CHAPTER 1

Introduction

1.1 *Vernonia scandens*

The genus *Vernonia*, in Compositae family, comprising about 1000 species is mainly distributed in the torrid zones of America, Asia and Africa. About 27 species of this genus grow in the southern part of China, many of them have potential applications in Chinese folklore medicine [1]. Several *Vernonia* species are widely used in native cultures as folklore remedies for a variety of human ailments. It should be noted that flavonoids, sesquiterpenoids, diterpenoids, triterpenoids, alkaloids and cardiac glycosides have also been reported from this genus [2, 3]. This class of compounds has been reported to be insect antifeedant, antifungal, cytotoxic and antitumor activities [4].

However, phytochemical and biological studies have not been done on *V. scandens*. Therefore, we will focus on the investigation of chemical composition and bioactive substances from this species.

1.1.1 The botany of *Vernonia scandens*

Kingdom:	*Plantae*
Phylum:	*Tracheophyta*
Class:	*Magnoliosida*
Order:	*Asterales*
Family:	*Asteraceae*
Genus:	*Vernonia* [5]

The aerial parts of *V. scandens*, commonly known as Gu-si-pah-doh (in Lua language), was collected from the northern part of Mae Jam District in Chiang Mai Province of Thailand, in March 2008, and identified by Maxwell J.F. and a voucher specimen (MAXWELL 08-54) was deposited in the herbarium of Department of Biology, Faculty of Science, Chiang Mai University, Thailand. The plant was identified and the specimen (BKF. No. 130768) has been deposited in the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of National Resources and Environment, Bangkok, Thailand as shown in Figure 1.1 [6].

Figure 1.1 Morphological illustration of *V. scandens*

1.2 Reviews of chemical constituents of *Vernonia* genus

Vernonia plants contains several types of chemical constituents. The compounds which had been reviewed are classified into two main groups *i.e.* terpenes (sesquiterpenes, diterpenes, and steroids) and phenols (flavonoids and phenylpropanoids) as follows;

1.2.1 Terpenes

The terpenes are among the most widespread and chemically diverse groups of natural products. Fortunately, despite their structural diversity, they have a simple unifying feature by which they are defined and by which they may be easily classified. Terpenes are a unique group of hydrocarbon-based natural products whose structure may be derived from isoprene, give rise to structures which may be divided into isopentane (2-methylbutane) units [7] (Figure 1.2).

isoprene (**1**)
(2-methyl-1,3-butadiene)

isopentane (**2**)
(2-methylbutane)
(isopentane)

Figure 1.2 The terpenes are comprised of isoprene units

Terpenes are thus classified by the number of 5-carbon units they contain: hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}) and tetraterpenes (C_{40}).

1) Sesquiterpenes

Derived from three isoprene units, C_{15} sesquiterpenes exist in a wide variety of forms, including linear, bicyclic, and tricyclic frameworks. Like the monoterpenes, most sesquiterpenes are considered to be essential oils because they belong to the steam distillable fraction often containing the characteristic odoriferous components of the plant. Some common sesquiterpenes are shown in Figure 1.3 [7].

The reports of sesquiterpenes from some *Vernonia* genus are shown in Table 1.1 and Figure 1.3, respectively.

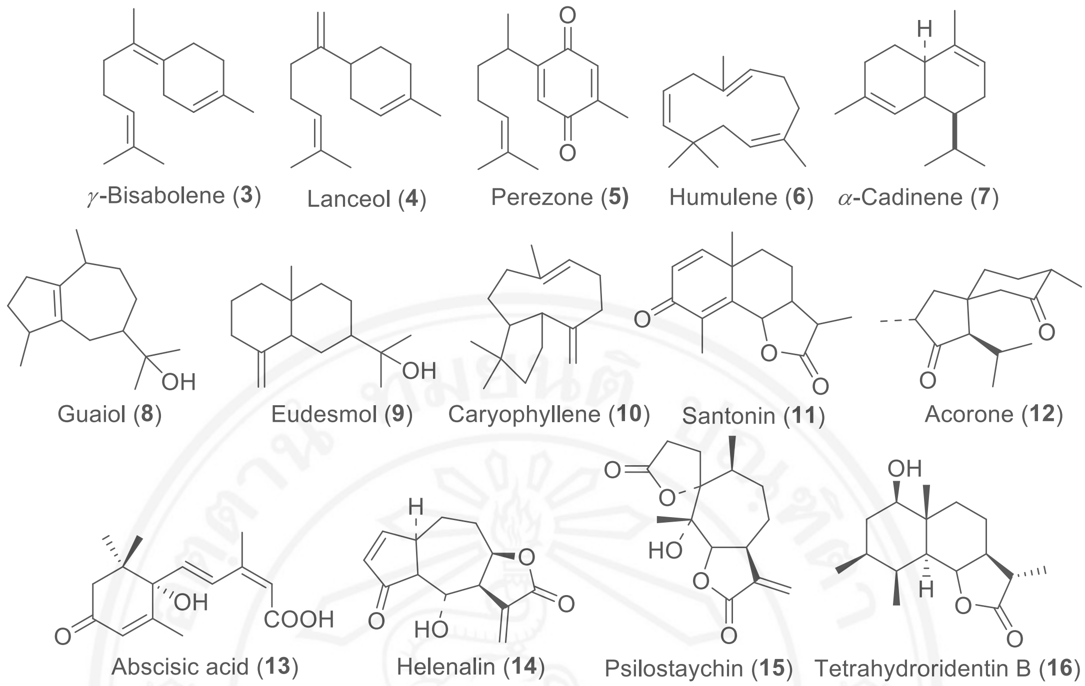


Figure 1.3 Some common sesquiterpenes in plants

Table 1.1 Sesquiterpenes from *Vernonia* genus

Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
Glaucolide B (**17**) [*V. fruticulosa*]	Aerial parts [hexane-EtOAc (4: 1) and EtOH]	-	1997[8]
1-Acetoxyhirsutinolide (**18**) [*V. fruticulosa*]	Aerial parts [hexane-EtOAc (4: 1) and EtOH]	-	1997[8]

Table 1.1 (Continued)

Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
Vernolide (**19**) [*V. colorata*][a,c] [*V. amygdalina*][b]	Leaves [a,b,c] [EtOAc][a] [99%EtOH][b] [Acetone] [c]	Antibacterial[a,b] Antifungal[b] Antiplasmodial activity [c] *P. falciparum* (D10) with IC_{50} 1.87 μg/ml Cytotoxicity (CHO) with IC_{50} 1.83 μg/ml	2002[9][a] 2006[4][b] 2009[10][c]
11*β*,13-Dihydrovernolide (**20**) [*V. colorata*]	Leaves [EtOAc]	Antibacterial	2002[9]
Vernodalin (**21**) [*V. colorata*][a,b]	Leaves[a,b] [EtOAc][a] [Acetone][b]	Antibacterial[a] Antiplasmodial activity[b] *P. falciparum* (D10) with IC_{50} 0.52 μg/ml Cytotoxicity (CHO) with IC_{50} 1.45 μg/ml	2002[9][a] 2009[10][b]
Zaluzanin D (**22**) [*V. arborea*]	Aerial parts	Antifungal	2003[11]
Vernolide A (**23**) [*V. cinerea*][a,b]	Stems[a] [EtOH][a] Whole plants[b] [95%EtOH][b]	-	2003[12][a] 2006[13][b]
Vernolide B (**24**) [*V. cinerea*][a,b]	Stems[a] [EtOH][a] Whole plants[b] [95%EtOH][b]	-	2003[12][a] 2006[13][b]
Glaucolide K (**25**) [*V. pachyclada*]	Leaves [EtOH]	-	2005[14]
Glaucolide L (**26**) [*V. pachyclada*]	Leaves [EtOH]	-	2005[14]

Table 1.1 (Continued)

Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
Glaucolide M (**27**) [*V. pachyclada*]	Leaves [EtOH]	-	2005[14]
8*β*-(3-Chloro-2-hydroxy-2-methylpropanoyloxy)-4*β*,6*α*,10*β*-trihydroxyl-13-ethoxy-7(11)-guaiaen-12,6-olide (**28**) [*V. cinerea*]	Whole plants [95%EtOH]	-	2006[13]
8*α*-[4-Hydroxytigloyloxy]-hirsutinolide-13-*O*-acetate (**29**) [*V. cinerea*]	Whole plants [95%EtOH]	-	2006[13]
8*α*-[4-Hydroxytigloyloxy]-10*α*-hydroxyhirsutinolide-13-*O*-acetate (**30**) [*V. cinerea*]	Whole plants [95%EtOH]	-	2006[13]
8*α*-Tigloyloxyhirsutinolide (**31**) [*V. cinerea*]	Whole plants [95%EtOH]	-	2006[13]
8*α*-Tigloyloxyhirsutinolide-13-*O*-acetate (**32**) [*V. cinerea*]	Whole plants [95%EtOH]	-	2006[13]
8*α*-[2-Methylacryloyloxy]-hirsutinolide (**33**) [*V. cinerea*]	Whole plants [95%EtOH]	-	2006[13]
8*α*-[2-Methylacryloyloxy]-hirsutinolide-13-*O*-acetate (**34**) [*V. cinerea*]	Whole plants [95%EtOH]	-	2006[13]

Table 1.1 (Continued)

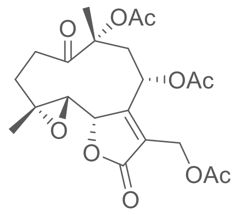
Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
8α-[2-Methylacryloyloxy]-1β,4β-epoxy-1α-methoxy-10βH-germacra-5*E*,7(11)-dien-12,6-olide (**35**) [*V. cinerea*]	Whole plants [95%EtOH]	-	2006[13]
8α-[2-Methylacryloyloxy]-1β,4β-epoxy-1α-methoxy-13-*O*-acetate-10βH-germacra-5*E*,7(11)-dien-12,6-olide **(36)** [*V. cinerea*]	Whole plants [95%EtOH]	-	2006[13]
Vernolide C (**37**) [*V. cinerea*]	Whole plants [CH_2Cl_2]	Antiplasmodial activity *P. falciparum* (W2) with IC_{50} 3.9 mM	2006[15]
Vernolide D (**38**) [*V. cinerea*]	Whole plants [CH_2Cl_2]	-	2006[15]
8α-Tigloyloxyhirsutinolide-13-*O*-acetate (**39**) [*V. cinerea*]	Whole plants [CH_2Cl_2]	-	2006[15]
8α-Tigloyloxyhirsutinolide (**40**) [*V. cinerea*]	Whole plants [CH_2Cl_2]	-	2006[15]
8α-(4-Hydroxylmethacryl-oyloxy)-hirsutinolide-13-*O*-acetate (**41**) [*V. cinerea*]	Whole plants [CH_2Cl_2]	-	2006[15]
8α-Epoxymethacryloyloxy hirsutinolide-13-*O*-acetate (**42**) [*V. cinerea*]	Whole plants [CH_2Cl_2]	-	2006[15]

Table 1.1 (Continued)

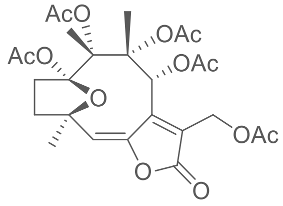
Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
Hirsutinolide-13-*O*-acetate (**43**) [*V. cinerea*]	Whole plants [CH_2Cl_2]	Antiplasmodial activity *P. falciparum* (W2) with IC_{50} 3.7 mM	2006[15]
Piptocarphin D (**44**) [*V. cinerea*]	Whole plants [CH_2Cl_2]	Antiplasmodial activity *P. falciparum* (W2) with IC_{50} 3.5 mM	2006[15]
Vernodalol (**45**) [*V. amygdalina*]	Leaves [99%EtOH]	Antibacterial Antifungal	2006[4]
8α-(4-Hydroxymethacryl- oyloxy)-10α-hydroxy-1,13- dimethoxyhirsutinolide (**46**) [*V. triflosculosa*]	Aerial parts [Methyl *t*-butyl ether (MTBE)- MeOH (8:2)]	Inhibition on IL-8 production in HeLa cells.	2006[3]
8α-(4-Hydroxymethacryl- oyloxy)-10α-hydroxy-13- methoxy hirsutinolide (**47**) [*V. triflosculosa*]	Aerial parts [Methyl *t*-butyl ether (MTBE)- MeOH (8:2)]	Inhibition on IL-8 production in HeLa cells.	2006[3]
8α-Methacryloyloxy)-10α- hydroxy-13-methoxy hirsutinolide (**48**) [*V. triflosculosa*]	Aerial parts [Methyl *t*-butyl ether (MTBE)- MeOH (8:2)]	Inhibition on IL-8 production in HeLa cells.	2006[3]
8α-(2-Methylacryloyloxy)-3- oxo-1-desoxy-1,2-dehydro- hirsutinolide-13-*O*-acetate (**49**) [*V. staehelinoides*]	Leaves [CH_2Cl_2]	Antiplasmodial activity *P. falciparum* (D10) with IC_{50} 260 ng/ml *P. falciparum* (K1) with IC_{50} 1,800 ng/ml Cytotoxicity (CHO) with IC_{50} 2.9 µg/ml	2007[16]

Table 1.1 (Continued)

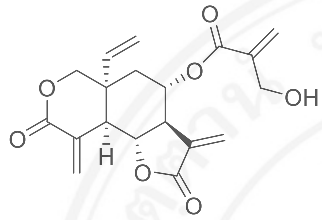
Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
8α-(5′-Acetoxysenecioyl-oxy)-3-oxo-1-desoxy-1,2-dehydro-hirsutinolide-13-*O*-acetate (**50**) [*V. staehelinoides*]	Leaves [CH_2Cl_2]	Antiplasmodial activity *P. falciparum* (D10) with IC_{50} 240 ng/ml *P. falciparum* (K1) with IC_{50} 2,600 ng/ml Cytotoxicity (CHO) with IC_{50} 0.9 µg/ml	2007[16]
Vernobockolides A (**51**) [*V. bockiana*]	Aerial parts [EtOH]	-	2008[1]
(1*S**,4*R**,8*S**,10*R**)-13-Acetyloxy-1,4-epoxy-1,10-dihydroxy-8-isobutyryloxy-germacra-5*E*,7(11)-dien-6,12-olide (**52**) [*V.* bockiana]	Aerial parts [EtOH]	-	2008[1]
Vernobockolides B (**53**) [*V. bockiana*]	Aerial parts [EtOH]	-	2008[1]
Piptocarphin C (**54**) [*V. bockiana*]	Aerial parts [EtOH]	-	2008[1]
Piptocarphin F (**55**) [*V. bockiana*]	Aerial parts [EtOH]	-	2008[1]
Piptocarphin A (**56**) [*V. bockiana*]	Aerial parts [EtOH]	-	2008[1]
Hirsutolide (**57**) [*V. bockiana*]	Aerial parts [EtOH]	-	2008[1]
β-D-Glucopyranosyl taraxinic ester (**58**) [*V. bockiana*]	Aerial parts [EtOH]	-	2008[1]



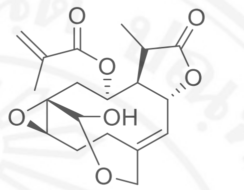
Glaucolide B (**17**)



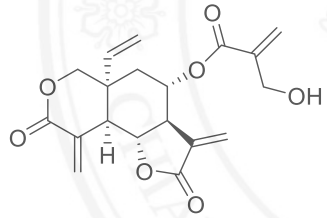
1-Acetoxyhirsutinolide (**18**)



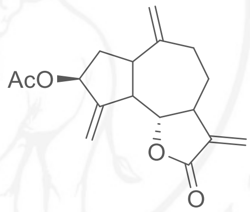
Vernolide (**19**)



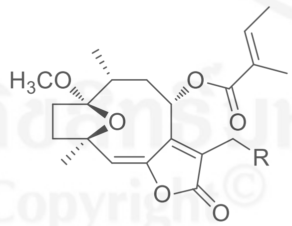
11β, 13-Dihydrovernolide (**20**)



Vernodalin (**21**)



Zaluzanin D (**22**)

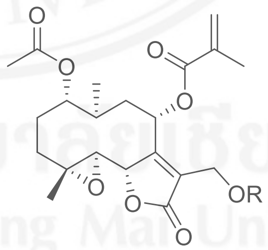


23: R = OH

24: R = OAc

Vernolide A (**23**)

Vernolide B (**24**)



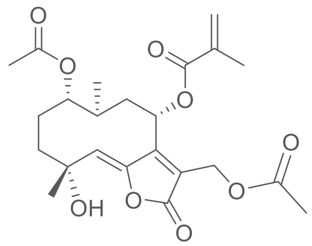
25: R = Ac

26: R = H

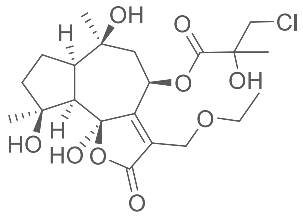
Glaucolide K (**25**)

Glaucolide L (**26**)

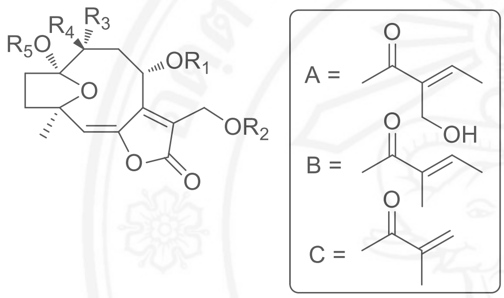
Figure 1.4 Sesquiterpenes from *Vernonia* genus



Glaucolide M (**27**)



8β-(3-Chloro-2-hydroxy-2-methylpropanoyloxy)-4β,6α,10β-trihydroxyl-13-ethoxy-7(11)-guaiaen-12,6-olide (**28**)



29: R_1 = A, R_2 = Ac, R_3 = CH_3, R_4 = H, R_5 = H

30: R_1 = A, R_2 = Ac, R_3 = OH, R_4 = CH_3, R_5 = H

31: R_1 = B, R_2 = H, R_3 = CH_3, R_4 = H, R_5 = H

32: R_1 = B, R_2 = Ac, R_3 = CH_3, R_4 = H, R_5 = H

33: R_1 = C, R_2 = H, R_3 = CH_3, R_4 = H, R_5 = H

34: R_1 = C, R_2 = Ac, R_3 = CH_3, R_4 = H, R_5 = H

35: R_1 = C, R_2 = H, R_3 = CH_3, R_4 = H, R_5 = CH_3

36: R_1 = C, R_2 = Ac, R_3 = CH_3, R_4 = H, R_5 = CH_3

8α-[4-Hydroxytigloyloxy]-hirsutinolide-13-*O*-acetate (**29**)

8α-[4-Hydroxytigloyloxy]-10α-hydroxyhirsutinolide-13-*O*-acetate (**30**)

8α-Tigloyloxyhirsutinolide (**31**)

8α-Tigloyloxyhirsutinolide-13-*O*-acetate (**32**)

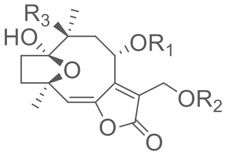
8α-[2-Methylacryloyloxy]-hirsutinolide (**33**)

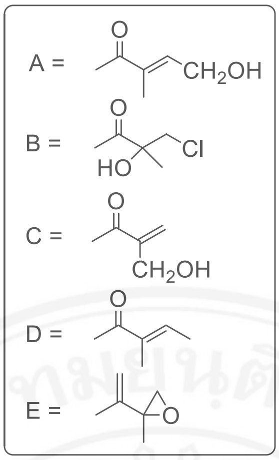
8α-[2-Methylacryloyloxy]-hirsutinolide-13-*O*-acetate (**34**)

8α-[2-Methylacryloyloxy]-1β,4β-epoxy-1α-methoxy-10βH-germacra-5*E*,7(11)-dien-12,6-olide (**35**)

8α-[2-Methylacryloyloxy]-1β,4β-epoxy-1α-methoxy-13-*O*-acetate-10βH-germacra-5*E*,7(11)-dien-12,6-olide **(36)**

Figure 1.4 (Continued)





37: R_1 = D; R_2 = Ac; R_3 = H

38: R_1 = E; R_2 = Ac; R_3 = H

39: R_1 = D; R_2 = H; R_3 = H

40: R_1 = B; R_2 = Ac; R_3 = H

41: R_1 = H; R_2 = Ac; R_3 = H

42: R_1 = H; R_2 = Ac; R_3 = OH

43: R_1 = C; R_2 = Ac; R_3 = H

44: R_1 = A; R_2 = Ac; R_3 = H

Vernolide C (**37**)

Vernolide D (**38**)

8α-Tigloyloxyhirsutinolide-13-*O*-acetate (**39**)

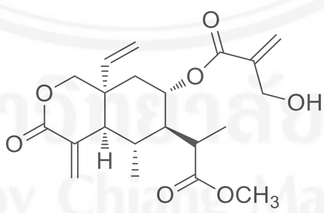
8α-Tigloyloxyhirsutinolide (**40**)

8α-(4-Hydroxylmethacryloyloxy)-hirsutinolide-13-*O*-acetate (**41**)

8α-Epoxymethacryloyloxy hirsutinolide-13-*O*-acetate (**42**)

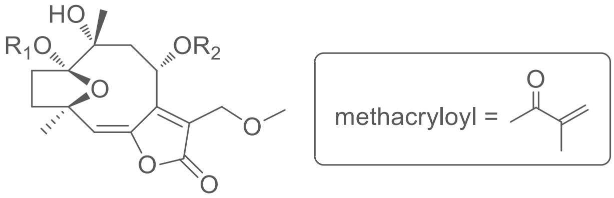
Hirsutinolide-13-*O*-acetate (**43**)

Piptocarphin D (**44**)



Vernodalol (**45**)

Figure 1.4 (Continued)



46: R_1 = CH_3, R_2 = 4-OH-methacryloyl

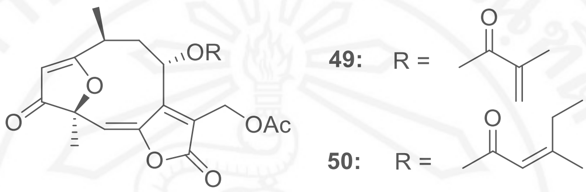
47: R_1 = H, R_2 = 4-OH-methacryloyl

48; R_1 = H, R_2 = methacryloyl

8α-(4α-Hydroxymethacryloyloxy)-10α-hydroxy-1,13-dimethoxyhirsutinolide (**46**)

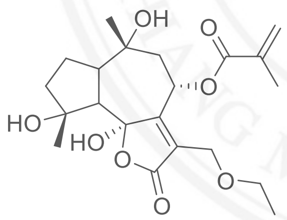
8α-(4-Hydroxymethacryloyloxy)-10α-hydroxy-13-methoxyhirsutinolide (**47**)

8α-(Methacryloyloxy)-10α-hydroxy-13-methoxyhirsutinolide (**48**)

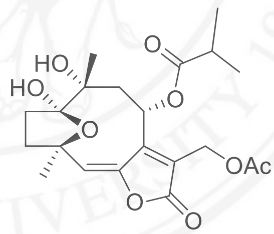


8α-(2-Methylacryloyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate (**49**)

8α-(5′-Acetoxysenecioyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate (**50**)

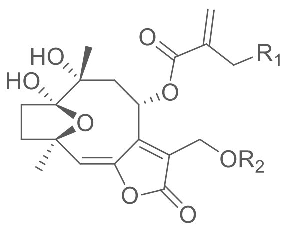


Vernobockolides A (**51**)



(1*S**,4*R**,8*S**,10*R**)-13-Acetyloxy-
1,4-epoxy-1,10-dihydroxy-8-isobutyryl-
oxygermacra-5*E*,7(11)-dien-6,12-olide (**52**)

Figure 1.4 (Continued)



53: R_1 = OH, R_2 = Et

54: R_1 = H, R_2 = H

55: R_1 = H, R_2 = Et

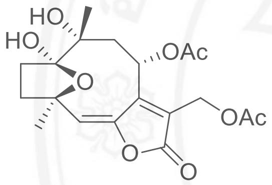
56: R_1 = H, R_2 = Ac

Vernobockolides B (**53**)

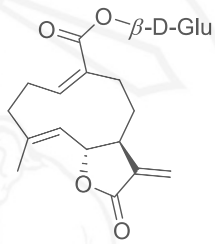
Piptocarphin C (**54**)

Piptocarphin F (**55**)

Piptocarphin A (**56**)



Hirsutolide (**57**)



β-D-Glucopyranosyltaraxinicester (**58**)

Figure 1.4 (Continued)

2) Diterpenes

The diterpenes are a widely varied group of compounds based on four isoprene groups, most of which are of limited distribution in the plant kingdom. Because of their higher boiling points, they are not considered to be essential oils. Instead, they are classically considered to be resins, the material that remains after steam distillation of a plant extract. Diterpenes exist in a variety of structural types some selections are shown is Figure 1.5 [7].

The reports of diterpenes from some *Vernonia* genus are summarized in Table 1.2 and Figure 1.6, respectively.

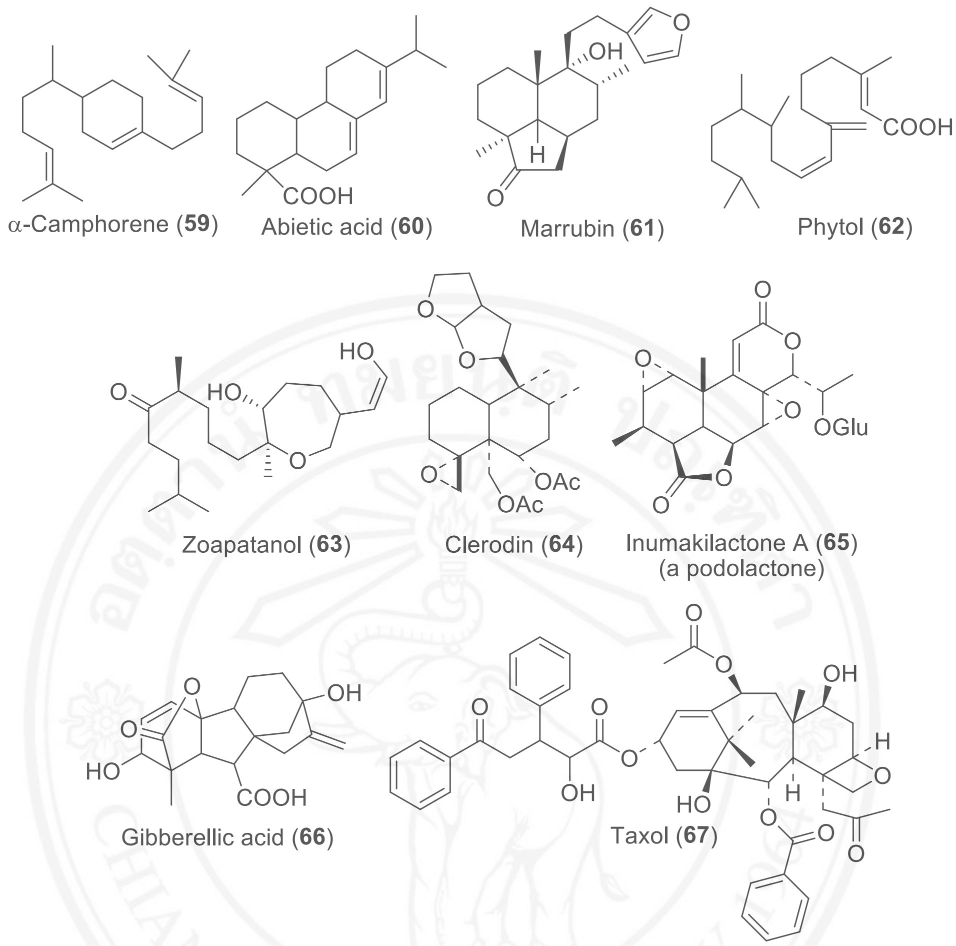
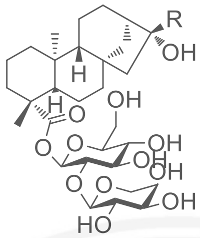


Figure 1.5 Some common diterpenes in plants

Table 1.2 Diterpene from *Vernonia* genus

Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
16*β*-Hydroxy-*ent*-kauran-19-oic acid -19-[*α*-L-arabinopyranosyl-(1→2)-*β*-D-glucopyranosyl] ester (**68**) [*V. triflosculosa*]	Aerial parts [Methyl *t*-butyl ether (MTBE)-MeOH (8:2)]	Inhibition on IL-8 production in HeLa cells.	2006[3]
16*β*-17-Dihydroxy-*ent*-kauran-19-oic acid-19-[*α*-L-arabinopyranosyl-(1→2)-*β*-D-glucopyranosyl] ester (**69**) [*V. triflosculosa*]	Aerial parts [Methyl *t*-butyl ether (MTBE)-MeOH (8:2)]	Inhibition on IL-8 production in HeLa cells.	2006[3]



68: R = CH_3

69: R = CH_2OH

16*β*-Hydroxy-*ent*-kauran-19-oic acid-19-[*α*-L-arabinopyranosyl-(1→2)-*β*-D-glucopyranosyl] ester (**68**)

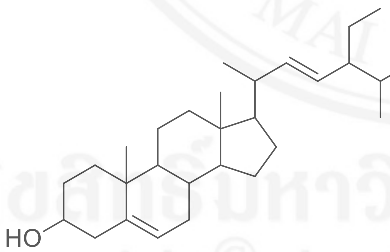
16*β*-17-Dihydroxy-*ent*-kauran-19-oic acid-19-[*α*-L-arabinopyranosyl-(1→2)-*β*-D-glucopyranosyl] ester (**69**)

Figure 1.6 Diterpenes from *Vernonia* genus

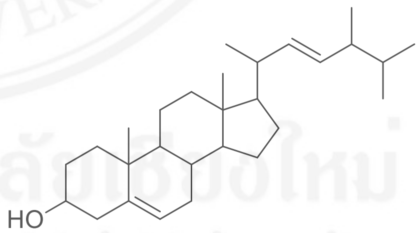
3) Steroids

Practically all plant steroids are hydroxylated at C-3 and are fact sterols. In the animal kingdom, the steroids have profound importance as hormones, coenzymes, and provitamins. The general steroid structure is shown in Figure 1.7 [7].

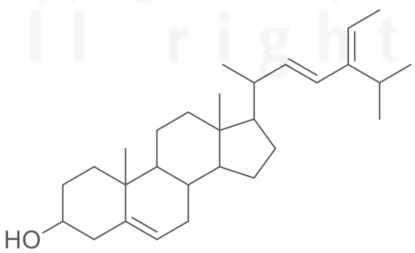
The reports of steroid compounds from some *Vernonia* genus are shown in Table 1.3 and Figure 1.8, respectively.



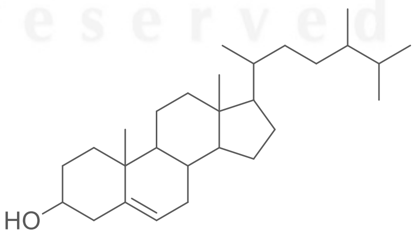
Stigmasterol (**70**)



Ergosterol (**71**)



Fucosterol (**72**)



Cholesterol (**73**)

Figure 1.7 Some plant sterol natural products

Table 1.3 Steroids from *Vernonia* genus

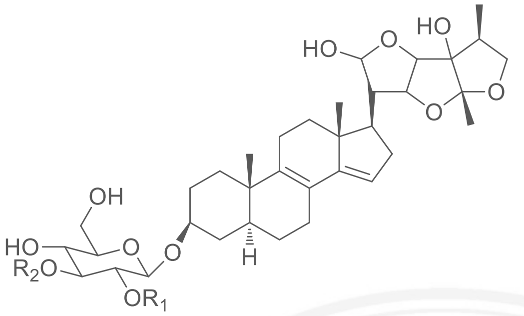
Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
Vernionioside D_1 (**74**) [*V. kotschyana*]	Roots -	-	1998[17]
Vernionioside D_2 (**75**) [*V. kotschyana*]	Roots -	-	1998[17]
Vernionioside D_3 (**76**) [*V. kotschyana*]	Roots -	-	1998[17]
Vernionioside F_1 (**77**) [*V. kotschyana*]	Roots -	-	1998[17]
Vernionioside F_2 (**78**) [*V. kotschyana*]	Roots -	-	1998[17]
Vernoguinosterol (**79**) [*V. guineensis*]	Stem barks [EtOH]	-	2002[18]
16β,22*R*;21,23*S*-Diepoxy-3β-O-β-D-glucopyranosyloxy-21*S*,24-dihydroxy-5α-stigmasta-8,14-dien-28-one (**80**) [*V. guineensis*]	Stem barks [CH_2Cl_2-MeOH (1:1)]	-	2003[2]
16β,22*R*;21,23*S*-Diepoxy-21*S*,24-dihydroxy-5α-stigmasta-8,14-dien-28-one (**81**) [*V. guineensis*]	Stem barks [CH_2Cl_2-MeOH (1:1)]	-	2003[2]
3-*O*-{β-D-Galactopyranosyl-(1-2)-[β-D-glucopyranosyl-(1$\rightarrow$6)]-β-D-glucopyranoside}-5α,14α-androst-8-ene (**82**) [*V. colorata*]	Leaves [Petroleum ether, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	Antiinflammatory activity	2004[19]

Table 1.3 (Continued)

Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
3-*O*-[β-D-Galactopyranosyl-(1→6)-β-D-glucopyranoside]-5α,14α-androst-8-ene (**83**) [*V. colorata*]	Leaves [Petroleum ether, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	Antiinflammatory activity	2004[19]
3β,21,24-Trihydroxy-21,23;22,28;26,28-triepoxy-5α-stigmasta-8(9),14(15)-dien-3-*O*-β-D-galactopyranosyl-(1→2)-β-D-glucopyranoside (**84**) [*V. colorata*]	Leaves [Petroleum ether, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	Antiinflammatory activity	2004[19]
3β,21,24-Trihydroxy-21,23;22,28;26,28-triepoxy-5α-stigmasta-8(9),14(15)-dien-3-*O*-β-D-galactopyranosyl-(1→2)-β-D-(6-acetyl)-glucopyranoside (**85**) [*V. colorata*]	Leaves [Petroleum ether, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	Antiinflammatory activity	2004[19]
3β,25,29-Trihydroxy-5α-stigmasta-8(9),14(15),24*Z*(28)-triene (**86**) [*V. colorata*]	Leaves [Petroleum ether, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	Antiinflammatory activity	2004[19]
3β,25,25-Trihydroxy-24,28-epoxy-5α-stigmasta-8(9),14(15)-diene (**87**) [*V. colorata*]	Leaves [Petroleum ether, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	Antiinflammatory activity	2004[19]
Vernocuminosides A (**88**) [*V. cumingiana*]	Stem barks [95% EtOH]	Antiinflammatory activities and cytotoxicities	2009[20]

Table 1.3 (Continued)

Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
Vernocuminosides B (**89**) [*V. cumingiana*]	Stem barks [95% EtOH]	Antiinflammatory activities and cytotoxicities	2009[20]
Vernocuminosides C (**90**) [*V. cumingiana*]	Stem barks [95% EtOH]	Antiinflammatory activities and cytotoxicities	2009[20]
Vernocuminosides D (**91**) [*V. cumingiana*]	Stem barks [95% EtOH]	Antiinflammatory activities and cytotoxicities	2009[20]
Vernocuminosides E (**92**) [*V. cumingiana*]	Stem barks [95% EtOH]	Antiinflammatory activities and cytotoxicities	2009[20]
Vernocuminosides F (**93**) [*V. cumingiana*]	Stem barks [95% EtOH]	Antiinflammatory activities and cytotoxicities	2009[20]
Vernocuminosides G (**94**) [*V. cumingiana*]	Stem barks [95% EtOH]	Antiinflammatory activities and cytotoxicities	2009[20]



74: $R_1 = R_2 = H$

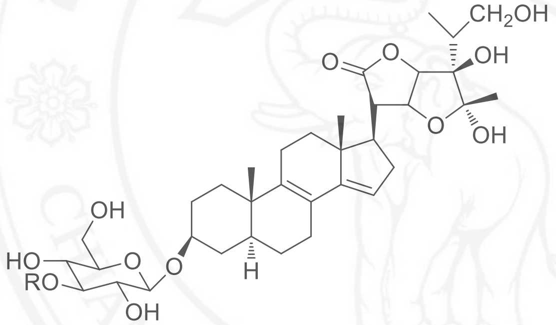
75: $R_1 = H$, $R_2 = \beta$-D-xylopyranosyl

76: $R_1 = \beta$-D-glucopyranosyl, $R_2 = H$

Vernioniside D_1 (**74**)

Vernioniside D_2 (**75**)

Vernioniside D_3 (**76**)

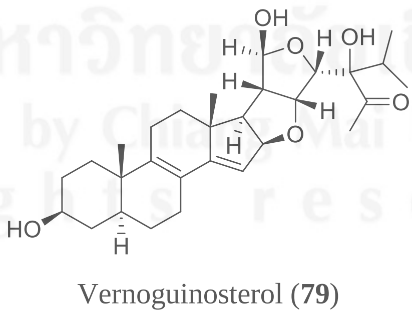


77: R = H

78: R = D-xylopyranosyl

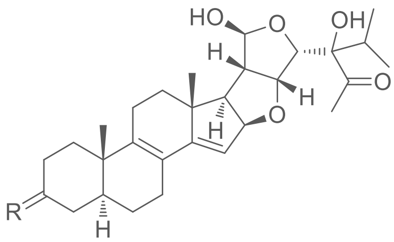
Vernioniside F_1 (**77**)

Vernioniside F_2 (**78**)



Vernoguinosterol (**79**)

Figure 1.8 Steroids from *Vernonia* genus

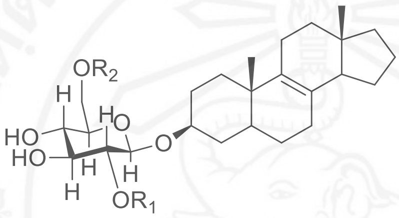


80: R = H, *β*-OGlc

81: R = O

16*β*,22*R*;21,23*S*-Diepoxy-3*β*-O-*β*-D-glucopyranosyloxy-21*S*,24-dihydroxy-5*α*-stigmasta-8,14-dien-28-one (**80**)

16*β*,22*R*;21,23*S*-Diepoxy-21*S*,24-dihydroxy-5*α*-stigmasta-8,14-dien-28-one (**81**)

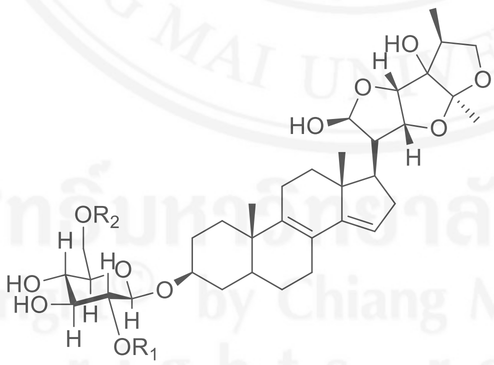


82: R_1 = Gal, R_2 = Glc

83: R_1 = H, R_2 = Glc

3-*O*-{*β*-D-Galactopyranosyl-(1-2)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside}-5*α*,14*α*-androst-8-ene (**82**)

3-*O*-[*β*-D-Galactopyranosyl-(1→6)-*β*-D-glucopyranoside]-5*α*,14*α*-androst-8-ene (**83**)



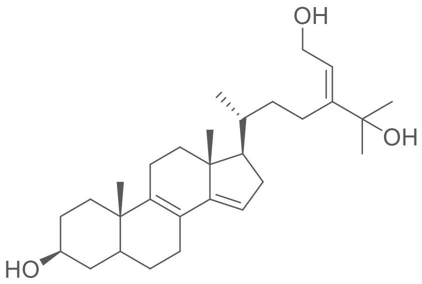
84: R_1 = Gal, R_2 = H

85: R_1 = Gal, R_2 = Ac

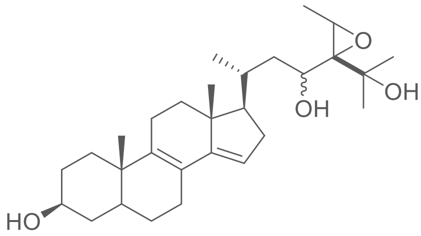
3*β*,21,24-Trihydroxy-21,23;22,28;26,28-triepoxy-5*α*-stigmasta-8(9),14(15)-dien-3-*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucopyranoside (**84**)

3*β*,21,24-Trihydroxy-21,23;22,28;26,28-triepoxy-5*α*-stigmasta-8(9),14(15)-dien-3-*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-(6-acetyl)-glucopyranoside (**85**)

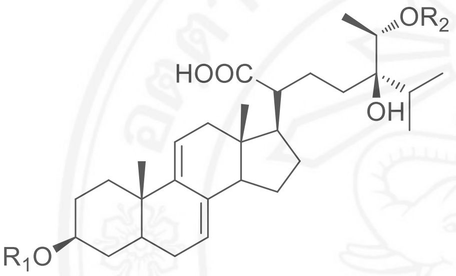
Figure 1.8 (Continued)



3β,25,29-Trihydroxy-5α-stigmasta-8(9),14(15),24*Z*(28)-triene (**86**)



3β,25,25-Trihydroxy-24,28-epoxy-5α-stigmasta-8(9),14(15)-diene (**87**)



88: R_1 = β-D-glc, R_2 = β-D-glc

89: R_1 = β-D-gal-(1→2)-β-D-glc, R_2 = β-D-glc

90: R_1 = β-D-glc, R_2 = H

91: R_1 = β-D-gal-(1→2)-β-D-glc, R_2 = H

92: R_1 = α-L-ara-(1→2)-β-D-glc, R_2 = H

93: R_1 = α-L-ara-(1→2)-β-D-glc, R_2 = β-D-glc

Vernocuminosides A (**88**)

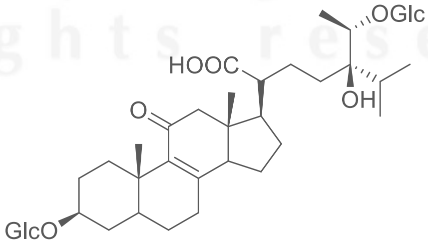
Vernocuminosides B (**89**)

Vernocuminosides C (**90**)

Vernocuminosides D (**91**)

Vernocuminosides E (**92**)

Vernocuminosides F (**93**)



Vernocuminosides G (**94**)

Figure 1.8 (Continued)

1.2.2 Phenol

The vast majority of the plant-based aromatic natural products are phenols. Numerous categories of these compounds exist; here we will consider the simple phenols, phenylpropanoids, flavonoids, tannins, and quinones [7].

1) Phenylpropanoids

Phenylpropanoids contain a three-carbon side chain attached to a phenol backbone. Common examples include hydroxycoumarins, phenylpropenes, and lignans which are also the representatives of this class of compounds as shown in Figure 1.9 [7].

The reports of phenylpropanoid compounds from some *Vernonia* genus are shown in Table 1.4 and Figure 1.10, respectively.

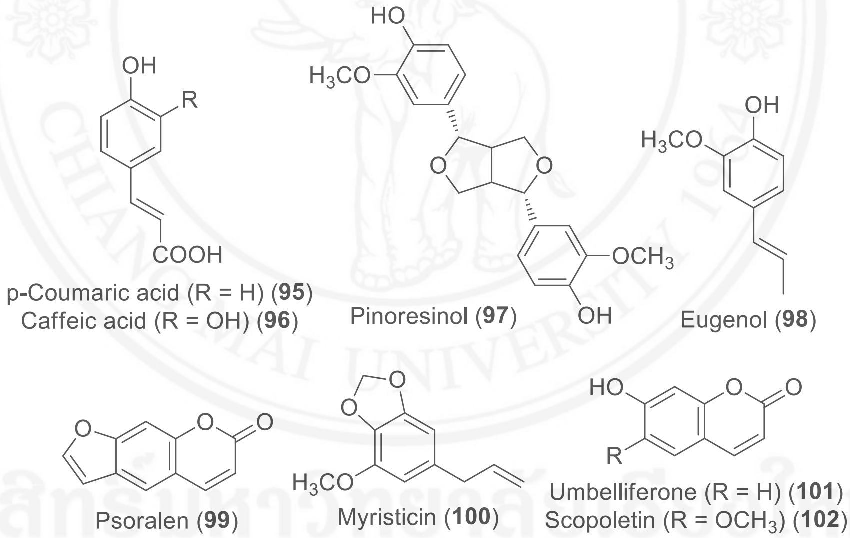
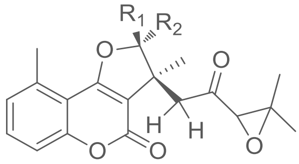


Figure 1.9 Examples of plant phenylpropaniods

Table 1.4 Phenylpropaniods from *Vernonia* genus

Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
2′-Epicycloisobrachycoumarinone epoxide (**103**) [*V. brachycalyx*]	Roots [Petroleum ether, $CHCl_3$-EtOAc (1:1), MeOH]	Against *Leishmania major* promastigotes, Against *P. falciparum* schizonts, Inhibition on the proliferation of human lymphocytes, which was significantly weaker than the antiparasitic effects.	1997[21]
Cycloisobrachycoumarinone epoxide (**104**) [*V. brachycalyx*]	Roots [Petroleum ether, $CHCl_3$-EtOAc (1:1), MeOH]	Against *Leishmania major* promastigotes, Against *P. falciparum* schizonts, Inhibition on the proliferation of human lymphocytes, which was significantly weaker than the antiparasitic effects.	1997[21]
4-Geranyl-5-*O*-*β*-D-glucopyranosyl-2(3*H*)-benzofuranone (**105**) [*V. mapirensis*]	Aerial parts [n-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	-	2007[22]
4-Isoprenyl-5-*O*-*β*-D-glucopyranosyl-2(3*H*)-benzofuranone (**106**) [*V. mapirensis*]	Aerial parts [*n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	-	2007[22]

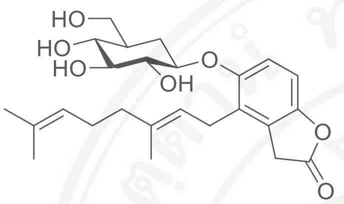


103: $R_1 = CH_3$, $R_2 = H$

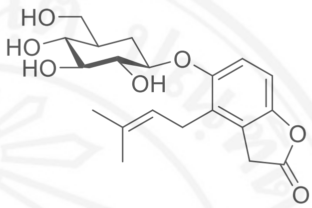
104: $R_1 = H$, $R_2 = CH_3$

2′-Epicycloisobrachycoumarinone epoxide (**103**)

Cycloisobrachycoumarinone epoxide (**104**)



4-Geranyl-5-*O*-*β*-D-glucopyranosyl-2(3*H*)-benzofuranone (**105**)



4-Isoprenyl-5-*O*-*β*-D-glucopyranosyl-2(3*H*)-benzofuranone (**106**)

Figure 1.10 Phenylpropanoids from *Vernonia* genus

2) Flavonoids

Flavonoids possess two benzene rings separated by a propane unit and are derived from flavone. They are generally water soluble. The more conjugated compounds often are bright colored. They are generally found in plants as their glycosides which can complicate structure determinations [7].

The different classes within the group are distinguished by additional oxygen containing heterocyclic rings and hydroxyl groups. These also include catechins, leucoanthocyanidins, flavanones, flavanonols, flavones, anthocyanidins, flavonols, chalcones, aurones, and isoflavones whose general structures are shown in Figure 1.11.

The reports of flavonoid compounds from some *Vernonia* genus are shown in Table 1.5 and Figure 1.12, respectively.

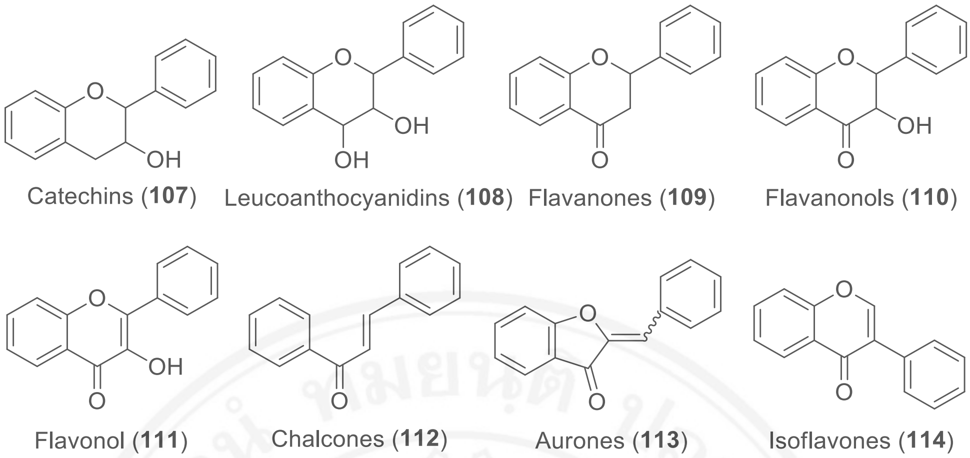


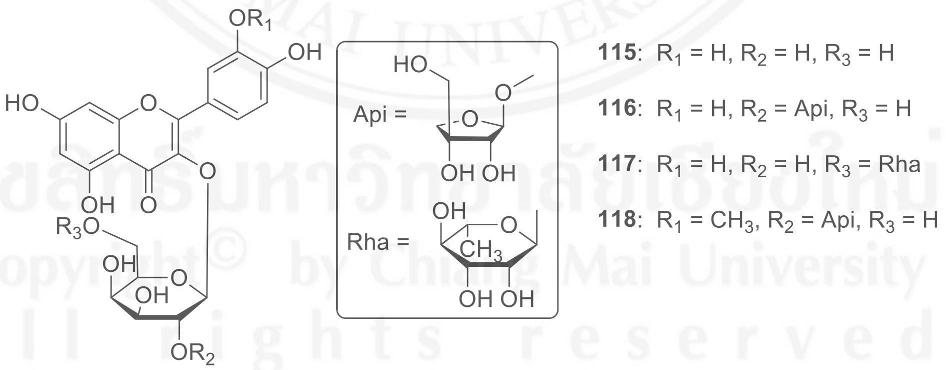
Figure 1.11 Common class of flavonoids

Table 1.5 Flavoniods from *Vernonia* genus

Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
Quercetin 3-*O*-*β*-D-galactopyranoside (**115**) [*V. galamensis*]	Leaves [MeOH]	-	1996[23]
Quercetin 3-*O*-*β*-D-apio-D-furanosyl (1→2)-*β*-D-galactopyranoside (**116**) [*V. galamensis*]	Leaves [MeOH]	-	1996[23]
Quercetin 3-*O*-*α*-L-rhamnopyranosyl (1→6)-*β*-D-galactopyranoside (**117**) [*V. galamensis*]	Leaves [MeOH]	-	1996[23]
Isorhamnetin 3-*O*-*β*-D-apio-D-furanosyl- (1→2)-*β*-D-galactopyranoside (**118**) [*V. galamensis*]	Leaves [MeOH]	-	1996[23]
2′,3,4,4′-Tetrahydroxychalcone (**119**) [*V. anthelmintica*]	Seeds [Petroleum, EtOH]	-	2004[24]
5,6,7,4′-Tetrahydroxyflavone (**120**) [*V. anthelmintica*]	Seeds [Petroleum, EtOH]	-	2004[24]
7,3′,4′-Trihydroxydihydroflavone (**121**) [*V. anthelmintica*]	Seeds [Petroleum, EtOH]	-	2004[24]

Table 1.5 (Continued)

Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
Quercetin 3-*O*-(6″-*p*-coumaroyl)-*β*-D-glucopyranoside-3′-*O*-*β*-D-glucopyranoside (**122**) [*V. mapirensis*]	Aerial parts [*n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	-	2007[22]
Kaempferol 4′- methyl ether 3-*O*-(6″-*p*-coumaroyl)-*β*-D-glucopyranoside (**123**) [*V. mapirensis*]	Aerial parts [*n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	-	2007[22]
Quercetin 3,5,7-trimethyl ether 3′-*O*-*β*-D-glucopyranoside (**124**) [*V. mapirensis*]	Aerial parts [*n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	-	2007[22]
3,7,3′,5′-Tetrahydroxy-4′-methoxyflavone 3-*O*-*β*-D-glucopyranoside (**125**) [*V. mapirensis*]	Aerial parts [*n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1), MeOH]	-	2007[22]



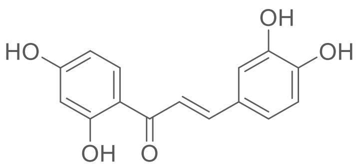
Quercetin 3-*O*-*β*-D-galactopyranoside (**115**)

Quercetin 3-*O*-*β*-D-apio-D-furanosyl(1→2)-*β*-D-galactopyranoside (**116**)

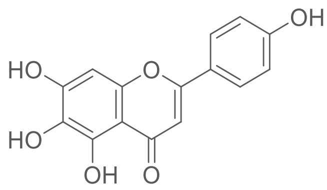
Quercetin 3-*O*-*α*-L-rhamnopyranosyl(1→6)-*β*-D-galactopyranoside (**117**)

Isorhamnetin 3-*O*-*β*-D-apio-D-furanosyl(1→2)-*β*-D-galactopyranoside (**118**)

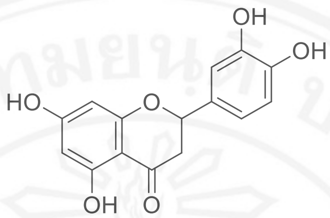
Figure 1.12 Flavonoids from *Vernonia* genus



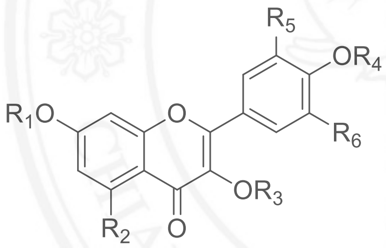
2′,3,4,4′-Tetrahydroxychalcone (**119**)



5,6,7,4′-Tetrahydroxyflavone (**120**)



7,3′,4′-Trihydroxydihydroflavone (**121**)



122: $R_1 = R_4 = R_6 = H$, $R_2 = OH$,
R_3 = (6 -p-coumaroyl)glc, R_5 = O-glc

123: $R_1 = R_5 = R_6 = H$, $R_2 = OH$,
R_3 = (6 -p-coumaroyl)glc, $R_4 = CH_3$

124: $R_1 = R_3 = CH_3$, $R_2 = OCH_3$,
R_4, R_6 = H, R_5 = O-glc

125: $R_1 = R_2 = H$, R_3 = glc, $R_4 = CH_3$,
R_5, R_6 = OH

Quercetin 3-*O*-(6″-*p*-coumaroyl)-*β*-D-glucopyranoside-3′-*O*-*β*-D-glucopyranoside (**122**)

Kaempferol 4′-methyl ether 3-*O*-(6″-*p*-coumaroyl)-*β*-D-glucopyranoside (**123**)

Quercetin 3,5,7-trimethyl ether 3′-*O*-*β*-D-glucopyranoside (**124**)

3,7,3′,5′-Tetrahydroxy-4′-methoxyflavone 3-*O*-*β*-D-glucopyranoside (**125**)

Figure 1.12 (Continued)

1.3 *Cleistocalyx nervosum* var. *paniala*

Cleistocalyx nervosum var. *paniala* (Makiang), an edible fruit found in some parts of Southeast Asia including Thailand, is found growing in scatter locations in some villages of the northern provinces of Thailand such as Chiang Rai, Chiang Mai, Lamphun, Lumpang and Mae Hong Son [25, 26]. Makiang fruit is sour and slightly astringent with scant smell. The rich purplish red color of Makiang is characterized by an anthocyanin profile [26]. Makiang seed is extracted for food flavors. The contribution of plant seed to a flavor can be related to terpene alcohol [27]. In *C. nervosum* var. *paniala* wine, the active compounds were identified as hydrolysable tannins and their derivative i.e. caffeoylquinic acid, gallic acid, ellagic acid and methoxymethylgallate [28].

For the *Cleistocalyx* genus, there were a few phytochemical investigations carried out on this genus. In this study, we will focus on investigation of chemical composition and bioactive substances from *C. nervosum* var. *paniala* seeds.

1.3.1 The botany of *Cleistocalyx nervosum* var. *paniala*

Kingdom:	*Plantae*
Phylum:	*Tracheophyta*
Class:	*Magnoliosida*
Order:	*Myrtineae*
Family:	*Myrtaceae*
Genus:	*Cleistocalyx* [29]

Fruits of *C. nervosum* var. *paniala* were collected from Chiang Mai Horticulture Research Center, Office of Agricultural Research and Development Region 1, Department of Agriculture, Lampang, Thailand, in July-August, 2009. Morphological illustration of *C. nervosum* var. *paniala* is shown in Figure 1.13 [30].

(a)

(b)

(c)

Figure 1.13 Morphological illustration of *C. nervosum* var. *paniala*;
a) Fruits, b) Flowers, and c) Leaves

1.4 Reviews of chemical constituents of *Cleistocalyx* genus

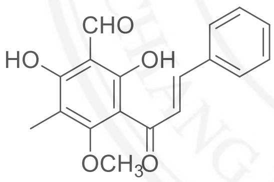
The phytochemical constituents have been reported from the *Cleistocalyx* genus in a few. The flavonoids were found that main group in this genus as shown in Table 1.6 and Figure 1.14, respectively.

Table 1.6 Flavoniods from *Cleistocalyx* genus

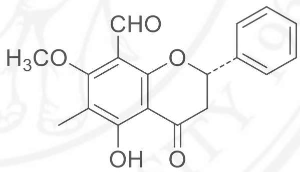
Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
3′-Formyl-4′,6′-dihydroxy-2′-methoxy-5′-methylchalcone (**126**) [*C. operculatus*]	Buds [MeOH-H_2O (7:3)]	-	2004[31]
(2*S*)-8-Formyl-5-hydroxy-7-methoxy-6-methylflavone (**127**) [*C. operculatus*]	Buds [MeOH-H_2O (7:3)]	-	2004[31]
2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**128**) [*C. operculatus*][a,b]	Buds[a,b] [MeOH-H_2O (7:3)][a,b]	Antitumor in SMMC-7721 with IC_{50} 32.3±1.13 μM and EC_{50} 9.0±0.36 μM, 8898, HeLa, SPC-A-1, 95-D and GBC-SD cell[a] Cytotoxic for K562 cell with IC_{50} 14.2±0.45 μM and EC_{50} 3.3±0.14 μM[b]	2004[32][a] 2005[33][b]
Cyanidine 3-glucoside (**129**) [*C. nervosum* var. *paniala*]	Ripe berries [EtOH]	Antioxidant	2008[26]
3′-Formyl-4′,6′,4-trihydroxy-2′-methoxy-5′-methylchalcone (**130**) [*C. operculatus*]	Buds [MeOH]	Exhibited DPPH radical scavenging activity with IC_{50} 22.8 μM	2008[34]

Table 1.6 (Continued)

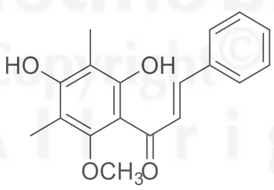
Compounds [Species]	Part of plants [Solvent]	Biological activities	Year [Ref.]
3′-Formyl-6′,4-dihydroxy-2′-methoxy-5′-methylchalcone 4′-*O*-*β*-D-glucopyranoside (**131**) [*C. operculatus*]	Buds [MeOH]	Exhibited DPPH radical scavenging activity with IC_{50} 117.2 μM	2008[34]
(2*S*)-8-Formyl-6-methyl naringenin (**132**) [*C. operculatus*]	Buds [MeOH]	Exhibited DPPH radical scavenging activity with IC_{50} 27.1 μM	2008[34]
(2*S*)-8-Formyl-6-methyl naringenin 7-*O*-*β*-D-gluco-pyranoside (**133**) [*C. operculatus*]	Buds [MeOH]	Exhibited DPPH radical scavenging activity with IC_{50} 105.8 μM	2008[34]



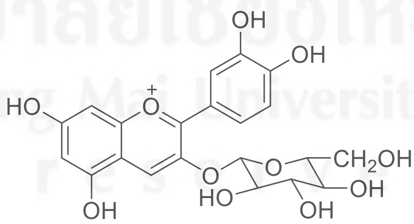
3′-Formyl-4′,6′-dihydroxy-2′-methoxy-5′-methylchalcone (**126**)



(2*S*)-8-Formyl-5-hydroxy-7-methoxy-6-methylflavone (**127**)

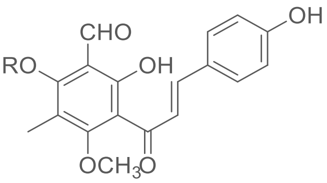


2′,4′-Dihydroxy-6′-methoxy -3′,5′-dimethylchalcone (**128**)



Cyanidine 3-glucoside (**129**)

Figure 1.14 Flavonoids from *Cleistocalyx* genus

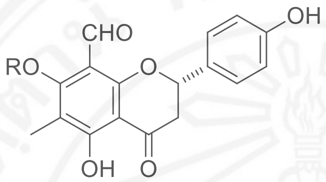


130: R = H

131: R = *β–D*-glucopyranosyl

3′-Formyl-4′,6′,4-trihydroxy-2′-methoxy-5′-methylchalcone (**130**)

3′-Formyl-6′,4-dihydroxy-2′-methoxy-5′-methylchalcone 4′-*O*-*β*-D-glucopyranoside (**131**)



132: R = H

133: R = *β–D*-glucopyranosyl

(2*S*)-8-Formyl-6-methyl naringenin (**132**)

(2*S*)-8-Formyl-6-methyl naringenin 7-*O*-*β*-D-glucopyranoside (**133**)

Figure 1.14 (Continued)

1.5 Objectives of the study

1.5.1 To isolate and identify the chemical constituents of *V. scandens* aerial parts and *C. nervosum* var. *paniala* seeds

1.5.2 To investigate the biological activities of crude extracts and the isolated constituents from *V. scandens* aerial parts and *C. nervosum* var. *paniala* seeds

1.5.3 To synthesize derivatives of 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone or DMC