/VTE4) to yield α-tocopherol.. For the production of tocotrienol, HGGT utilizes HGA as prenyl acceptor and transfers prenyl group from GGPP to HGA resulting in formation of the first intermediate which is 2-methyl-6-geranylgeranyl-1,4-benzoquinone (MGGBQ). Hence, methylation of MGGBQ by VTE3 yields 2,3-dimethyl-5-phytyl-1,4-geranylgeranyl benzoquinone (DMGGBQ) and VTE1 cyclizes DMGGBQ to form γ-tocotrienol and then methylation of γ-tocotrienol finally yield α-tocotrienol. In plastoquinone biosynthesis pathway, HST catalyzes prenylation reaction by transferring solanesyl diphosphate (a prenyl group) to HGA to form 2-methyl-6-solanesyl-1,4-benzoquinol (MSBQ) intermediate. The biosynthesis of tocopherol, tocotrienol and platoquinone occur in chloroplast and HPT, HGGT and HST are embedded in chloroplast membrane. Under oxidative stress conditions induced by high light, drought or infection, these compounds are increasely produced to protect cell membrane from free radical or reactive oxygen species (ROS) (Sharma, et al., 2012).

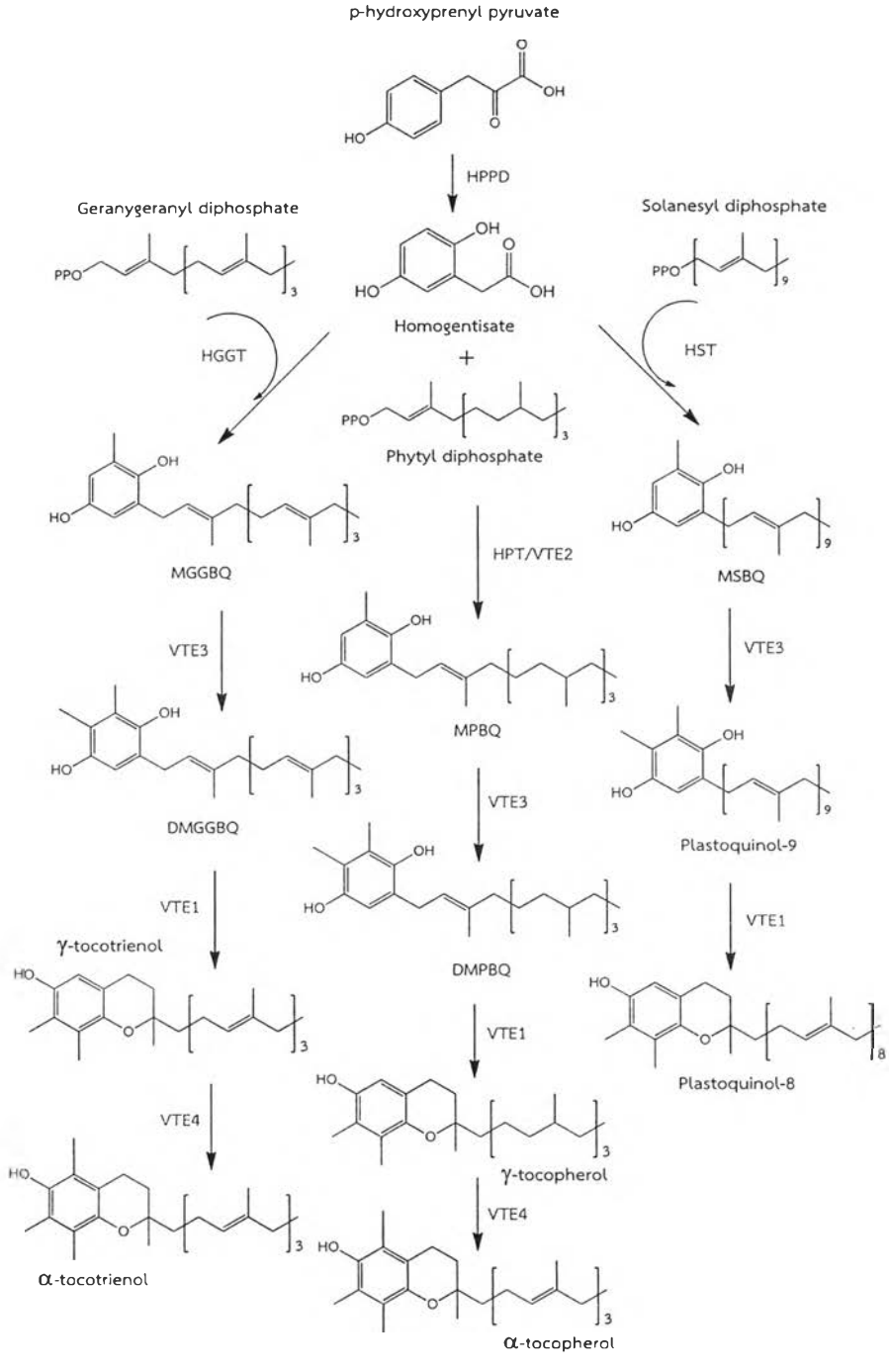

Figure 6 Biosynthesis of tocopherol, tocotrienol and platoquinone in plants.

2.3 Characterize and function of aromatic PTases

The HPT, HGGT and HST are a group of enzymes that transfer different forms of prenyl group to HGA. Although these enzymes catalyze prenylation reaction and their structures are very similar (transmembrane α-helix), their amino sequences are not quite different. The phylogenetic analysis showed the amino acid sequences of flavonoid PTases shared sequence similarity with HPT. It is implied that the flavonoid PTases may evolve from HPT (Figure 7).

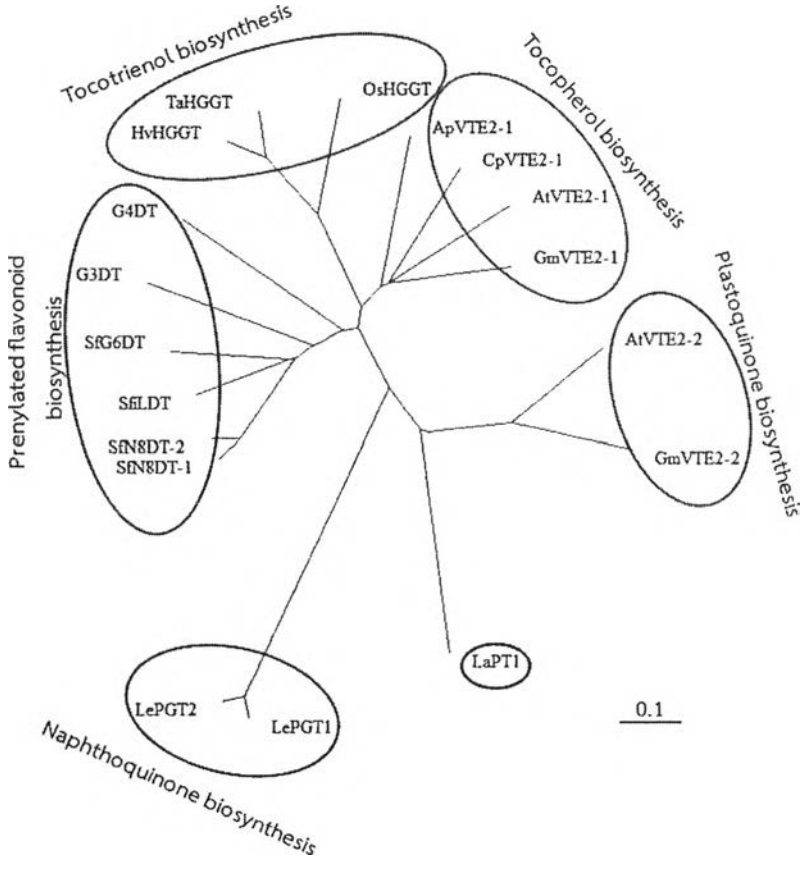

Figure 7 Phylogenetic relationship of aromatic prenyltransferases. A rooted phylogram was generated using a ClustalW alignment. Ap, *Allium porrum*; At, *Arabidopsis thaliana*; Cp, *Cuphea pulcherrima*; Gm, *Glycine max*; Hv, *Hordeum vulgare*; Os, *Oryza sativa*; Ta, *Triticum aestivum*.

Aromatic PTases in plant are membrane-bound proteins of UbiA superfamily localized in plastid membrane. They contains two aspartate-rich regions for binding of the prenyl diphosphate substrate via chelating Mg^{2+} ion required for enzyme activity (Table 1) (Huang, et al., 2014). In *E. coli*, the UbiA protein catalyzes cleavage of pyrophosphate from polyprenyl diphosphate and transfer prenyl chain to PHB (Ashby, et al., 1992). Generally, identified aromatic PTases have 7 – 9 transmembrane α-helixes and N-signaling transit peptide to localize at chloroplast membrane. Expression of flavonoid PTase proteins was successfully done in yeasts such as strain W303-1A-Δcoq2 (Sasaki, et al., 2009) and strain YPH499 (Wang, et al., 2014). It has been found that PTases activity from Leguminosae expressed in yeast microsomal fractions prefered DMAPP as prenyl substrate while PTase activity from Moraceae can use DMAPP and GPP in prenylation The enzyme assay required Mg^{2+} for catalyzing reaction with the optimal pH of 7 -10. Functional study of aromatic PTase can be tested in plant system. The overexpression of aromatic PTases has been reported in several plant systems such as Arabidopsis (Mene-Saffrane, et al., 2010), tobacco (Harish, et al., 2013a), tomato and lettuce (Lee, et al., 2007). Overexpression of *SfN8DT* gene encoding naringenin 8-dimethylallyltransferase in *Arabidopsis* showed the accumulation of 8-prenylated kaempferol which was not detected in *in vitro* enzyme assay (Sasaki, et al., 2008).

The PPT enzyme involve in ubiquinone biosynthesis are located in the inner membrane of mitochondria but LePG1 that member of PPT involved in naphthoquinone biosynthesis is localized to the endoplasmic reticulum (Ohara, et al., 2010; Ohara, et al., 2006; Okada, et al., 2004). In addition, the HPT and HST catalyzing the prenylation in tocopherol biosynthesis and plastoquinone, respectively are located at the plasmid (Block, et al., 2013; Hunter and Cahoon, 2007). Same as flavonoid PTases, the study of PPT protein expression was done in yeast stain W303-

1A-Δcoq2 and tobacco and the protein activity was detected from microsomal fraction (Ashby, et al., 1992; Ohara, et al., 2006; Okada, et al., 2004) while LePG1 expression was performed in insect cell (sf9 cell) system.

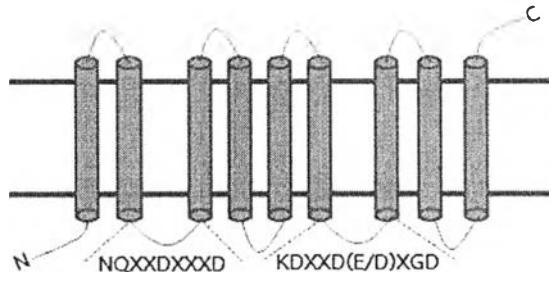

Table 1 Aspartate rich regions of plant aromatic PTases.

Protein	Motif I	Motif II	Protein	Motif I	Motif II
ApVTE2-1	NQLFDIEID	KDIPDIDGD	OsHGGT	NQLYDIQID	KDIPDIDGD
AtVTE2-1	NQLSDVEID	KDIPDIEGD	TaHGGT	NQLYDIQID	KDIPDVDGD
CpVTE2-1	NQLSDIDID	KDIPDIEGD	SfN8DT-1	NQLCDIEID	KDIPDMEGD
GmVTE2-1	NQLSDVEID	KDIPDIEGD	SfN8DT-2	NQLCDIEID	KDIPDMEGD
TaVTE2-1	NQLFDIEID	KDIPDIEGD	G4DT	NQLYDLEID	KDIPDVEGD
MdVTE2-1	NQLSDIDID	KDIPDIDGD	SfiLDT	NELCDVELD	KDIPDIEGD
GmVTE2-2	NQIYDISID	KDLPDVEGD	SfG6DT	NQLCDIEID	DIPDTEGD
AtVTE2-2	NQIYDIGID	KDLPDVEGD	G3DT	NQLCDLEID	KDIPDMEGD
LaPT1	NQIFDMDID	KDLSDINGD	MalDT	NQIYDADID	KDLTDMEGD
HvHGGT	NQLYDIQID	KDIPDVDGD	LePGT1	NDYFDRNFD	YAHQDKVDD

2.4 *Artocarpus lakoocha* Rox

2.4.1 Plant description

Artocarpus lakoocha Rox or known in Thai as Mahaad (Figure 8) is a deciduous plant belonging to Moraceae family. It is widely distributed in South and South-east Asia such as India, Nepal, Malaysia, and Thailand. *A. lakoocha* is a tree that can reach to 15 – 18 m in height and its elliptical pointed leave is 10 – 25 cm long. Its fruits are yellow at maturity and changed to reddish brown later and its seeds have thin white seed coat and sticky latex.

Figure 8 Leaves of *Artocarpus lakoocha* Rox

2.4.2 Chemical constituents and biological activities of *A. lakoocha*

A. lakoocha is a plentiful source of secondary metabolites, especially a group of flavonoids, stilbenes, and their derivative compounds. In phytochemical studies, artocarpin **(19)**, norartocarpin **(20)**, cycloartocarpin **(21)**, resorcinol **(22)**, and oxyresveratrol **(23)** has been found in the heartwood of *A. lakoocha* (Tunsaringkarn, et al., 2007) and prenylated 2-arybenzofurans consist of artolakoochol **(24)**, 4-hydroxyartolakoochol **(25)** and cyclo-artolakoochol **(26)** (Sritularak, et al., 2010) as well as prenylated stilbene (lakoochin A **(27)** and lakoochin B **(28)**) were found in the root (Puntumchai, et al., 2004). Moreover, the *A. lakoocha* callus culture produces prenylated flavones and stilbenes (Maneechai, et al., 2012) (Figure 9).

Several biological activities have been reported from the extracts of *A. lakoocha*. The crude extracted from heartwood showed antityrosinase activity by inhibiting melanin production, antimicrobial, antibiofilm activity from oral pathogen and *Candida*. The leave extracted has been found activity against inflammatory, analgesic and CNS depressant (Nesa, et al., 2015). The root extract showed antiherpetic and anticancer activities (Arung, et al., 2006; Dej-adisai, et al., 2014; Shimizu, et al., 2002).

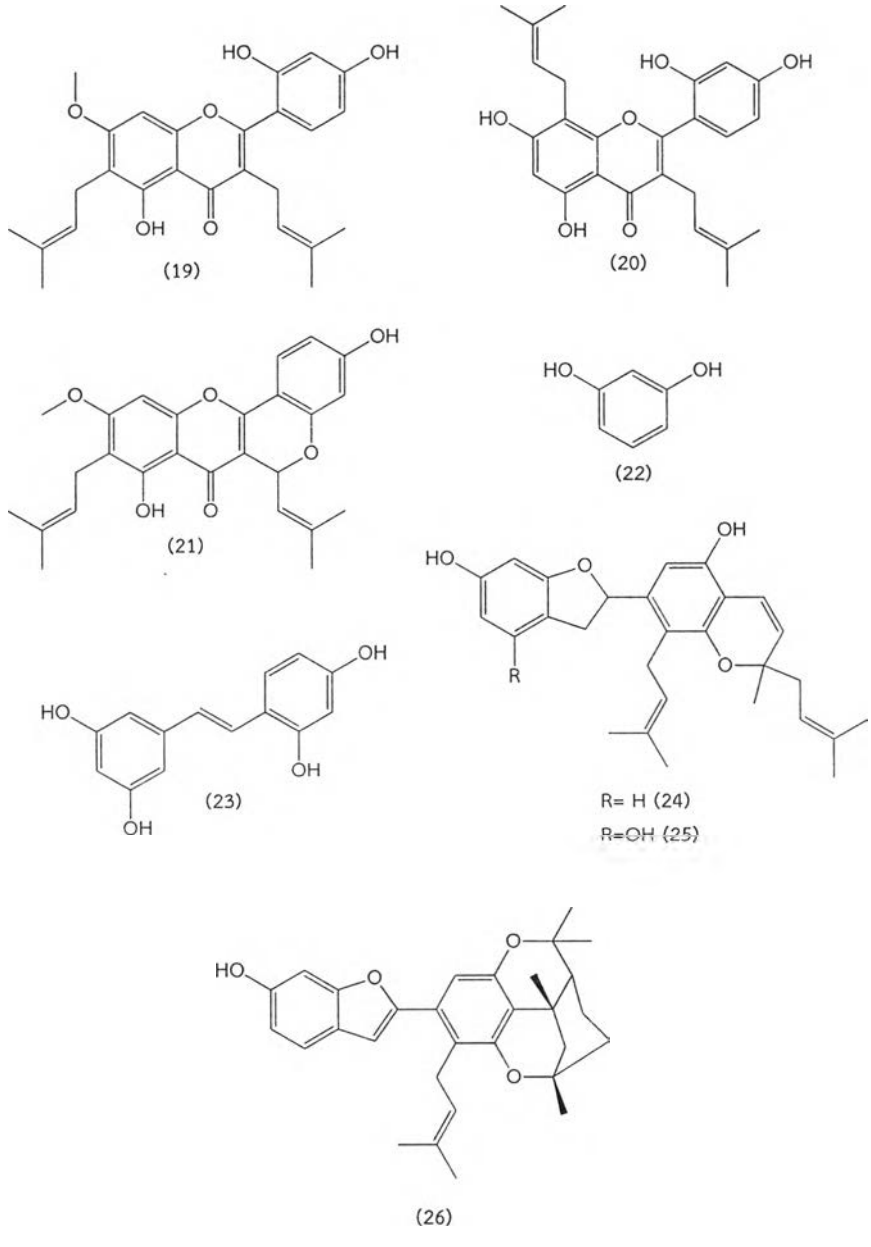

Figure 9 The secondary metabolites from *A. lakoocha*.

2.5 *Clitoria ternatea* L.

2.5.1 Plant description

Clitoria ternatea L. or Un-chann in Thai name is climbing plant belonging to Fabaceae family. It is distributed in India, Philippines, and other tropical Asian

countries. The plant leave is imparipinnate with five to seven leaflets, 6 - 13 cm long and ovate. The seeds are yellowish-brown or blackish in color and oval in shape. Its flower color is blue or white (Figure 10).

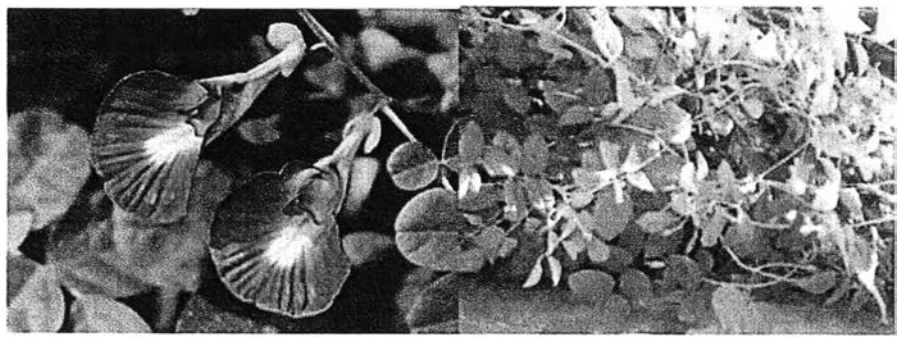

Figure 10 Plant and flower of *Clitoria ternatea* L.

2.5.2 Chemical constituents and biological activities in *C. ternatea*

C. ternatea is a medicinal plant which has many biologically active compounds. The taraxerol and taraxerone were isolated from root. A number of anthocyanins and flavonoids were separated from flower. Leaves contain essential oils and flavone glycosides. *C. ternatea* has been screened for biological activities. It has an effect on learning and memory enhancing by which it increased acetylcholine content (Rai, et al., 2002). It also showed antidepressant, anti-inflammatory, anticancer, and anti-platelet aggregation (Devi, et al., 2003; Jacob and Latha, 2012; Jain, et al., 2003; Kelemu, et al., 2004; Nithianantham, et al., 2011).