CHAPTER II

LITERATURE REVIEWS

2.1 General characteristic of *Croton oblongifolius* Roxb.

Croton oblongifolius Roxb. is a middle sized medicinal plant, in the Euphorbiaceae family **[1]**. In this family, there are 326 generas and 7,750 species. The genus *Croton* has 750 species. The magnitudes of leaves are between 5.6–12.0 cm to 13.0–24.0 cm and crowed toward the end of the branchlets. The flat–edged leaf is oblong–lanceolate shaped and the base is usually acute with no apparent glands above the petiols which are 1.3–6.0 cm long. The flowers are solitary or existing alone and pale yellowish–green and solitary in the axials of minute bracts on long erect racemes. The female flowers are situated in dept less than the part of the raceme, while the male flowers are located in higher in the part of the raceme. The male flowers are slender and have the length of pedicels of 4.0 mm. Calyx are more than 6.0 mm and have segments that are ovate, obtuse, blunt and more than 2.5 mm long. The six male petals have covered as woolly texture, are 3.0 mm long and elliptic–lanceolate shaped. The female flowers have the pedicels are short, physically strong and are vigorous. Its sepals are more acute or shrewd than the sepals in the male flower and have relatively high density ciliated margins. The fruits have a diameter less than 1.3 cm, are frailly 3 lobed which are small orbicular scales. In addition, inside each fruit there are three seeds, each seed approximately 6.0 mm long, are spherical or circular and smooth to get to back completely **[11].**

The pictures of stem bark, tree, leaves, flowers and fruits of *Croton oblongifolius* Roxb. are shown in Figure 1.

Figure 1 *Croton oblongifolius* Roxb.

2.2 Previous work on chemical constituents of genus Croton

The genus Croton has nearly 700 species and belongs to family Euphorbiaceae. According to literature search, Croton has been widely study in chemical constituents and their biological activity. The chemical constituents, especially diterpenoid compounds were found in Croton genus.

In 1979, Kitazawa and Ogiso **[12]** found two new diterpenelactones, plaunol A and B exhibiting anti–Shay ulcer activity from Thai medicinal plant, *Croton sublyratus*. Later five novel furanoid diterpene of the *ent* -clerodane type, plaunol A, B, C, D and E, from acetone extract and it had potent anti–Shay ulcer activity, were described **[10]**. In 1981, two diterpene alcohols, *ent*-3α-hydroxy–13–epimanool and *ent*–16β, 17–dihydroxykaurane were found from the the bark of *Croton sublyratus* **[13].**

In 1982, Silveria **[14]** isolated sonderianol (12–hydroxy–3–oxo–cleistanth–8,11,13,15–tetraene) and 3,4–seco–sonderianol (methyl–12–hydroxy–3,4-seco-cleistanth–8,11,13,15,18 (4)–penten–3–oate), two new diterpenes with cleistanthane skeletons, from heartwood of *Croton sonderianus*.

In 1989, McChesney and Silveria **[15]** found two new neo–clerodane diterpenes, 12–hydroxyhardwikiic acid and sonderianial, from the haxane extract of the roots of *Croton sonderianus*.

In 1991, McChesney and Clark **[16]** reported the new 3,4–secotrachylobanoic acid from hexane extracted resin of the root of *Croton sonderianus,* and it showed the antimicrobial activity against Gram–positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Fungi (*Candida albicans* and *Trichophyton mentagrophytes*). In addition, (-)–hardwikiic acid and some of its derivatives, isolating from this plant had significant qualitative antimicrobial activity as well.

In 1993, Cai and coworkers **[17]** isolated 1,3,5-trimethoxybenzene,2,4,6–trimethoxyphenol, 3,4-dimethoxyphenol,3,4–dimethoxybenzyl alcohol, 4-hydroxyphenethyl alcohol and its acetate, sitosterol, sitosterol-β-D-glucopyranoside and β - sitosteronone, from chloroform extract of bark of *Croton lachleri* .

In 1994, Chen, Cai and Philipson **[18]** isolated taspine **(1),** 3,4–*O*-dimethylcedrucin **(2)**, hardwickiic acid **(3)**, bincatriol **(4)**, crolechinol **(5)**, crolechinic acid**(6)**, korberin A **(7)**, korberin B **(8)** from the blood–red sap of *Croton lachleri* from Ecuador. The investigation of biological activity of these compounds showed that compounds **3,4,6-8** had cytotoxicity against KB cell and compounds **5-8** had anti-bacterial activity against *Bacillus subtilis.* It was observed that the constituents of *Croton lachleri* sap collected in different locations varied to a great extent **[17]**.

In 1997, Peres and coworkers **[19]** isolated known compounds, such as acetyl aleuritolic acid, stigmasterol, β-sitosterol, campesterol, sitosterol 3-*O*-β-glucoside, sonderianin, catechin and gallocathechin, from methanolic extract of *Croton urucurana* Baillon. Acetyl aleuritonic acid has been reported to exhibit the best minimum inhibitory cocentration against *Staphylococcus aureus* and *Salmonella typhimurium.*

In 1998, Piacente and coworkers **[20]** reported that the methanolic extract of the aerial parts of *Croton ruizinus* afforded two new pregnane glycosides **(1**and **2)**, together with the morphinandienone alkaloids flavinantine **(3)** and *O*–methylflavinantine **(4)**. The pregnane glycosides were found to promote platelet aggregation, while **(3)** and **(4)** showed only slight activity.

In 1999, Ngadjui and coworkers **[21]** found a new labdane diterpene, crotonadiol, from the stem bark of *Croton zambesicus* Muell.Arg., together with the known clerodane crotocorylifuran and two trachylobanes: 7β-acetoxytrachyloban–18–oic acid and trachyloban-7β, 18 diol. Lupeol, β-sitosterol and its derivatives.

In 2000, Maciel and coworkers **[22]** found numerous chemical components from the bark of *Croton cajucara* Benth., *trans*–dehydrocrotonin **(1)**, acetyl aleuritolic acid **(2)**, two novel clerodanes; *trans*-cajucarin B **(3)** and sacacarin **(4)**, *trans*–crotonin **(5)**, *cis*–cajucarin B **(6)**, cajucarin A **(7)**, cajucarnolide **(8)**, two flavonoids; kaempferol 3,4′,7–trimethyl ether **(9)** and 3,7–dimethyl ether **(10)**, three steroids: β-sitosterol **(11)**, stigmasterol **(12)** and sitosterol 3-*O*-β-glucoside **(13)**. From the biological activity test of these compounds indicated that **(1)** produced anti-inflammatory and antinociceptive effects and significant hypoglycemia in alloxanthine-induced diabetic rats. Moreover **(1)** was also evaluated for possible antioestrogenic activity.

In 2001, Vigor and coworkers **[23]** found three furanoid clerodanes from the stem bark of *Croton eluteria* Bennett. The compounds were named cascarillin B (7α-acetoxy –3,4,15,16-diepoxy–12-oxo–cleroda–13(16),14–dien–20-al), cascarillin C (7α-acetoxy –15,16,12,20-diepoxy –20-hydroxy–cleroda–3,4,13(16),14–triene) and cascarillin D (7α -acetoxy–3,4,15,16-diepoxy–cleroda–13(16), 14–dien–20-al).

2.3 **Previous work on chemical constituents of *Croton oblongifolius* Roxb.**

In 1968, Rao and coworkers **[24]** found a new diterpene alcohol, oblongifoloiol together with β-sitosterol from the bark of *Croton oblongifolius*.

In 1969, Aiyar and Seshadri **[25]** isolated deoxyoblongifoliol from the stem bark of *Croton oblongifolius*.

In 1970, Aiyar and Seshadri **[26]** investigated the structure of oblongifolic acid, (+)- isopimara–7(8), 15-diene-19-oic acid, the major diterpene acid component of the bark.

In 1971, Aiyar and Seshadri **[27]** found three new components from the stem bark, *ent*–isopimara-7,15-diene**(1)**, 19-hydroxy-ent-isopimara-7,15-diene **(2)** and *ent*–isopimara-7,15-diene-19-aldehyde **(3)**. In the same year **[28]**, two compounds have been elucidated and identified as *ent*–isopimara-7,15-diene-3β-ol and *ent*–isopimara-7,15-diene-3β,19-diol. In addition, they found acetyl aleuritolic acid, 3β-acetoxy-olean-14(15)-ene-28-oic acid from the stem bark also **[29]**.

In 1972, Aiyar and Seshadri **[30,31]** discovered two furanoid diterpenes from the bark and were named as *ent*-15,16-epoxy-3,11,13(16), 14-clerodatetraene-19-oic acid or dehydro(-)-hardwikiic acid and (-)-hardwickiic acid. In addition, leaves parts of *Croton oblongifolius* gave only waxy materials.

In 1998, Roengsumran and coworkers **[32]** found two new cembranoid diterpenes, crotocembraneic acid and neocrotocembraneic acid, from the stem bark of *C. oblongifolius* collected from Petchaboon province.

In 1999, Roengsumran and coworkers **[33]** investigated the stem bark of *C. oblongifolius* collected from Prachuabkhirikhan province, and discovered four new labdane diterpenes, labda–7,12(*E*),14–triene **(1)**, labda–7,12(*E*),14–triene–17–ol **(2)**, labda–7,12(*E*),14–triene–17–al **(3)** and labda–7,12(*E*),14–triene–17–oic acid **(4)**. According to cytotoxicity test, **(2)** and **(3)** gave effective cytotoxicity against cancer cell lines HS–27, Hep–G2, SW 620, Chago, Kato–3 and BT 474 with the IC_{50} values were 7.4, 6.3, 5.7, 5.8, 5.8 and 5.4μg/ml, respectively, for **(2)** and 7.1, 5.2, 5.5, 4.8, 4.2 and 5.0 μg/ml, respectively, for **(3)**. They also found a new cembranoid diterpene, neocrotocembranal, from the stem bark. This compound showed inhibition of platelet aggregation induced by thrombin and exhibited cytotoxicity against P-388 cell line with an IC_{50} value of 6.48 μg/ml **[34].**

In 2001 Roengsumran and coworkers **[35]** reported that three labdane diterpenoids, 2-acetoxy-3-hydroxy-labda-8(17),12(*E*)-14-triene **(1)**, 3-acetoxy-2-hydroxy-labda-8(17),12(*E*)-14-triene **(2)** and 2,3-dihydroxy-labda-8(17),12(*E*)-14-triene **(3)** were isolated from the stem bark of *Croton oblongifolius* from Loei province. Compound **(3)** showed moderate cytotoxicity against human cancer cell lines; Kato–3, SW 620, BT 474, Hep–G2 and Chago with the IC_{50} value were 2.2, 2.7, 4.6, 3.7 and 3.3 μg/ml, respectively, whereas **(1)** and **(2)** were less active.

From literature reviews, it is found that *Croton oblongifolius* Roxb. has been widely studied. Many different compounds, especially diterpenoids, have been found and are summarized in Table 1. The structures of these compounds are shown in Figure 2.

Table1 Previous studies of chemical constituents from the stem bark of *Croton oblongifolius* Roxb.

Organic compound	Location	Reference
Oblongifoliol	India	**[24]**
Oblongifolic acid	India	**[25]**
Ent-isopimara-7,15-diene	India	**[26]**
3-Deoxyoblongifoliol	India	**[27]**
Ent–isopimara-7,15-diene-19-aldehyde	India	**[27]**
Acetyl aleuritonic acid	India	**[29]**
Labda-7,12-(*E*),14diene	Prachuabkhirikhan	**[36]**
Labda-7,12-(*E*),14triene-17al	Prachuabkhirikhan	**[36]**
Labda-7,12-(*E*),14triene-17-ol	Prachuabkhirikhan	**[36]**
Labda-7,12-(*E*),14triene-17-oic acid	Prachuabkhirikhan	**[36]**

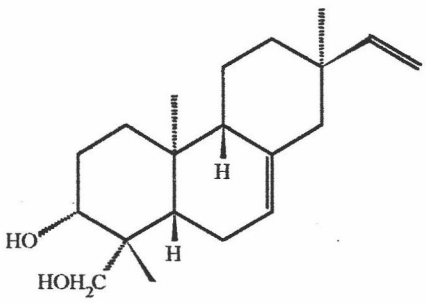
Table1 continued

Organic compounds	Location	Reference
Crotocembraneic acid	Petchaboon	[37]
Neocrotocembraneic acid	Petchaboon	[37]
Neocrotocembranal	Petchaboon	[37]
Crotohalimaneic acid	Nakornrachasima	[37]
Benzoyl crotohalimanolic acid	Nakornrachasima	[37]
Crotohalimoneic acid	Nakornrachasima	[37]
Crovatin	Kanchanaburi	[37]
Isokolavenol	Kanchanaburi	[37]
Nidorellol	Loei,Sakolnakorn	[37]
Poilaneic acid	Chaingmai	[37]
Hardwickiic acid	India,Chonburi	[38]
11-Dehydrohardwickiic acid	Udonthani	[39]
Labda-7,13(*Z*)-diene-17,12-olide	Udonthani	[39]
Labda-7,13(*Z*)-diene-17,12-olide-5-ol	Udonthani	[39]
(-)-20-benzyloxyhardwickiic acid	Udonthani	[39]
(-)-Pimara-9(11),15-diene-19-oic acid	Uttaradit	[40]
(-)-Pimara-9(11),15-diene-19-ol	Uttaradit	[40]

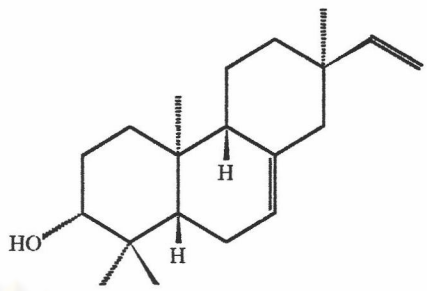
Table1 continued

Organic compounds	Location	Reference
(2*E*,7*E*,11*E*)-1-Isopropyl-1,4-dihydroxy-4,8-dimethylcyclotetradeca-2,7,11-triene-12-carboxylic acid	Uttaradit	**[40]**
3-Acetoxy-labda-8(17),12(*E*)-triene-2-ol	Loei	**[38]**
2-Acetoxy-labda-8(17),12(*E*)-triene3-ol	Loei	**[38]**
Labda-8(17),12(*E*)-triene-2,3-ol	Loei	**[38]**
Kaur-16-en-19-oic acid	Prachuabkhirikhan	**[41]**
Abeita-7,13-diene-3-one	Prachuabkhirikhan	**[43]**
Cleistantha-4,13(17),15-triene-3-oic acid	Prachuabkhirikhan	**[43]**
Cleistantha-4(18),13(17),15-triene-3-oic acid	Loei	**[42]**

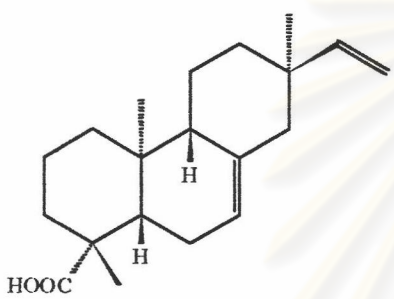
Isopimarene group



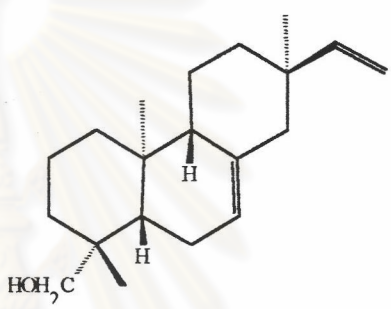
Oblongifoliol



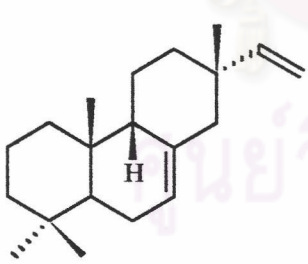
19-Deoxyoblongifoliol



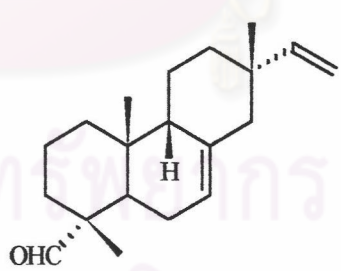
Oblongifolic acid



3-Deoxyoblongifoliol



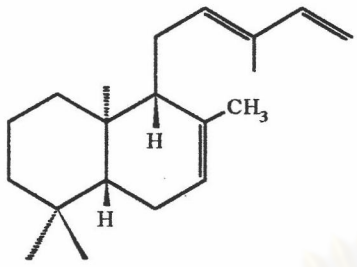
e*nt*–Isopimara-7,15-diene



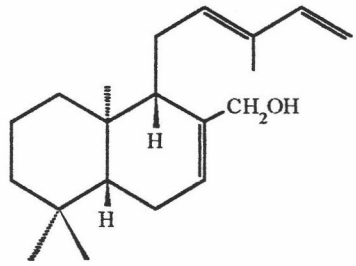
e*nt*–Isopimara-7,15-diene-19-aldehyde

Figure 2 Structures of the chemical constituents of *C. oblongifolius* Roxb.

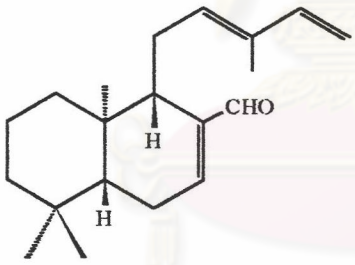
Labdane group



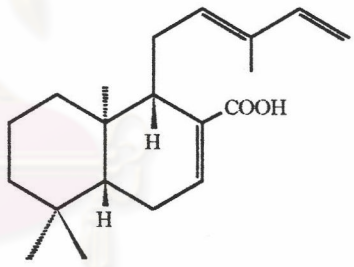
Labda-7,12(*E*),14-triene



Labda-7,12(*E*),14-triene-17-ol

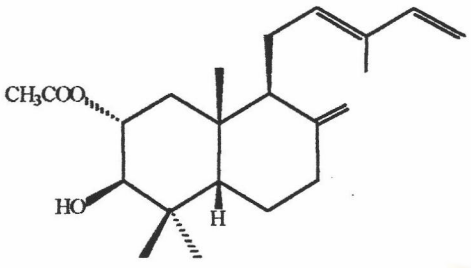


Labda-7,12(*E*),14-triene-17-al

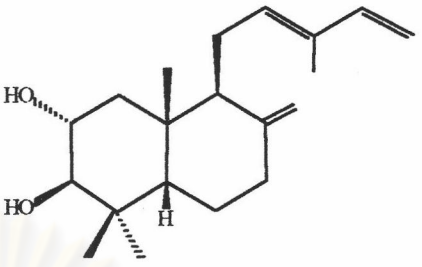


Labda-7,12(*E*),14-triene-17-oic acid

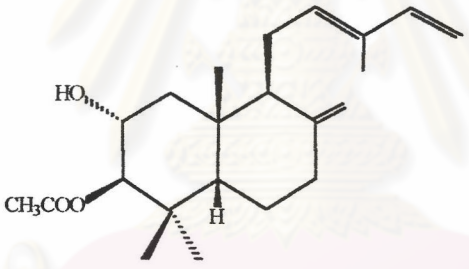
Figure 2. continued



2-Acetoxy-labda-8(17),12(E),14-triene-3-ol



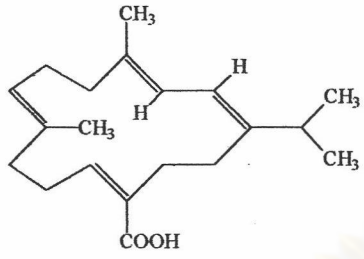
Labda-8(17),12(E),14-triene-2,3-ol



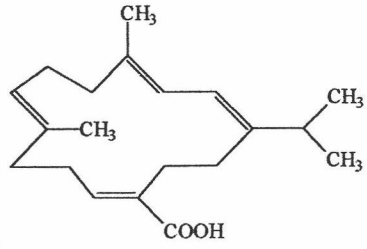
3-Acetoxy-labda-8(17),12(E),14-triene-2-ol

Figure 2 continued

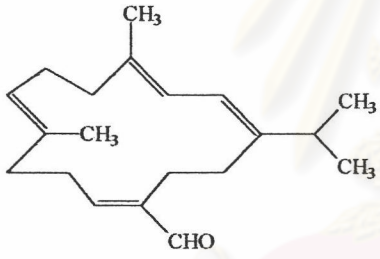
Cembrane group



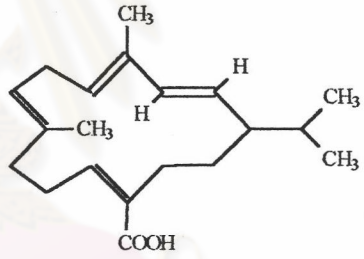
Crotocembraneic acid



Neocrotocembraneic acid



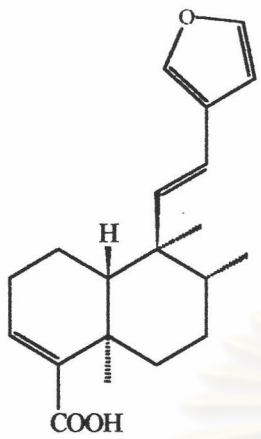
Neocrotocembranal



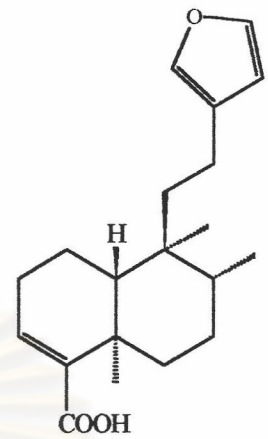
Poilaneic acid

Figure 2 continued

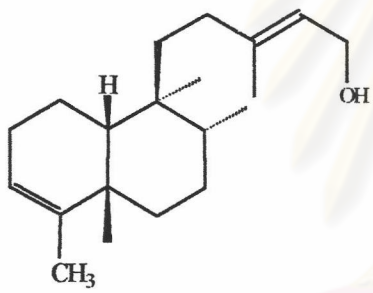
Clerodane group



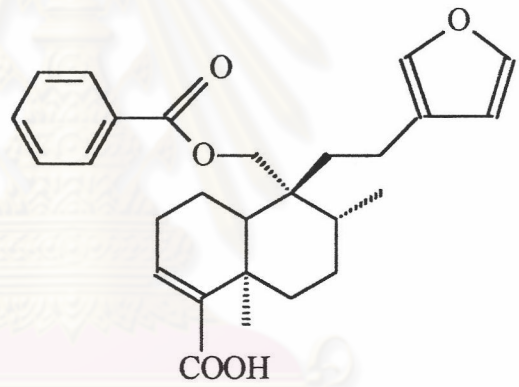
11-Dehydro-(-)-hardwickiic acid



(-)-Hardwickiic acid



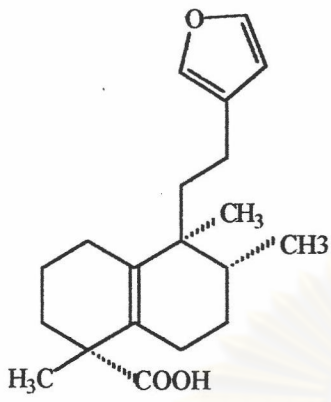
Isokolavenol



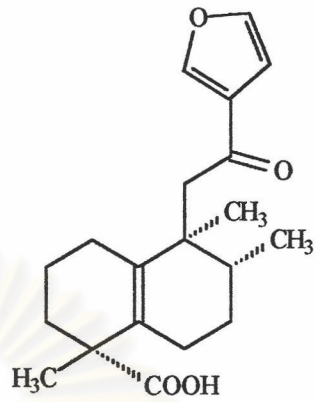
(-)-20-benzyloxyhardwikiic acid

Figure 2 continued

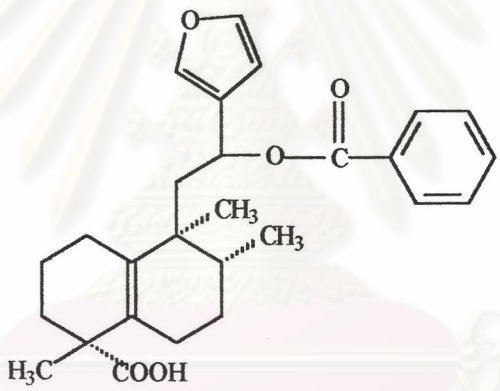
Halimane group



Crotohalimaneic acid



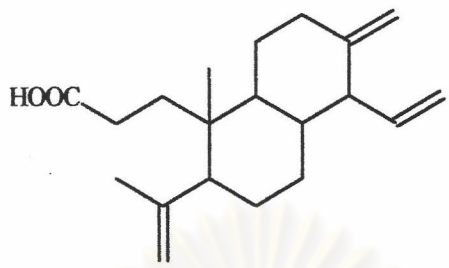
Crotohalimoneic acid



Benzoyl crotohalimonolic acid

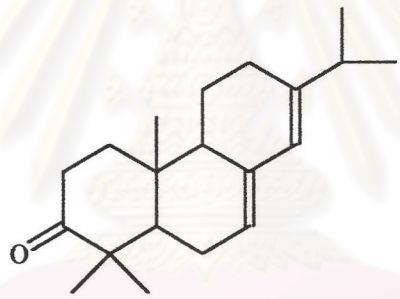
Figure 2. continued

Cleistantane group



Cleistantha-4,13(17),15-triene-3-oic acid

Abeitane group



Abeita-7,13-diene-3-one

Figure 2. continued

2.4 Cytotoxic activity of some isolated compounds of *Croton oblongiffolius* Roxb.

Table 2 shows the cytotoxicity of some compounds isolated from the stem bark of *Croton oblongifolius* against 6 human cancer cell lines: Hs 27 (fibroblast), Hep-G2 (hepatoma), SW 620 (colon), Chago (lung), Kato (gastric) and BT 474 (breast).

Table2 Cytotoxicity against human cancer cell lines of some compounds isolated from *Croton oblongifolious*

Compound	%Survival (10 μg/ml)					
	HS27 fibroblast	HepG2 hepatoma	SW620 colon	Chago lung	Kato gastric	BT474 breast
(-)-20-benzyloxyhardwickiic acid **[39]**	100	74	58	100	65	82
Labda-7,12-(*E*),14triene-17al **[36]**	6	7	3	3	7	13
Labda-7,12-(*E*),14triene-17-oic acid **[36]**	73	57	88	59	70	91
Labda-7,12-(*E*),14diene **[36]**	100	61	73	72	47	75
Labda-7,12-(*E*),14triene-17-ol **[36]**	64	7	3	82	6	11
Crotocembraneic acid **[37]**	82	71	6	3	6	7
Neocrotocembraneic acid **[37]**	46	37	96	97	90	95
Neocrotocembranal **[37]**	82	71	8	12	10	45
Crotohalimaneic acid **[37]**	64	7	3	82	6	11

Table2 continued

Compound	%Survival (10 µg/ml)					
	HS27 fibroblast	HepG2 hepatoma	SW620 colon	Chago lung	Kato gastric	BT474 breast
Crotohalimoneic acid **[37]**	91	86	0	0	70	0
Crovatin **[37]**	18	29	8	0	30	16
Isokolavenol **[37]**	36	93	97	18	94	89
Nidorellol **[37]**	9	21	12	27	30	16
(-)-Hardwickiic acid **[44]**	104	79	112	104	67	115
Kaur-16-en-19-oic acid **[41]**	108	77	42	52	73	80
(-)-Pimara-9(11),15-diene-19-ol **[40]**	89	14	62	66	16	43