CHAPTER II
LITERATURE REVIEW

2.1 *Thunbergia laurifolia* Linn.

2.1.1 Botanical description

Thunbergia laurifolia Linn. is a plant which belongs to the family Acanthaceae. *T. laurifolia* as a woody vine; stem terete, twining to the left, glabrous green; leaves opposite 2 – 4 times as long as wide, ovate – oblong to oblong – lanceolate, from a cuneate, obtuse, round, or subcordate 3 - 5 nerved base, acutely acminate, entire or slightly crenate – dentate, penninerved, glabrous 7.5 – 18 cm by 2.5 – 6 cm; petiole sparsely hispidulous to nearly glabrous 1 – 6.5 cm long. Terminal raceme 4 – 30 cm, dense sometimes with axillary flowers; pedicels 1.75 – 3 cm; basal bracteoles of calyx green with violet blotches, persistent till long after the fall of the corolla 4 -5 cm long; calyx outside pubscent and nectariferous only along the outer margin of the lop; corolla – tube 3.25 – 4.75 cm long, inside without a ring of hairs; limb 6 – 8 cm across, dark blue – violet, rarely white; tube constricted above the conical, much thickened base, above the constriction turbinately widened, with a dorsal longitudinal bulge embrancing stamens and style, yellowish inside; filaments in their lower part very much thickened; 1 cell of posterior anthers ecalcarate, all other cells longitudinally hairly and provided with a subulate spur; pollen grians not echinate; dark yellowish white, including the lower half of the ovary; style thin, apically curved forward; stigmatic lobes broad, flat, anterior one patent, posterior one erect with a pinched upper half; capsule often produced, its basal part 12 – 16 mm diameter; beak 2.25 – 3 cm; seeds ventrally smooth, dorsally tuberculate (Backer and Bakhuizen van Den Brink 1965) (**Figure 2**).

Figure 2 *Thunbergia laurifolia* Linn.

2.1.2 Chemical constituents

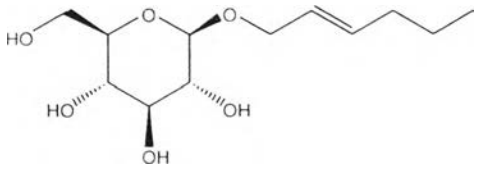
Several phytochemical investigations of *T. laurifolia* leaves have been reported. Iridoid glycosides, 8-*epi*-grandifloric acid and 3'-*O*-ß-glucopyranosyl-stilbericoside, have been isolated from the methanolic extract from leaves of *T. lourifolia* along with glycosides of grandifloric acid, apigenin-7-*O*-ß-D-glucopyranoside, (*E*)-2-hexenyl-ß-glucopyranoside, benzyl-ß-glucopyranoside, benzyl-ß-(2'-*O*-ß-glucopyranoside)-glucopyranoside, hexanol-ß-glucopyranoside, and 6,8-di-*C*-glucopyranosyl apigenin (Kanchanapoom, *et al.* 2002). The aqueous extract of leaves of *T. laurifolia* have also been found to contain phenolics such as caffeic acid, gallic acid, chlorogenic acid, and protocatechuic acid (Chan, *et al.* 2011). Flavonoids, apigenin and chlorogenic acid, were isolated from the aqueous extract of *T. laurifolia* leaves (Purnima 1978, Oonsivilai, *et al.* 2007). Distributions of these compounds in *T. laurifolia* are summarized in **Table 1** and chemical structures are shown in **Figure 3 – 8**.

Table 1 Distribution of chemical constituents reported in *T. laurifolia*.

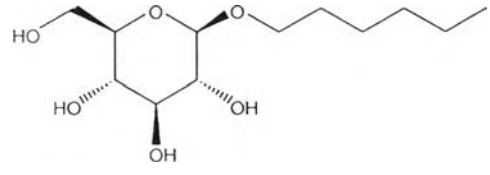
Secondary metabolites	Compound	Plant part	References
Aliphatic alcohol glucosides	(*E*)-2-hexenyl-ß-glucopyranoside [1]	Aerial parts	(Kanchanapoom, *et al.* 2002)
	Hexanol-ß-glucopyranoside [2]	Aerial parts	(Kanchanapoom, *et al.* 2002)
Benzyl alcohol glucosides	Benzyl-ß-glucopyranoside [3]	Aerial parts	(Kanchanapoom, *et al.* 2002)
	Benzyl-ß-(2'-*O*-ß-glucopyranosyl)-glucopyranoside [4]	Aerial parts	(Kanchanapoom, *et al.* 2002)
Flavonoids	Apigenin [5]	Leaves	(Oonsivilai, *et al.* 2007)
	Apigenin-7-*O*-ß-D-glucopyranoside [6]	Aerial parts	(Kanchanapoom, *et al.* 2002)

Table 1 (continued).

Secondary metabolites	Compound	Plant part	References
Flavonoids	Delphinidin [7]	Leaves and Flowers	(Chan, *et al.* 2011)
	Delphinidin-3-5-di-*O*-ß-D-glucoside [8]	Flowers	(Thongsaard and Marsden 2002)
	6-*C*-glucopyranosyl apigenin [9]	Aerial parts	(Kanchanapoom, *et al.* 2002)
	6,8-di-*C*-glucopyranosyl apigenin [10]	Aerial parts	(Kanchanapoom, *et al.* 2002)
Iridoids	8-*epi*-grandifloric acid [11]	Aerial parts	(Kanchanapoom, *et al.* 2002)
	3'-*O*-ß-glucopyranosyl-stilbericoside [12]	Aerial parts	(Kanchanapoom, *et al.* 2002)
	Grandifloric acid [13]	Aerial parts	(Kanchanapoom, *et al.* 2002)
Phenolics	Caffeic acid [14]	Leaves	(Oonsivilai, *et al.* 2007)
	Chlorogenic acid [15]	Leaves and flowers	(Purnima 1978)
	Gallic acid [16]	Leaves	(Oonsivilai, *et al.* 2007)
	Protocatechuic acid [17]	Leaves	(Oonsivilai, *et al.* 2007)
	Rosmarinic acid [18]	Leaves	(Suwanchaikasem 2011)
Terpenoids	Lutein [19]	Leaves	(Oonsivilai, *et al.* 2007)

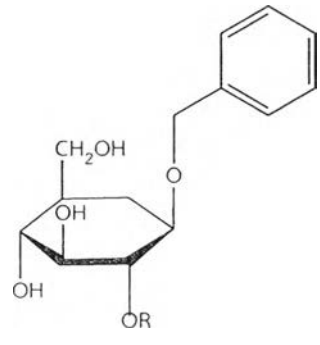


(*E*)-2-hexenyl-ß-glucopyranoside [1]



Hexanol-ß-glucopyranoside [2]

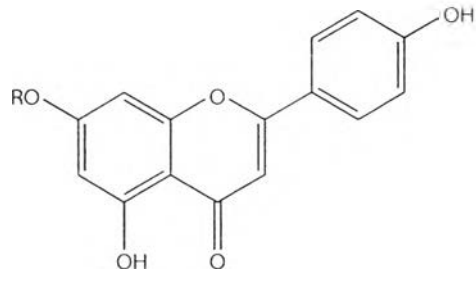
Figure 3 Structures of aliphatic alcohol glucosides isolated from *T. laurifolia*



R = H; Benzyl-ß-glucopyranoside [3]

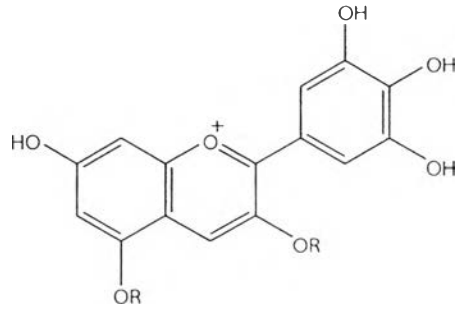
R = ß-D-glucose; Benzyl-ß-(2'-*O*-ß-glucopyranosyl)-glucopyranoside [4]

Figure 4 Structures of benzyl alcohol glucosides isolated from *T. laurifolia*



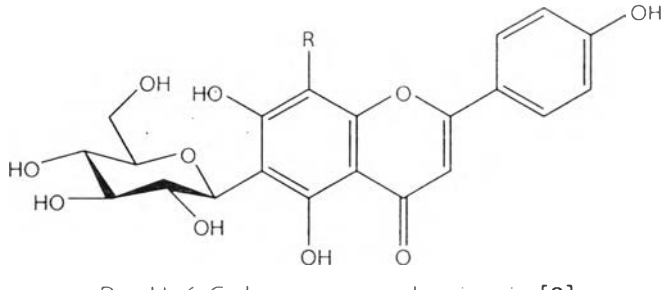
R = H; Apigenin [5]

R = ß-D-glucose; Apigenin-7-*O*-ß-D-glucopyranoside [6]



R = H; Delphinidin [7]

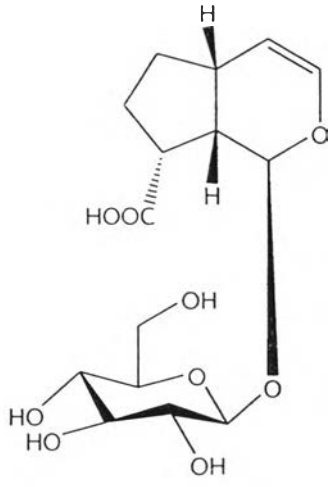
R = ß-D-glucose; Delphinidin-3-5-di-*O*-ß-D-glucoside [8]



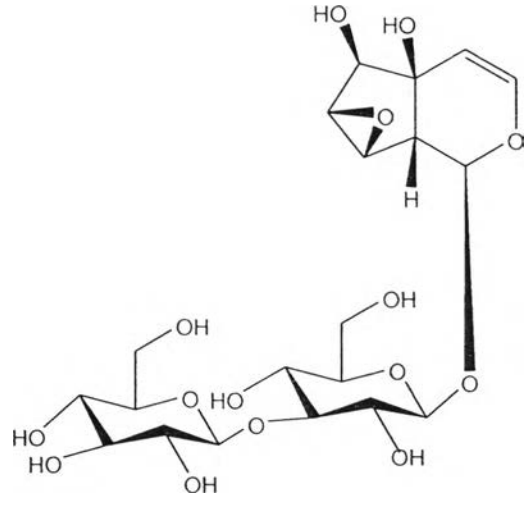
R = H; 6-*C*-glucopyranosyl apigenin [9]

R = glucose; 6, 8-di-*C*-glucopyranosyl apigenin [10]

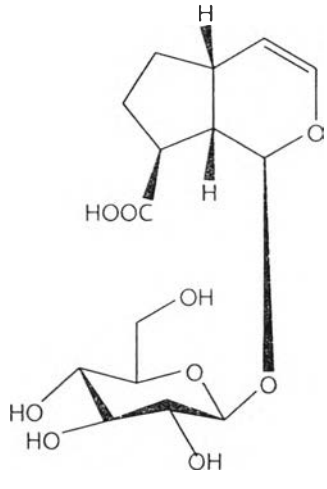
Figure 5 Structures of flavonoids isolated from *T. laurifolia*



8-*epi*-grandifloric acid **[11]**

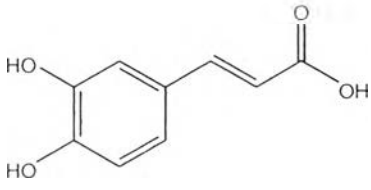


3'-*O*-β-glucopyranosyl-stilbericoside **[12]**

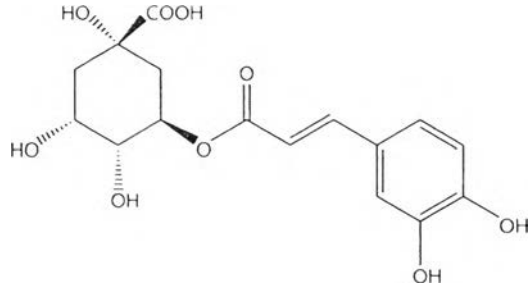


Grandifloric acid **[13]**

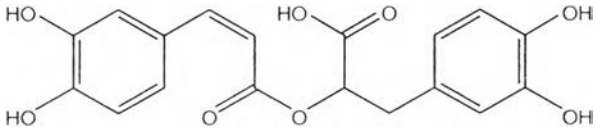
Figure 6 Structures of iridoids isolated from *T. laurifolia*



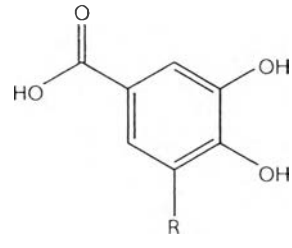
Caffeic acid **[14]**



Chlorogenic acid **[15]**



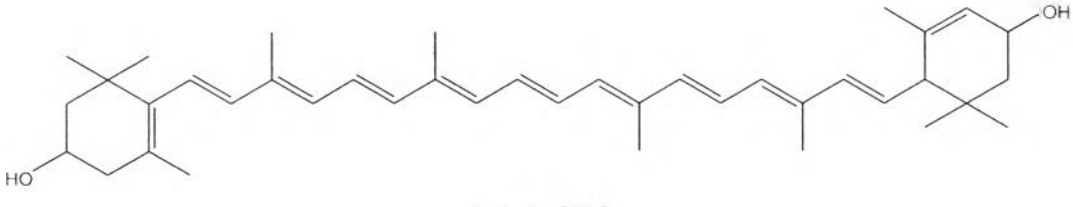
Rosmarinic acid **[16]**



R = OH; Gallic acid **[17]**

R = H; Protocatechuic acid **[18]**

Figure 7 Structures of phenolics isolated from *T. laurifolia*



Lutein **[19]**

Figure 8 Structures of terpenoids isolated from *T. laurifolia*

2.1.3 Bioactivities of *T. laurifolia*

In Thailand, different parts of *T. laurifolia* are used for various medicinal purposes. For example, the fresh leaves, dried leaves, roots, and bark are used as detoxification and as antidotes for poisoning with insecticide (Tejasen and Thongthapp 1980), lead (Palipoch, *et al.* 2011), cadmium (Chattaviriya, *et al.* 2010), and ethanol (Pramyothin, *et al.* 2005). The dried leaves and roots have been applied as antipyretic (Tachakittirungrod, *et al.* 2007) and anti-inflammatory agents (Wonkchalee, *et al.* 2012). Various pharmacological activities of *T. laurifolia* have been investigated in earlier studies such as anti-diabetic, antioxidant, and hepatoprotective.

Antidote activity

T. laurifolia is a herbal medicine used as an antidote for several poisonous agents in Thai traditional medicine. The aqueous crude extract of *T. laurifolia* leaves increased the potassium-stimulated dopamine release from rat striatal slices in a similar manner as amphetamine, which determined whether the treatment of drug addiction (Thongsaard and Marsden 2002). The aqueous extract from leaves of *T. laurifolia* was found to alleviate lead poisoning in male mice (Tangpong and Satarug 2010). Moreover, the aqueous extract of *T. laurifolia* leaves could reduce toxicity from high cadmium in rat kidney (Chattaviriya, *et al.* 2010).

Anti-inflammatory activity

The alcohol and hexane extracts from leaves of *T. laurifolia* were found to possess anti-inflammatory activity against carrageenin-induced paw edema in mice (Charumanee, *et al.* 1998). Fresh and dried leaves of *T. laurifolia* solutions reduced the inflammatory cells surrounding the hepatic bile ducts in Syrian hamsters induced by *Opisthorchis viverrini* infection and *N*-nitrosodimethylamine (NDMA) administration (Wonkchalee, *et al.* 2012).

Anti-diabetic activity

Hypoglycemic properties of the aqueous extract from the leaves of *T. laurifolia* were evaluated in normoglycemic and alloxan-induced diabetic rat. Results showed that the 15-day-treatment with the extract (60mg/mL/day) decreased the levels of blood glucose in diabetic rats. The recovery of β-cells was also found in diabetic rats treated with the extract (Aritajat, *et al.* 2004).

Antioxidant activity

Antioxidant activities and the total phenolic content of *T. laurifolia* extracts were evaluated using the Folin-Ciocalteu method and ferric reducing antioxidant power (FRAP) assay. The results showed that the water extraction of phenolic compounds was the most efficient (2433.9mg GAE/100g) compared to ethanol and acetone extraction. In addition, the aqueous extract of *T. laurifolia* leaves also showed the highest antioxidant activities using free radical scavenging and total antioxidant activity using FRAP assay (Oonsivilai, *et al.* 2008). In the DPPH analysis, the scavenging capacity of the ethanolic extract from leaves of *T. laurifolia* (EC_{50} value = 119.97μg/mL) showed the higher than the aqueous extract (Suwanchaikasem, *et al.*).

Hepatoprotective activity

The aqueous extract from leaves of *T. laurifolia* protected mice from hepatic injury induced by ethanol (Chanawirat 2000). The hepatoprotective activity of aqueous extract from *T. laurifolia* leaves against ethanol induced liver injury in male Wistar rats and in primary cultures of rat hepatocytes has also been reported (Pramyothin, *et al.* 2005). Furthermore, the aqueous extract of *T. laurifolia* leaves clearly demonstrated the reduction of inflammatory cells in hepatic tissue, showing decreased serum ALT and decreased liver cell damage (Wonkchalee, *et al.* 2012).

2.2 *Phyllanthus amarus* Schum. and Thonn.

2.2.1 Botanical description

Phyllanthus amarus Schum. and Thonn. (Euphorbiaceae) is a small tropical herb found in tropical and subtropical countries, including the United States, Brazil, India, and Thailand. *P. amarus* are erect annual herbs, 10 – 60 cm tall; main stem simple or branched, terrete smooth or scabridulous in younger parts. Cataphylls, stipules 1.5 – 1.9 mm long, deltoid acuminate blade 1 – 1.5 mm long, subulate acuminate. Leaves 3 – 11 × 1.5 – 6 mm elliptic oblong obovate, oblong, or even obovate, obtuse, or minutely apiculate at apex, obtuse or slightly inequilateral at base, petioles 0.3 – 0.5 mm long, stipules 0.8 – 1.1 mm long triangular accumate. Flowers minutes, proximal 2 – 3 axis with unisexual cymules, each consisting of 1 male and 1 female or 2 -3 males and female or 1 male and 2 females flower or combination. The seed capsules on stalks are 1 – 2 mm long, round, smooth, 2 mm wide, with 6 seeds. When the fruits burst open the seeds are hurled away. Seeds are triangular (like an orange segment); light brown, 1 mm long, with 5 – 6 ribs on the back (Patel, *et al.* 2011) (**Figure 9**).

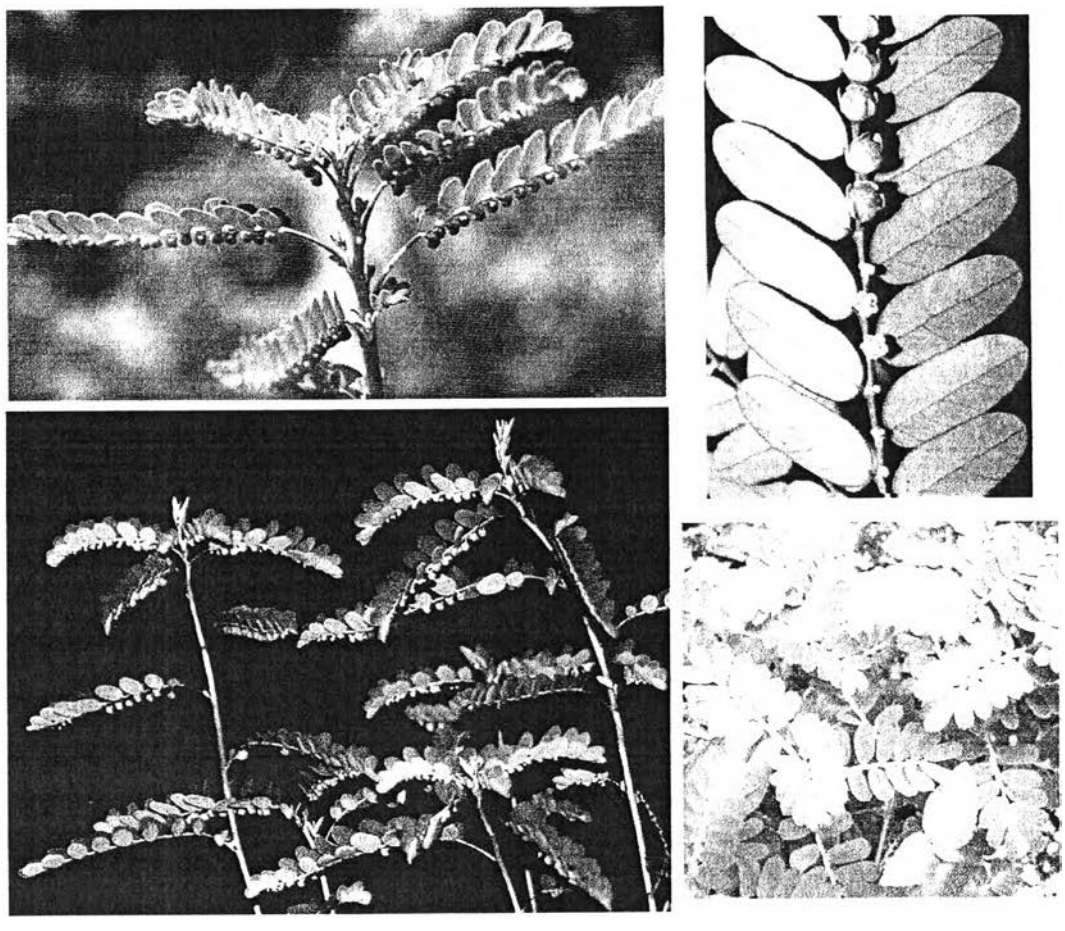


Figure 9 *Phyllanthus amarus* Schum. and Thonn.

2.2.2 Chemical constituents

In a previous phytochemical study on the aerial parts of *P. amarus*, hydrolysable tannins, such as phyllanthusiin D, ellagitannin 1, and corilagin were isolated (Foo and Wong 1992). The whole *P. amarus* plant includes alkaloids, such as securinine, isobubbialine, and epibubbialine (Houghton, *et al.* 1996). The flavonoids kaempferol, rutin, and quercetin were isolated from whole *P. amarus* plants (Londhe, *et al.* 2008). The lignan groups, phyllanthin, hypophyllanthin, niranthin, phyltetralin, and nirtetralin, were also characterized (Maciel, *et al.* 2007). Distributions of these compounds in this plant are summarized in **Table 2** and chemical structures are shown in **Figure 10 - 17**.

Table 2 Distribution of chemical constituents reported in *P. amarus*.

Secondary metabolites	Compound	Plant part	References
Alkaloids	Allo-securinine [20]	Whole plant	(Singh, *et al.* 2008)
	Dihydrosecurinine [21]	Aerial parts	(Kassuya, *et al.* 2006)
	Epibubbialine [22]	Leaves	(Houghton, *et al.* 1996)
	Isobubbialine [23]	Leaves	(Houghton, *et al.* 1996)
	4-methoxydihydrosecurinine [24]	Aerial parts	(Foo and Wong 1992)
	4-methoxy-nor-securinine [25]	Aerial parts	(Foo and Wong 1992)
	4-methoxytetrahydrosecurinine [26]	Aerial parts	(Foo and Wong 1992)

Table 2 (continued).

Secondary metabolites	Compound	Plant part	References
Alkaloids	Nor-securinine [27]	Leaves	(Houghton, *et al.* 1996)
	Phenazine [28]	Aerial parts	(Foo 1995)
	Phenazine of amariine [29]	Aerial parts	(Foo 1993)
	Phyllanthine [30]	Leaves	(Houghton, *et al.* 1996)
	Securinine [31]	Leaves	(Houghton, *et al.* 1996)
	Securinol A [32]	Aerial parts	(Kassuya, *et al.* 2006)
	Tetrahydrosecurinine [33]	Aerial parts	(Kassuya, *et al.* 2006)
Fatty alcohol and analogues	Dotricontanyl docosanoate [34]	Whole plant	(Ali, *et al.* 2006)
	Triacontanol [35]	Whole plant	(Ali, *et al.* 2006)
Flavonoids	Astragalin [36]	Aerial parts	(Foo and Wong 1992)
	Quercetin [37]	Whole plant	(Kiran, *et al.* 2011)
	Quercetin-3-*O*-ß-D-glucopyranosyl-(1-4)-α-L-rhamnopyranoside [38]	Stem	(Kiran, *et al.* 2011)
	Quercetin-3-*O*-glucoside [39]	Aerial parts	(Londhe, *et al.* 2008)
	Quercitrin [40]	Whole plant	(Kiran, *et al.* 2011)
	Rutin [41]	Aerial parts	(Foo 1993)

Table 2 (continued).

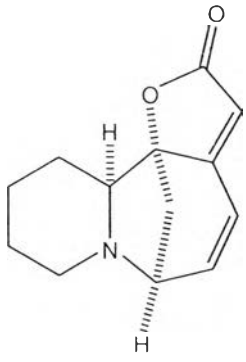
Secondary metabolites	Compound	Plant part	References
Lignans	5-demethoxy niranthin [42]	Whole plant	(Maciel, *et al.* 2007)
	Demethylenedioxyniranthin [43]	Whole plant	(Maciel, *et al.* 2007)
	5-methoxy bursehernin [44]	Aerial parts	(Singh, *et al.* 2009)
	7-hydroxy niranthin [45]	Aerial parts	(Singh, *et al.* 2009)
	Heliobupthalmin lactone [46]	Leaves	(Shanker, *et al.* 2011)
	Hinokinin [47]	Whole plant	(Huang, *et al.* 2003)
	Hypophyllanthin [48]	Aerial parts	(Somanabandhu, *et al.* 1993)
	Isolintetralin [49]	Whole plant	(Maciel, *et al.* 2007)
	Isonirtetralin [50]	Aerial parts	(Kassuya, *et al.* 2003)
	Lintetralin [51]	Whole plant	(Patel, *et al.* 2011)
	Niranthin [52]	Aerial parts	(Kassuya, *et al.* 2003)
	Nirtetralin [53]	Aerial parts	(Kassuya, *et al.* 2003)
	Niranthin [52]	Aerial parts	(Kassuya, *et al.* 2003)
	Nirtetralin [53]	Aerial parts	(Kassuya, *et al.* 2003)
	Phyllanthin [54]	Aerial parts	(Somanabandhu, *et al.* 1993)

Table 2 (continued).

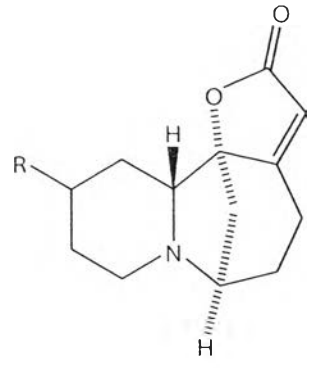
Secondary metabolites	Compound	Plant part	References
Lignans	Phyltetralin [55]	Aerial parts	(Kassuya, *et al.* 2003)
	Virgatusin [56]	Leaves	(Shanker, *et al.* 2011)
Tannins	Amariin [57]	Aerial parts	(Foo 1993)
	Amariinic acid [58]	Aerial parts	(Foo 1995)
	Amarulone [59]	Aerial parts	(Foo 1995)
	Corilagin [60]	Aerial parts	(Foo and Wong 1992)
	1,6-digalloylglucopyranose [61]	Aerial parts	(Foo 1993)
	Elaeocarpusin [62]	Aerial parts	(Foo 1995)
	Ellagic acid [63]	Whole plant	(Dhalwal, *et al.* 2006)
	Furosin [64]	Aerial parts	(Foo 1995)
	Gallic acid [17]	Aerial parts	(Foo 1993)
	Gallocatechin [65]	Aerial parts	(Foo 1993)
	1-galloyl-2,3-DHHDP-glucose [66]	Aerial parts	(Londhe, *et al.* 2008)
	Geraniin [67]	Aerial parts	(Foo and Wong 1992)
	Geraniinic acid B [68]	Aerial parts	(Foo 1995)
	Isocorilagin [69]	Aerial parts	(Foo 1995)
	4-*O*-galloylquinic acid [70]	Aerial parts	(Foo 1995)
	Phyllanthusiin A – D [71 - 74]	Aerial parts	(Foo and Wong 1992)
	Repandusinic acid B [75]	Aerial parts	(Foo 1995)

Table 2 (continued).

Secondary metabolites	Compound	Plant part	References
Terpenoids	2Z,6Z,10Z,14E,18E,22E-farnesyl farnesol [76]	Whole plant	(Maciel, *et al.* 2007)
	Lupeol [77]	Aerial parts	(Foo and Wong 1992)
	Oleanolic acid [78]	Whole plant	(Ali, *et al.* 2006)
	Phyllanthenol [79]	Aerial parts	(Foo and Wong 1992)
	Phyllanthenone [80]	Aerial parts	(Foo and Wong 1992)
	Phyllantheol [81]	Aerial parts	(Foo and Wong 1992)
	Ursolic acid [82]	Whole plant	(Ali, *et al.* 2006)
Sterols	Estradiol [83]	Root and bark	(Kiran, *et al.* 2011)
	Amarosterol A [84]	Whole plant	(Ahmad and Alam 2003)
	Amarosterol B [85]	Whole plant	(Ahmad and Alam 2003)
Volatile oils	Linalool [86]	Shrubs	(Moronkola, *et al.* 2009)
	Phytol [87]	Shrubs	(Moronkola, *et al.* 2009)

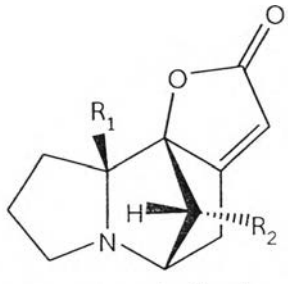


Allo-securinine [20]



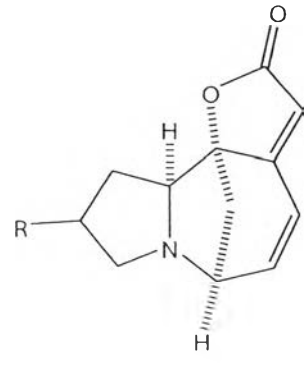
R = H; Dihydrosecurinine [21]

R = OCH_3; 4-methoxydihydrosecurinine [24]



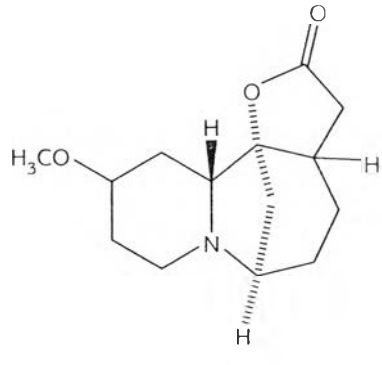
R_1 = OH, R_2 = H; Epibubbialine [22]

R_1 = H, R_2 = OH; Isobubbialine [23]



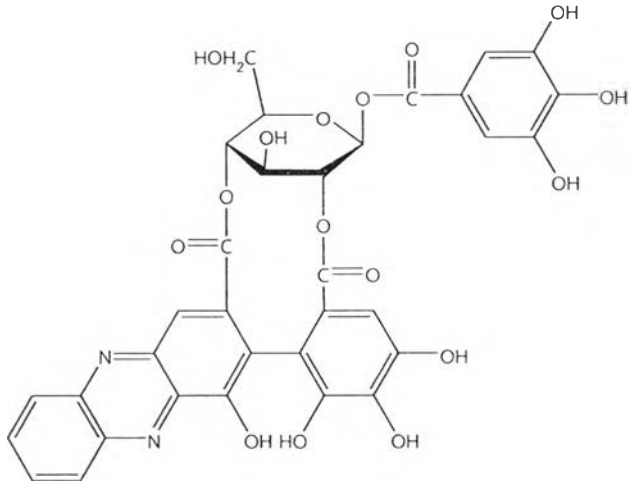
R = OCH_3; 4-methoxy-nor-securinine [25]

R = H; Nor-securinine [27]

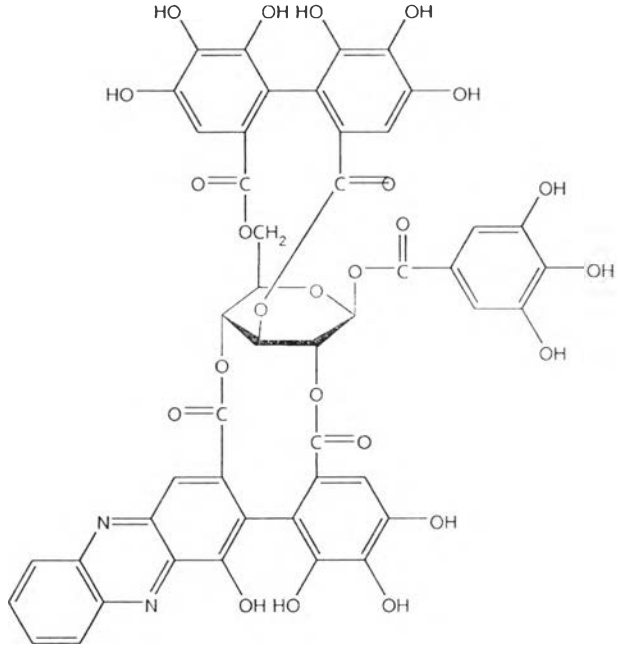


4-methoxytetrahydrosecurinine [26]

Figure 10 Structures of alkaloids isolated from *P. amarus*

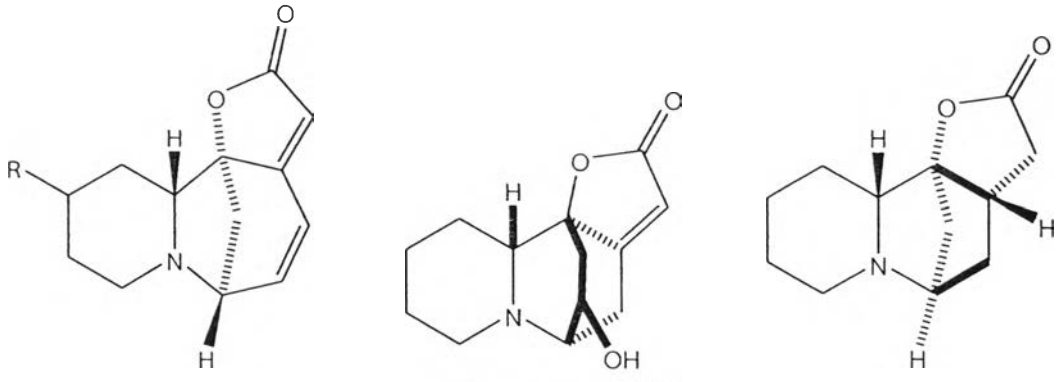


Phenazine [28]



Phenazine of amariine [29]

Figure 10 Structures of alkaloids isolated from *P. amarus* (continued).

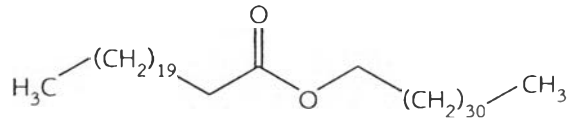


R = OCH_3; Phyllanthine **[30]**
R = H; Securinine **[31]**

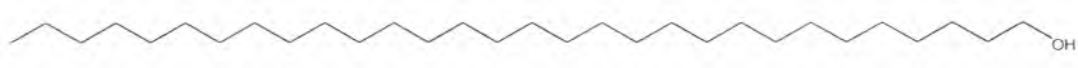
Securinol A **[32]**

Tetrahydrosecurinine **[33]**

Figure 10 Structures of alkaloids isolated from *P. amarus* (continued).

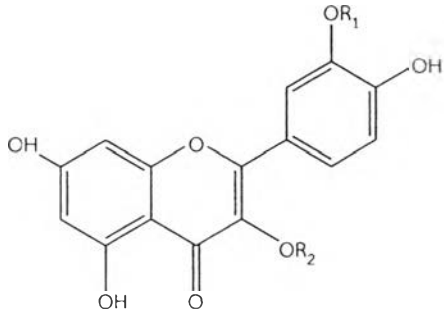


Dotricontanyl docosanoate **[34]**

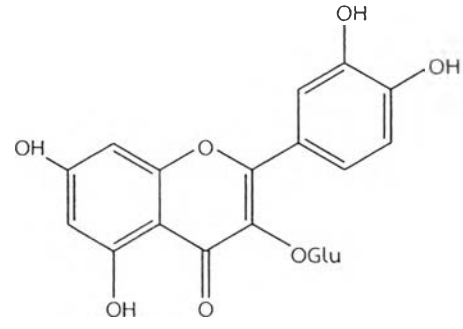


Triacontanol **[35]**

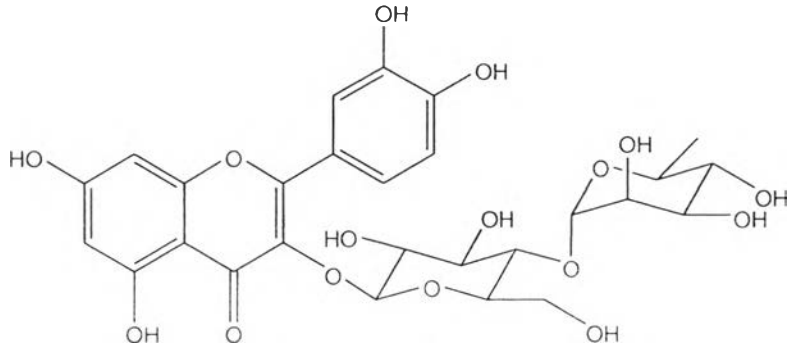
Figure 11 Structures of Fatty alcohol and analogues isolated from *P. amarus*



R_1	R_2	
H	Glu;	Astragalin **[36]**
H	H;	Quercetin **[37]**
H	Rha;	Quercitrin **[40]**
H	Rha-glu;	Rutin **[41]**



Quercetin-3-*O*-glucoside **[39]**



Quercetin-3-*O*-ß-D-glucopyranosyl-(1-4)-α-L-rhamnopyranoside **[38]**

Figure 12 Structures of flavonoids isolated from *P. amarus*

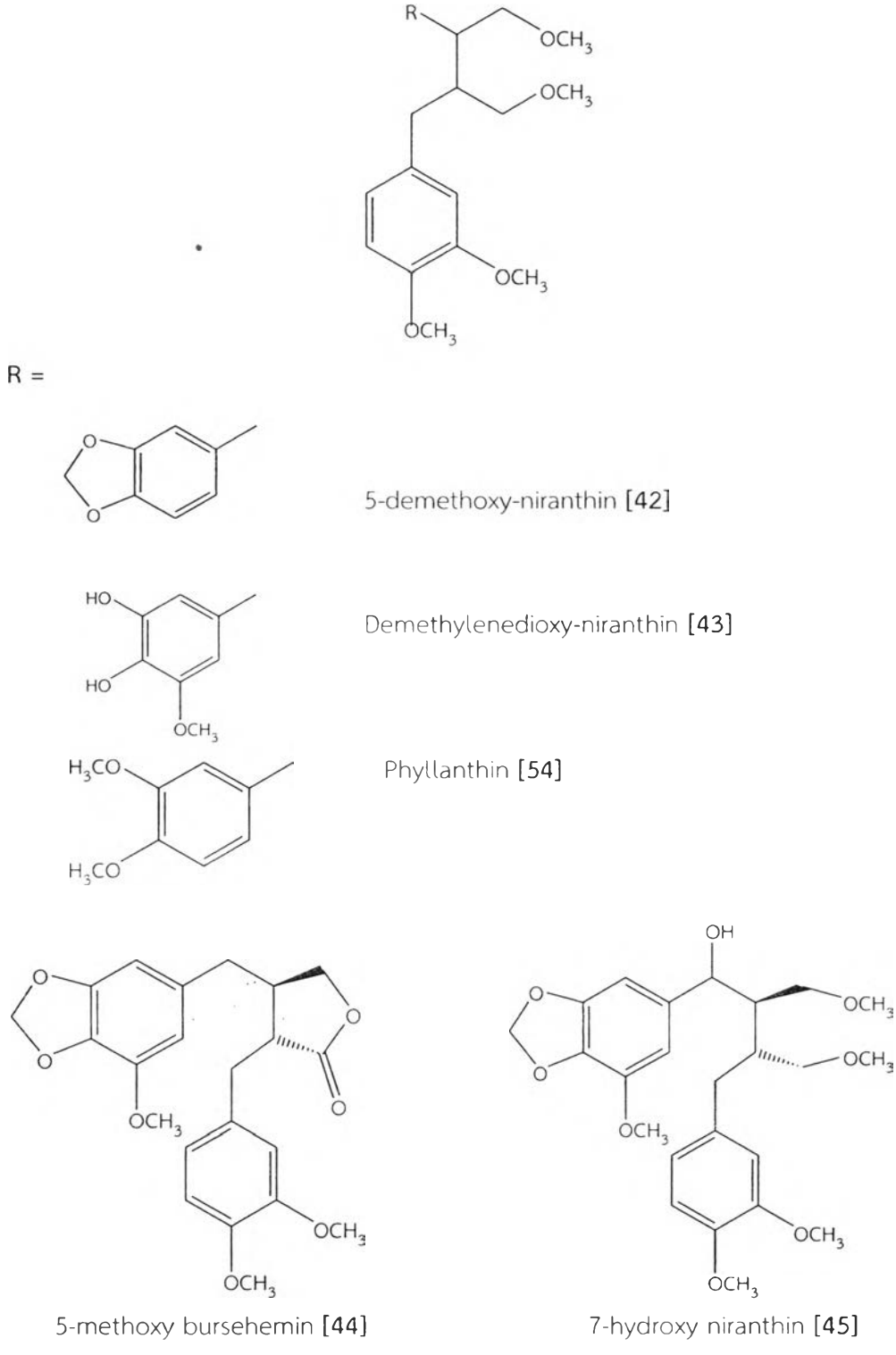
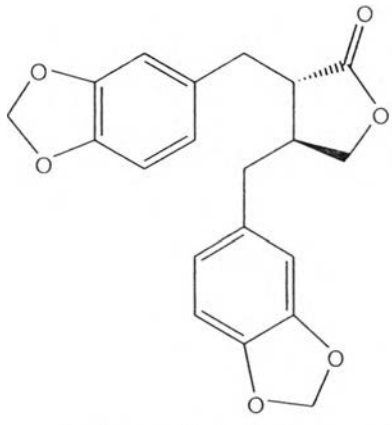
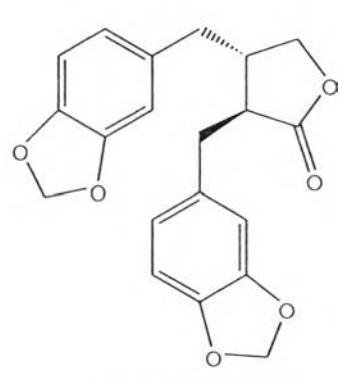


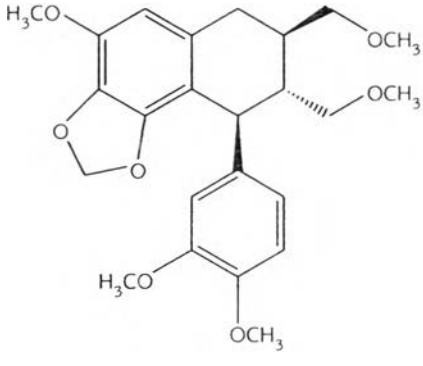
Figure 13 Structures of lignans isolated from *P. amarus*



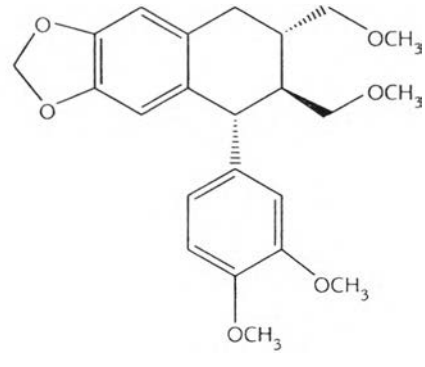
Heliobupthalmin lactone [46]



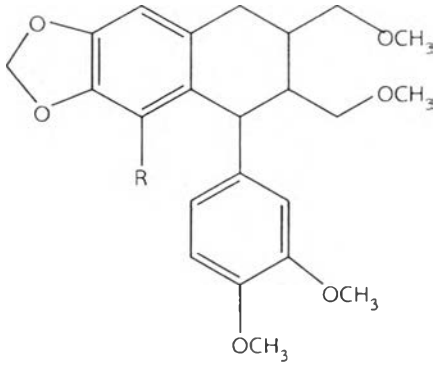
Hinokinin [47]



Hypophyllanthin [48]

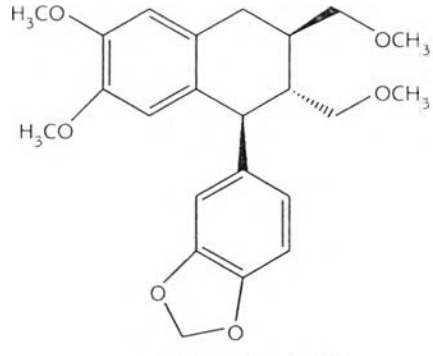


Isolintetralin [49]



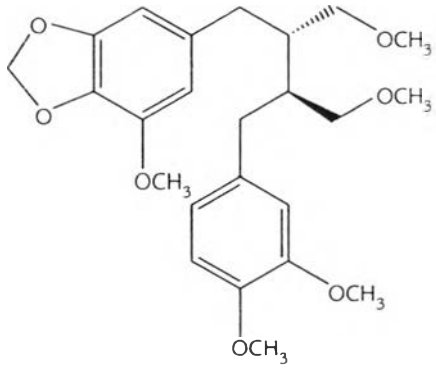
R = H; Isonirtetralin [50]

R = OCH_3; Phyltetralin [55]

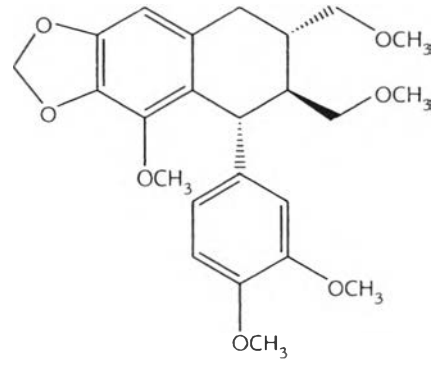


Lintetralin [51]

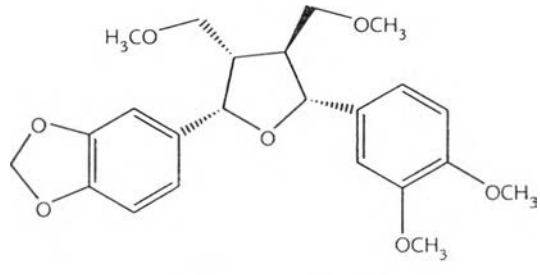
Figure 13 Structures of lignans isolated from *P. amarus* (continued).



Niranthin [52]

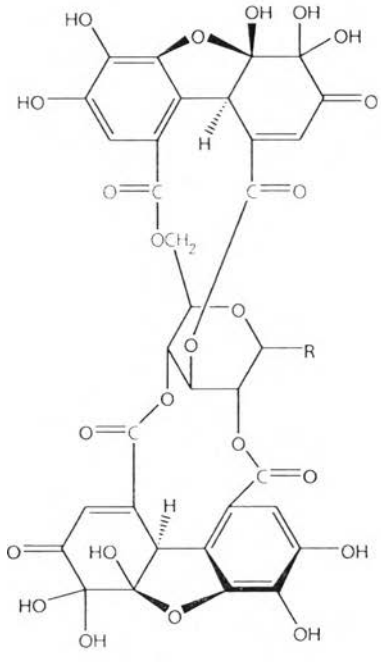


Nirtetralin [53]

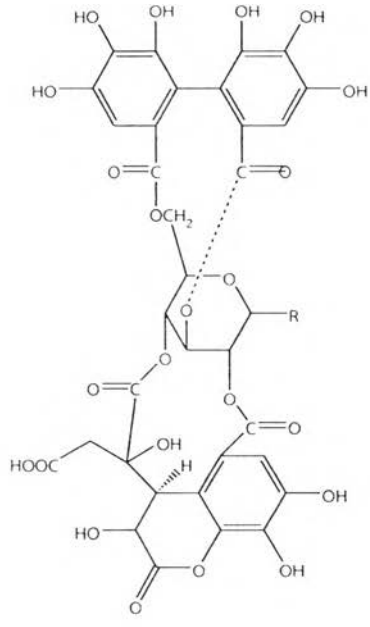


Virgatusin [56]

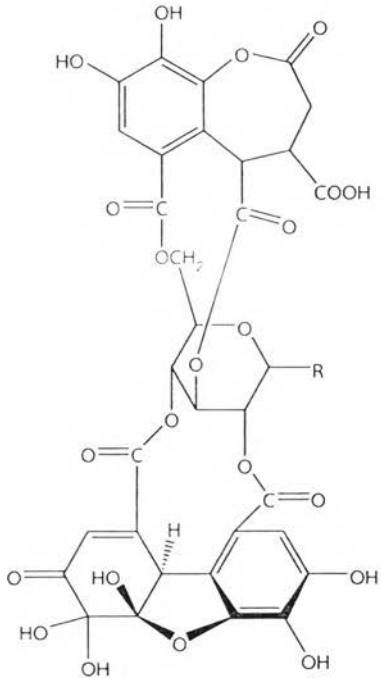
Figure 13 Structures of lignans isolated from *P. amarus* (continued).



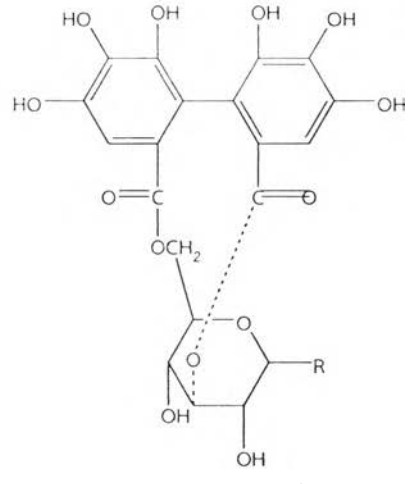
Amariin [571]



Amariinic acid [58]



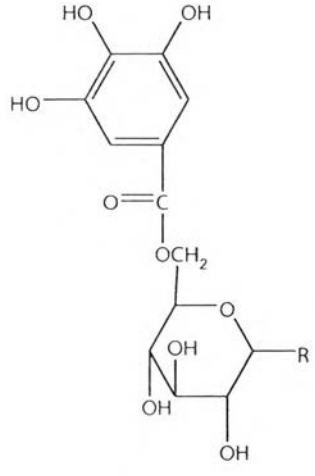
Amarulone [59]



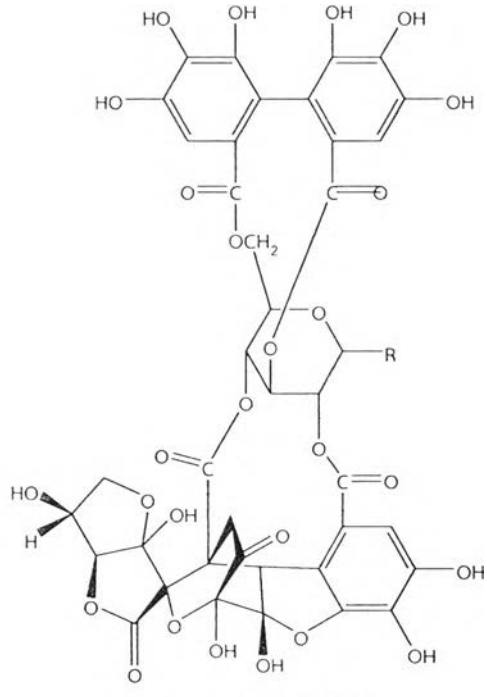
Corilagin [60]

R = Gallic acid

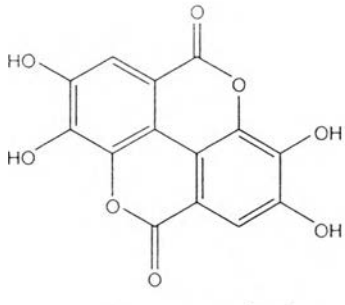
Figure 14 Structures of tannins isolated from *P. amarus*



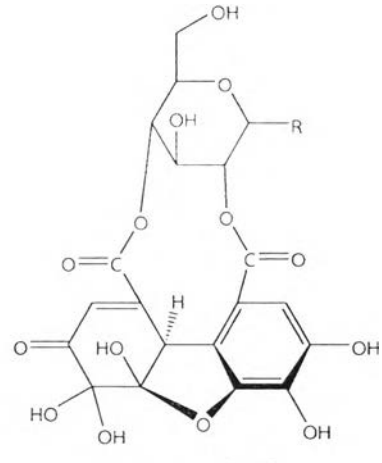
1,6-digalloylglucopyranose [61]



Elaeocarpusin [62]



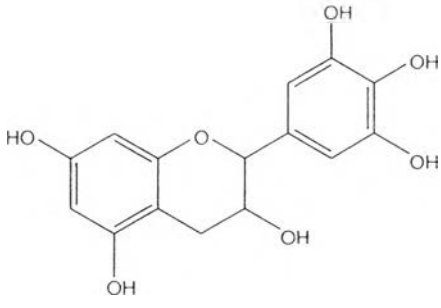
Ellagic acid [63]



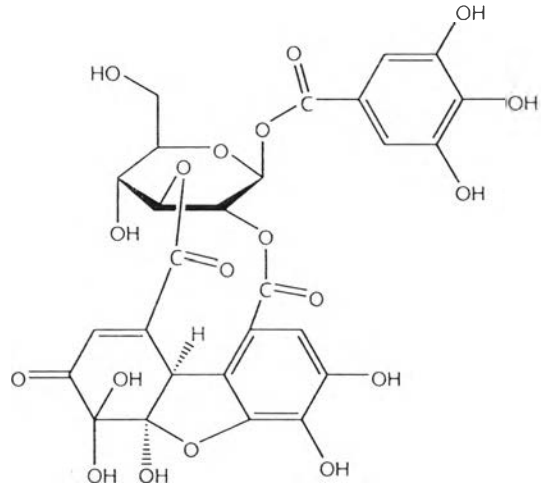
Furosin [64]

R = Gallic acid

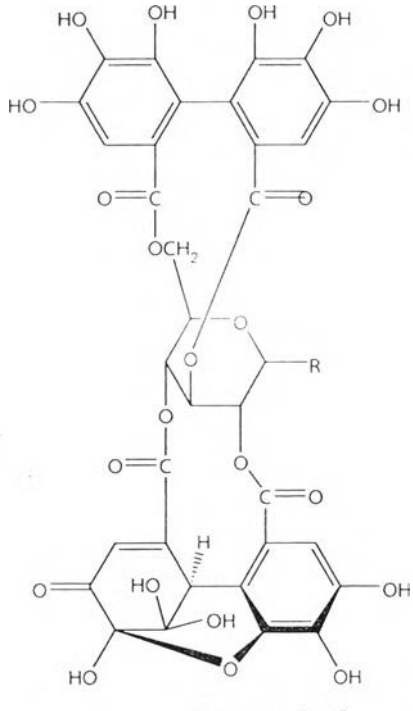
Figure 14 Structures of tannins isolated from *P. amarus* (continued).



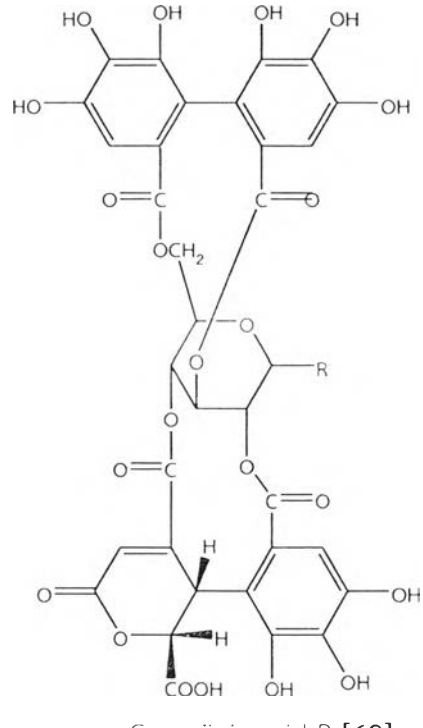
Gallocatechin [65]



1-galloyl-2, 3-DHHDP-glucose [66]



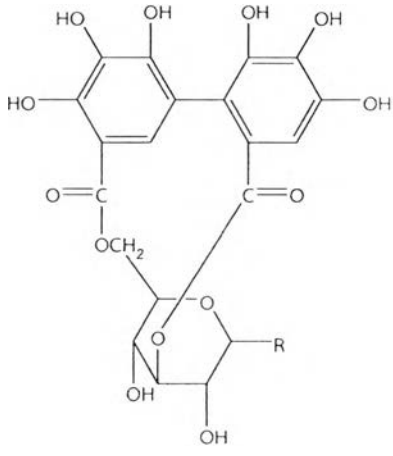
Geraniin [67]



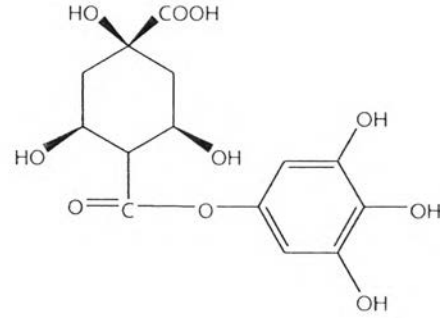
Geraniinic acid B [68]

R = Gallic acid

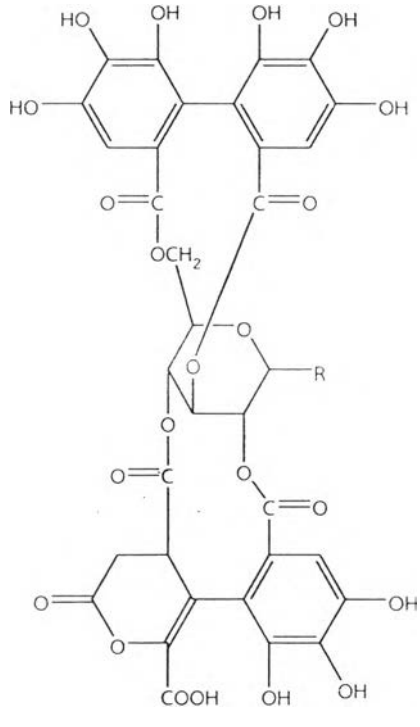
Figure 14 Structures of tannins isolated from *P. amarus* (continued).



Isocorilagin **[69]**



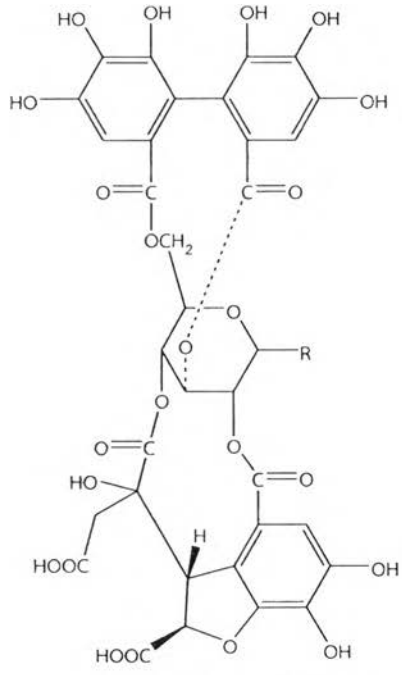
4-O-galloylquinic acid **[70]**



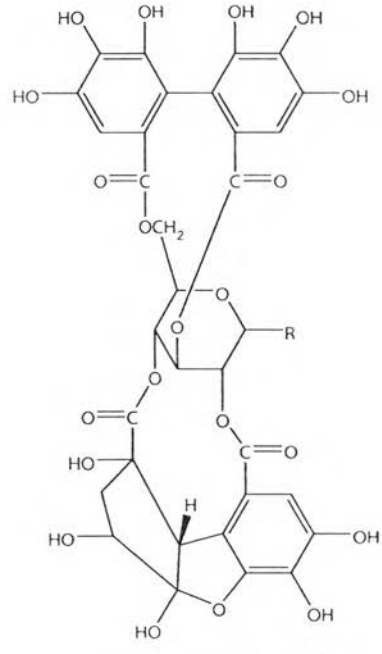
Phyllanthusiin A **[71]**

R = Gallic acid

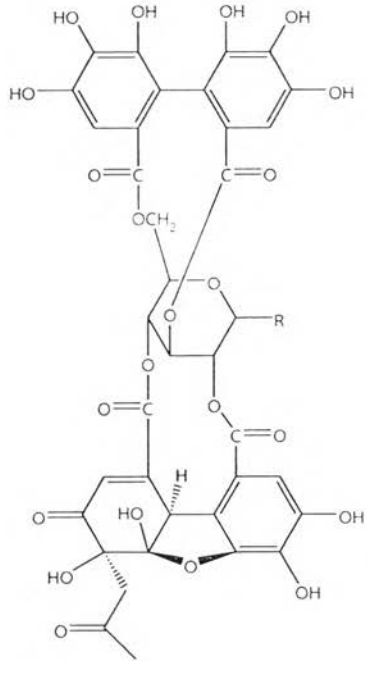
Figure 14 Structures of tannins isolated from *P. amarus* (continued).



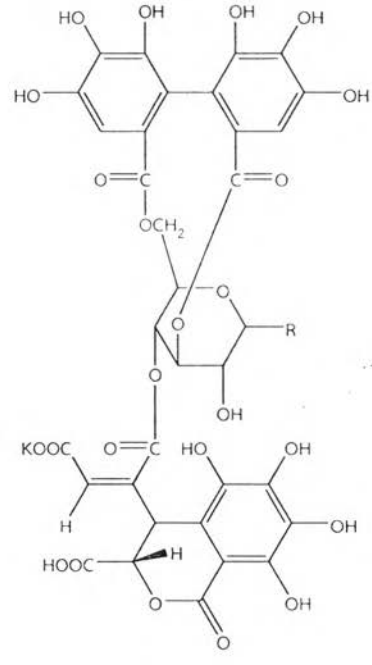
Phyllanthusiin B [72]



Phyllanthusiin C [73]



Phyllanthusiin D [74]



Repandusinic acid A (Potassium salt) [75]

R = Gallic acid

Figure 14 Structures of tannins isolated from *P. amarus* (continued).

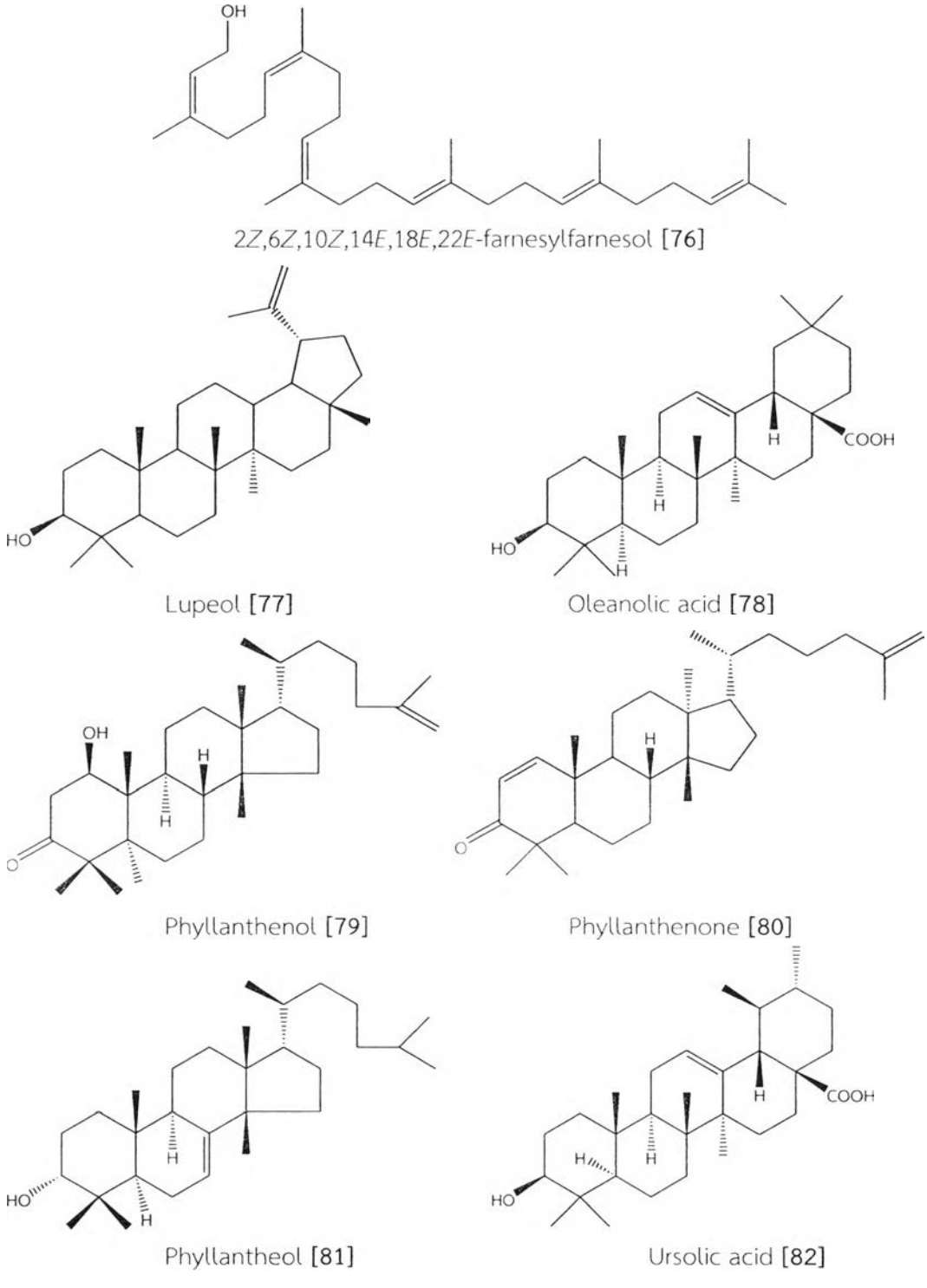
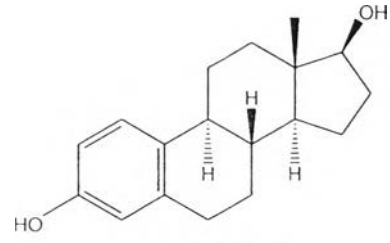
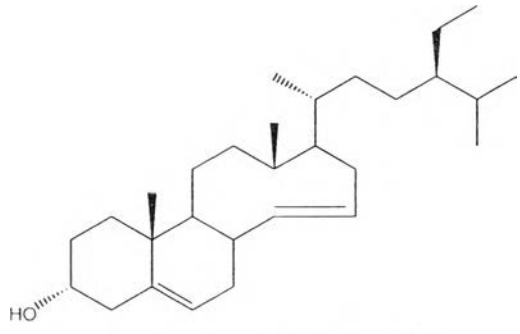


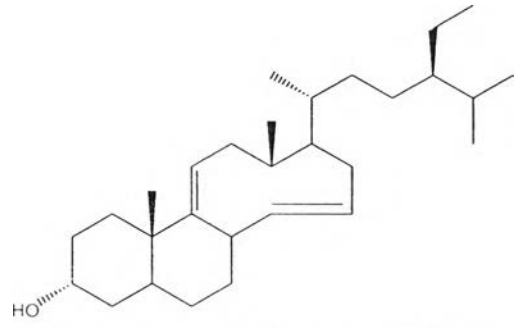
Figure 15 Structures of terpenoids isolated from *P. amarus*



Estradiol **[83]**

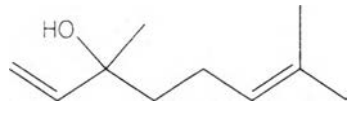


Amarosterol A **[84]**



Amarosterol B **[85]**

Figure 16 Structures of sterols isolated from *P. amarus*



Linalool **[86]**

Phytol **[87]**

Figure 17 Structures of volatile oils isolated from *P. amarus*

2.2.3 Bioactivities of *P. amarus*

P. amarus is a widely distributed medicinal plant with many beneficial activities. Different parts of *P. amarus* have been used for various treatments. For example, the whole plants are used for treating malaria, migraine, and jaundice (Shanmugam, *et al.* 2009). Leaves and roots of *P. amarus* are used for the treatment of chronic dysentery, diabetes, anorexia, and urinary tract infection (Sarin, *et al.* 2014). In addition, reports on *P. amarus* indicate that it has various pharmacological properties, including anti-inflammatory, antiplasmodial, antioxidant, and hepatoprotective activities.

Anti-inflammatory activity

The effects of 75% methanolic extract of *P. amarus* whole plant on different phases of inflammation were performed using phlogistic agents-induced paw oedema, carrageenan-induced air-pouch inflammation, and cotton pellet induced granuloma model in male Wister rats. The extract (250mg/kg body weight p.o.) inhibited carrageenan, bradykinin, serotonin, and prostaglandin E_1-induced paw oedema (Mahat and Patil 2007). The aqueous extract of *P. amarus* leaves was investigated for anti-inflammatory activity in rats. The extract increased the inhibition of the carrageenan- induced paw oedema in rats. The inhibition produced by 200mg/kg of the extract was significantly higher than that of acetylsalicylic acid, anti-inflammatory medicine, the reference drug (Iranloye, *et al.* 2011).

Antiplasmodial activity

The methylene chloride, methanolic, and ethanolic extracts of *P. amarus* entire plant showed significant activity against the chloroquine-sensitive strain of *Plasmodium falciparum* 3D7. The IC_{50} value of the methanolic and methylene chloride extract was 5 and 14.53 µg/mL, respectively (Adjobimey, *et al.* 2004). The aqueous and ethanolic extracts of *P. amarus* whole plant were administered to Swiss albino mice to investigate the antiplasmodial effect of the extract against *P. yoelii* infection and compared with the standard prophylactic and chemotherapeutic drugs. The drugs were used in chloroquine resistant plasmodium infection. The results indicated that both extracts of *P. amarus* possess repository and chemotherapeutic effects against resistant strains of *P. yoelii* in mice (Ajala, *et al.* 2011).

Antioxidant activity

The free – radical scavenging activity of 50% ethanolic extract of the aerial parts of *P. amarus* extract and phyllanthin were examined using DPPH assay. The results indicated that phyllanthin exhibited the antioxidant activity higher than *P. amarus* crude extract, which is clearly evident by a low IC_{50} value of 7.4µmol/mL (Krithika, *et al.* 2009). Amariin, repandusinic acid, and phyllanthusiin D showed high ability to scavenge free radicals in a range of systems including DPPH (2, 2-dipheyl-2-picrylhydrazyl), ABTS (2, 2-azobis-3-ethylbenzthiazoline-6-sulfonic acid)/ ferrylmyoglobin FRAP (ferric reducing antioxidant power), and pulse radiolysis and were compared with flavonoids compound, rutin and quercetin 3-*O*-glucoside, antioxidant compounds (Londhe, *et al.* 2008).

Anticancer activity

MNNG (N-methyl N'-nitro-N-nitrosoguanidine) induced stomach cancer in male Wistar rats was significantly inhibited by the administration of 75% methanolic extract of *P. amarus* aerial parts at a 150 and 750mg/kg body weight. The enzyme level in the stomach was found to reduce by the extract treatment (Raphael and Kuttan 2003). Cytotoxicity of the aqueous and methanolic crude extracts of *P. amarus* were screened using the 3-(4, 5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reduction assay. The aqueous extract inhibited MCF-7 (breast carcinoma) and A549 (lung carcinoma) cells growth with IC_5 values range from 56 to 126 µg/mL while the methanolic extract was showed with range from 150 to 240 µg/mL (Lee, *et al.* 2011).

Hepatoprotective activity

The whole plant has been widely used in traditional medicine to treat liver diseases. Hepatoprotective properties for the aqueous extract in the aerial part of the plant were demonstrated using animal models for paracetamol- (Wongnawa, *et al.* 2005), CCl_4- (Walaiphachara 1994, Yadav, *et al.* 2008), and ethanol-induced liver toxicity (Pramyothin, *et al.* 2007). The methanolic extract from *P. amarus* leaves protects the liver against ethanol in adult male rats (Faremi, *et al.* 2008). In addition, silymarin, which is a hepatoprotective drug, and the ethanolic extract from the whole *P. amarus* plant have been combined and exhibit a positive synergistic

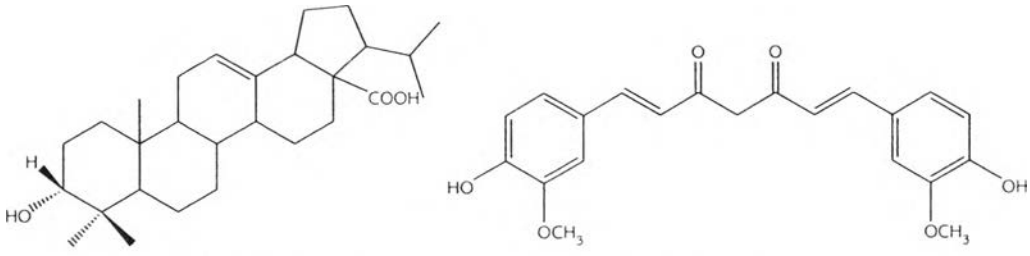
hepatoprotection effect, which was demonstrated through significant changes in various liver parameters for male mice (Yadav, *et al.* 2008).

2.3 Anti-hepatotoxic activity of some medicinal plants

Liver disease remains a worldwide health problem; thus the search for new medicines is still needed. A lot of medicinal plants have been reported for anti-hepatotoxic activity. The aqueous and methanolic extracts derived from 5 plants used in Rwanda for hepatoprotective activity including *Crassocepholum vitellinum*, *Guizotia scabro*, *Microglossa pyrifolia*, *Ocimum lamiifolium*, and *Vernonia lasiopus* leaves were tested *in vivo* on CCl_4-treated guinea pigs by the barbiturate-induced sleep modification method. *In vivo* results, the aqueous extracts of *O. lamiifolium*, *C. vitellinum*, and *G. scabra* leaves allowed the recovery of the sleeping time by 88.2%, 77.9%, and 61.4%, respectively. The results confirm those obtained on rat PCLS (Precision-cut liver slices) with the methanolic leaves extracts of *O. lamiifolium* and *C. vitellinum* protecting the liver slices against acetaminophen hepatotoxicity (Mukazayire, *et al.* 2010).

The plant *Crotalaria emarginella* Vatke (Leguminosae) is distributed in the tropical East Africa and used in traditional medicine for fever, impetigo, scabies, and as a colic remedy (Chaudhary, *et al.* 2010). A triterpene compound, crotalic acid, was isolated from the aerial parts of *C. emarginella*, which also showed anti-hepatotoxic activity comparable with standard drug silymarin against CCl_4 induced toxicity in Wistar rats (Ahmed, *et al.* 2006).

Curcumin, a polyphenol compound in the rhizome of *Curcuma longa*, could protect the liver against injury and fibrogenesis by suppressing hepatic inflammation, attenuating hepatic oxidative stress (Mathuria and Verma 2007, Verma and Mathuria 2008), and inhibiting hepatic stellate cells activation (Zheng, *et al.* 2007). In addition, curcumin was shown as a potential protective agent against liver damage induced by heavy metal such as arsenic, cadmium, copper, lead, and mercury (García-Niño and Pedraza-Chaverri 2014).



Crotalic acid Curcumin

Figure 18 Anti-hepatotoxic compounds from some medicinal plant.