CHAPTER II

LITERATURE REVIEWS

2.1 Cytotoxicity against cancer cell lines of selected plants used as anticancer agents.

There have been many studies around the world which are concentrated on the investigation of plants used against cancer and purification of the active compounds to treat cancer. Over 3000 species of plants which had been reported to have anticancer properties, was published serially over a period of 5 years by Hartwell in 1967, 1968, 1969a,b,c, 1970a,b, 1971a,b,c,d and added about 350 species in the year 2000 (Hartwell, J., 2000). From several studies in this field, they have found many cytotoxic agents active *in vitro* against cell cultures and become lead compounds as antitumour agents. Examples of these compounds are presented in Table 1.

Table 1 Natural compounds which are under development as anticancer drugs.

Kind /Name	**Source** Species (Family)	**Anticancer properties**
Alkaloids		
Ellipticine and their derivative	*Ochrosia elliptica* (Apocynaceae)	Pretty well against some kind of breast cancer
Camptothecin 9-Amino-camptothecin	*Camptotheca acuminata* (Nyssaceae)	Fairly well in human clinical trials level
Indicine-N-oxide	*Heliotropium indicum* (Compositae)	Phase II: good effectively against acute leukemia but toxic for liver
Maytansine	*Maytenus serrate* (Celastraceae)	Phase II: little effective

Table 1 continued

Kind /Name	**Source** Species (Family)	**Anticancer properties**
Homoharringtonine	*Cephalotaxus harringtonia* (Cephalotaxaceae)	Pretty well in leukemia patient
Quassinoids		
Bruceantin	*Brucea antidysenterica* (Simaroubaceae)	Phase II: little effective
Diterpenes		
Taxol	*Taxus brevifolia* (Taxaceae)	Phase II: very effectively against ovaries cancer and breast cancer
Tripdiolide	*Tripterygium wilfordii* (Celastraceae)	Under drug formalar developing
Steroidal lactone		
4-β-OH-withanolide E	*Acnistus arborescens* (Solanaceae)	Preclinical evalutation
Sesquiterpenes		
Phyllanthoside Phyllanthostatin I	*Phyllanthus acuminatus* (Euphorbiaceae)	Preclinical evalutation
Baccharin	*Baccharis megapotamica* (Compositae)	Preclinical evalutation

2.2 General characteristics of the plants in the Genus Croton.

The genus Croton comprises 700 species of trees or shurbs. Leaves are usually alternate with 2-glandular stipule at the base. Their flowers are solitary or clustered in the rhachis of a terminal raceme and bracts are small. Male flowers contain 5-calyx, 5-petals. There are many stamens inserted on a hairy receptacle. In female flowers, sepals are usually more ovate than the male, petals are smaller than the sepals or missing and disk annular of 4-6 glands are opposite the sepals. There are three ovary with solitary ovule in each cell. Seeds are smooth, albumen copious and broad cotyledons (Blatter, E., Caius, J. F. and Mhaskar, K. S., 1975).

2.3 General characterization of *Croton oblongifolius* Roxb.

Croton oblongifolius Roxb. is a medium sized deciduous tree in the Euphorbiaceae family. There are about 750 species in this family. In Thailand, it is commonlly called Plao Yai (cental) or Plao Luang (Northern). Its calyx and ovary are clothed with minute orbicular silvery scales. Leaves simple, alternate, oblong, elliptic-oblong, ovate or lanceolate, 5-10 cm wide,9-30 cm long. Young leave is brownish. Inflorescence in terminal raceme or panicle, unisexual, monoecious or dioecious. Flowers are pale geenish yellow and solitary in the axials of minute bracts on long erect racemes. The male flowers locate in the upper part of the raceme and the females in the lower part. Male flowers are slender and have the length of pedicels of 4.0 mm. Calyx is more than 6.0 mm long and segments are ovate, obtuse and more than 2.5 mm long. Petals are 3.0 mm long, elliptic-lanceolate and woolly. The twelve stamens are inflexed in bud and the length of filaments are 3.0 mm. Infemals flowers, the pedicels are short and stout. Its sepals are more acute than in the male with densely ciliated margins. Diameter of fruit is less than 1.3 cm, slightly 3-lobed and clothed with small orbicular scales In each fruit, the mumber of seeds are eight which are 6.0 mm long rounded and quite smooth on the back(เต็ม สมิตินันท์, 2523 ; ลีนา ผู้พัฒนพงศ์, 2530).

The picture of stem-barks, tree, leaves, flowers and fruits of *Croton oblongifolius* Roxb. are shown in Figure 1.

Figure 1. *Croton oblongifolius* Roxb.

2.4 Previous studies of diterpenoid compounds from *Croton oblongifolius* Roxb.

In 1968, Rao and coworkers found a new diterpene alcohol, oblongifoloiol together with β-sitosterol from the bark of *Croton oblongifolius* (Rao, P.S., Sachdev, T.R. and Singh, H.B., 1968).

In 1969, Aiyar and coworkers isolated deoxyoblongifoliol from the stem bark of *Croton oblongifolius* (Aiyar, V.N., et al., 1969).

In 1970, Aiyar and Seshadri investigated the structure of oblongifolic acid, (+)-isopimara-7(8), 15-diene-19-oic acid, the major diterpene acid component of the bark (Aiyar, V.N. and Seshadri, T.R., 1970).

In 1971, Aijar and Seshadri 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, according to detailed chemical and spectral data of oblongifoliol. Two compounds have been elucidated their structures as *ent*-isopimara-7,15-diene-3β-ol and *ent*-isopimara-7,15-diene-3β,19-diol. Moreover, they found acetyl aleuritolic acid, 3β-acetoxy-olean-14(15)-ene-28-oic acid from the stem bark also (Aiyar, V.N. and Seshadri, T.R., 1971).

In 1972, Aijar and Seshadri 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, the leaf parts of *Croton oblongifolius* gave only wax materials (Aiyar, V.N. and Seshadri, T.R., 1972).

In 1998, Roengsumran and coworkers found two new cembranoid diterpenes, crotocembraneic acid and neocrotocembraneic acid, from the stem bark of *Croton oblongifolius* collected from Petchaboon province (Roengsumran, S., et al., 1998).

In 1999, Roengsumran and coworkers investigated the stem bark of *Croton 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)**. Consequently 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 5.4 μg/ml, respectively, for **(2)** and 7.1, 5.2, 5.5, 4.8, 4.2 and 5.0 μg/ml, respectively, for **(3)** (Roengsumran, S., et al., 1999). They also found a new cembranoid diterpene, neocrotocembranal, from the stem bark. This compound showed inhibiton of platelet aggregation induced by thrombin and exhibited cytotoxicity against P-388 cells *in vitro*, with an IC_{50} value of 6.48 μg/ml (Roengsumran, S., et al., 1999).

In 2001, Roengsumran and coworkers 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)** gave less active (Roengsumran, S., et al., 2001).

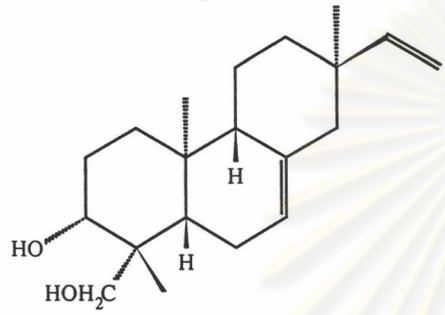
From the literature surveys, *Croton oblongifolius* Roxb. have been widely studied and many diterpenoid compounds have been isolated and characterized in Table 2. The structures of these compounds are shown in Figure 2.

Table 2 Chemical constituents from stem bark of *Croton oblongifolius* Roxb.

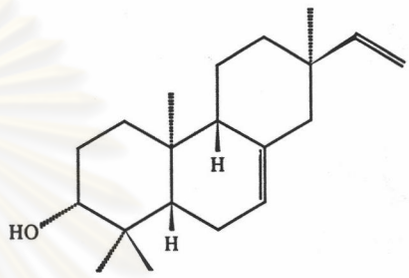
Group	Substaneces	Area	References
Pimarane	Oblongifoliol	India	Rao et al.,1968
	19-Deoxyoblingifoliol	India	Aiyar et al., 1969
	3-Deoxyoblingifoliol	India	Aiyar and Seshadri, 1971
	Oblongifolic acid	India	Aiyar et al., 1969; Aiyar and Seshadri, 1970
Isopimarane	*Ent*-Isopimara-7,15-diene	India	Aiyar and Seshadri, 1971
	Ent-Isopimara-7,15-diene-19-aldehyde	India	Aiyar and Seshadri, 1971
	19-Hydroxy-ent-isopimara-7,15-diene	India	Aiyar and Seshadri, 1971
Clerodanes	11-Dehydro(-)-hardwickiic acid	India	Aiyar and Seshadri, 1972
	(-)-Hardwickiic acid	India, Loei	Aiyar and Seshadri, 1971; Kutiyanuwat. N,1999
	Acetyl aleuritolic acid	India	Aiyar and Seshadri, 1971
	Crovatin	Kanchanaburi	Singtothong, P.,1999
	Isokolavenol	Kanchanaburi	Singtothong, P.,1999
Cembrane	Crotocembraneic acid	Petchaboon	Roengsumran et al.,1998
	Neo-Crotocembraneic acid	Petchaboon	Roengsumran et al.,1998
	Neocrotocembranal	Petchaboon	Singtothong, P.,1999
	Poilaneic acid	Prachubkhirikhan	Singtothong, P.,1999
Labdane	Labda-7,12(*E*),14-triene	Prachubkhirikhan	Roengsumran et al.,1999
	Labda-7,12(*E*),14-triene-17-al	Prachubkhirikhan	Roengsumran et al.,1999
	Labda-7,12(*E*),14-triene-17-ol	Prachubkhirikhan	Roengsumran et al.,1999
	Labda-7,12(*E*),14-triene-17-oic acid	Prachubkhirikhan	Roengsumran et al.,1999
	3-Acetoxy-labda-8(17),12(*E*)-triene-2-ol	Loei	Kuptiyanuwat, N., 1999
	2-Acetoxy-labda-8(17),12(*E*)-triene3-ol	Loei	Kuptiyanuwat, N., 1999
	Labda-8(17),12(*E*)-triene-2,3-ol	Loei	Kuptiyanuwat, N., 1999
Halimane	Crotohalimoneic acid	Nakornrachsima	Singtothong, P.,1999
	Benzoyl crotohalimanolic acid	Nakornrachsima	Singtothong, P.,1999
	Crotohalimoneic acid	Nakornrachsima	Singtothong, P.,1999

Table 2 Continued			
Group	Substaneces	Area	References
Cleistantane	Cleistantha-4,13(17),15-triene-3-oic acid	Prachuabkhirikhan	(Sriyangnok, S., 2000)
	Cleistantha-4(18),13(17),15-triene-3-oic acid	Loei	(Siriwat, K., 1999)
Abitane	Abeita-7,13-diene-3-one	Prachuabkhirikhan	(Sriyangnok, S., 2000)

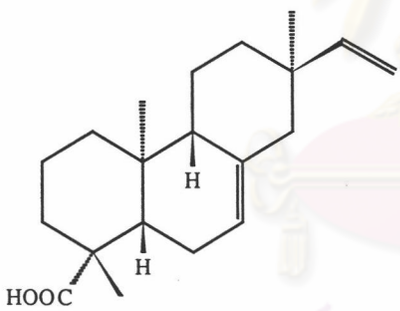
Pimarane and Isopimarane group



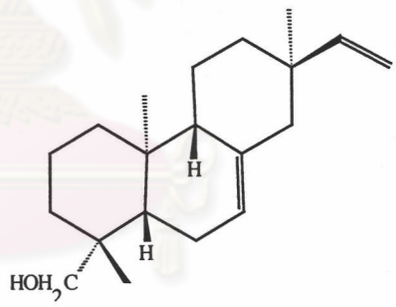
Oblongifoliol



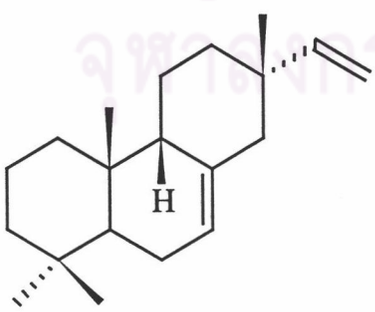
19-Deoxyoblongifoliol



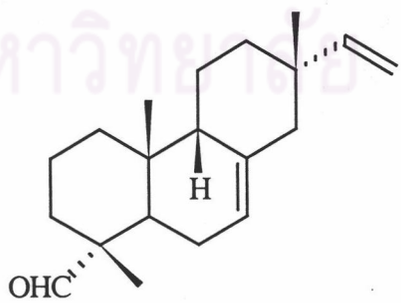
Oblongifolic acid



3-Deoxyoblongifoliol



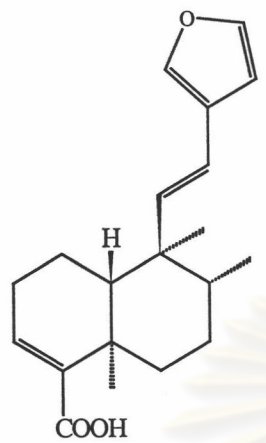
ent – Isopimara-7,15-diene



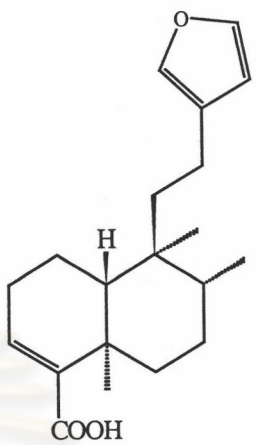
ent-Isopimara-7,15-diene-19-aldehyde

Figure 2 The structures of the diterpenoid compounds from *Croton oblongifolius* Roxb.

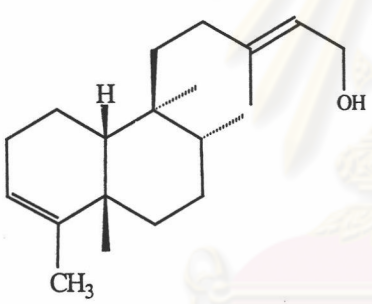
Clerodane group



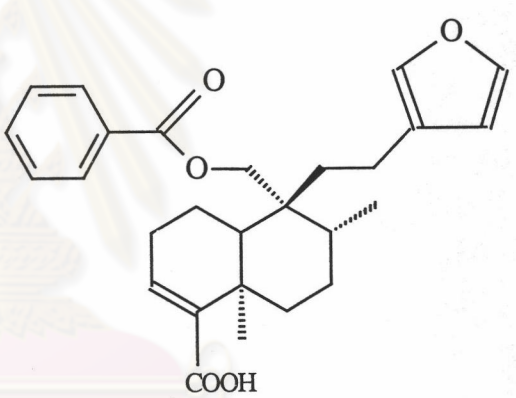
11-Dehydro(-)-hardwickiic acid



(-)-Hardwickiic acid



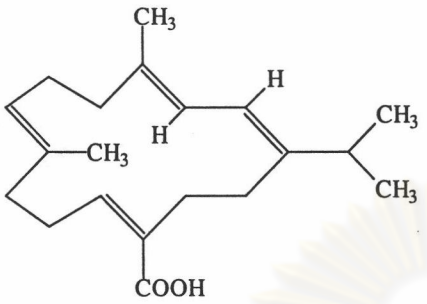
Isokolavenol



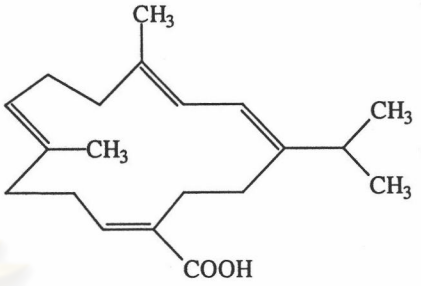
(-)-20-benzyloxyhardwikiic acid

Figure 2 continued

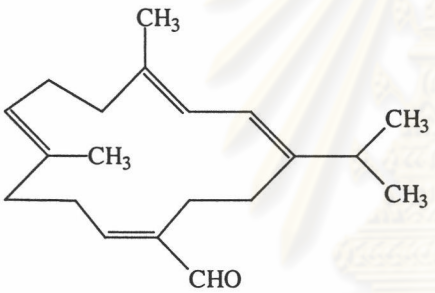
Cembrane group



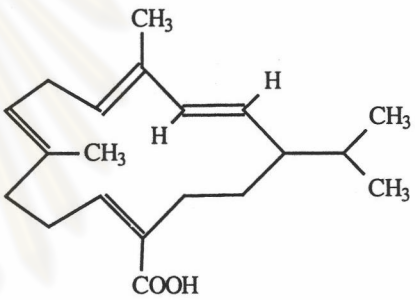
Crotocembraneic acid



Neocrotocembraneic acid



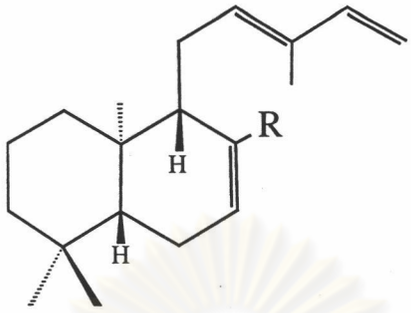
Neocrotocembranal



Poilaneic acid

Figure 2 continued

Labdane group

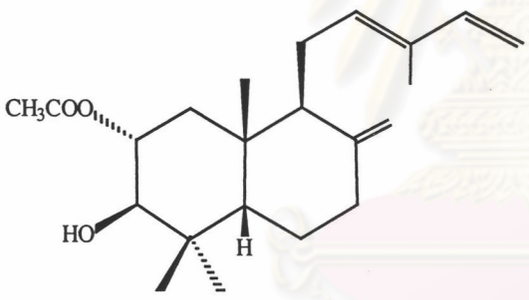


R = CH_3 = Labda-7,12(*E*),14-triene

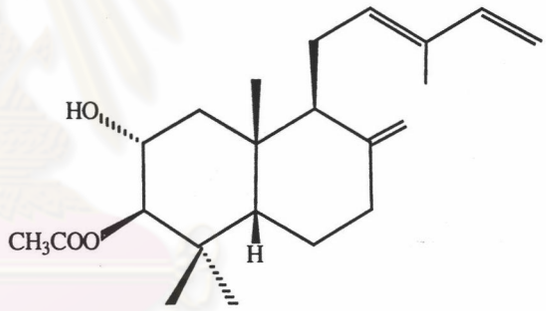
R = CHO = Labda-7,12(*E*),14-triene-17-al

R = CH_2OH = Labda-7,12(*E*),14-triene-17-ol

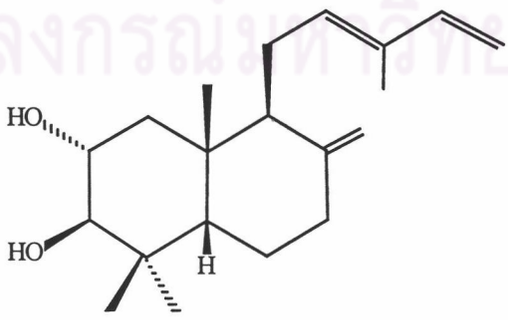
R = CO_2H = Labda-7,12(*E*),14-triene-17-oic acid



2-Acetoxy-labda-8(17),1 2(E),14-triene-3-ol



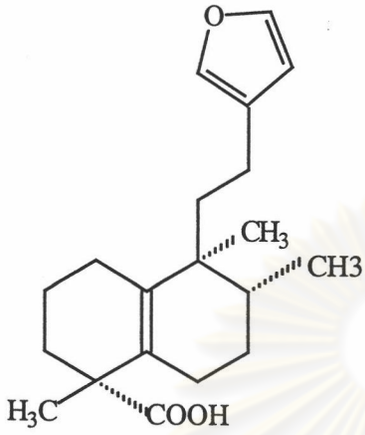
3-Acetoxy-labda-8(17),12(E),14-triene-2-ol



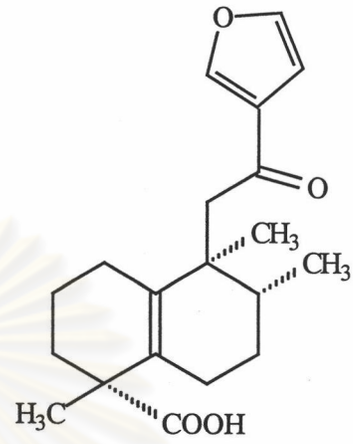
Labda-8(17),12(E),14-triene-2,3-ol

Figure 2 continued

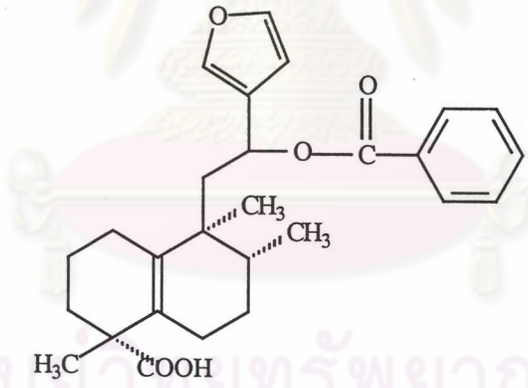
Halimane group



Crotohalimaneic acid



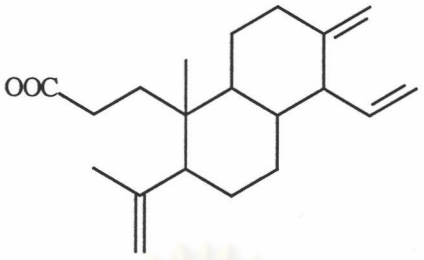
Crotohalimoneic acid



Benzoyl crotohalimonolic acid

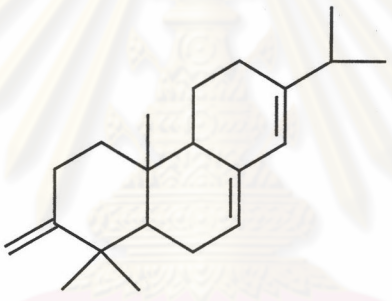
Figure 2 continued

Cleistantane group



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

Abeitane group



Abeita-7,13-diene-3-one

Figure 2 continued

2.5 The cytotoxic activity of some isolated compounds of *Croton oblongiffolius* Roxb.

The previous studies in cytotoxicity of some isolated compounds from the stem bark of *Croton oblongifolious* against six human cancer cell lines: L929 (fibroblast), Hep-G2 (hepatoma), SW 620 (colon), Chago (lung), KATO(gastric) and BT 474 (breast) are summarized in Table 3.

Table 3 Cytotoxicity against six human cancer cell lines of some isolated compounds from *Croton oblongifolious*

Compounds	% survival					
	L929 fibroblast	HepG2 hepatoma	SW620 colon	Chago lung	Kato gastric	BT474 breast
(-)-20-benzyloxyhardwickiic acid (Baigern, S., 1999)	100	74	58	100	65	82
Labda-7,12-(*E*),14triene-17al (Sommit, D., 1996)	6	7	3	3	7	13
Labda-7,12-(*E*),14triene-17-oic acid (Sommit, D., 1996)	73	57	88	59	70	91
Labda-7,12-(*E*),14diene (Sommit, D., 1996)	100	61	73	72	47	75
Labda-7,12-(*E*),14triene-17-ol (Sommit, D., 1996)	64	7	3	82	6	11
Crotocembraneic acid (Singtothong, P., 1999)	82	71	6	3	6	7
Neocrotocembraneic acid (Singtothong, P., 1999)	46	37	96	97	90	95
Neocrotocembranal (Singtothong, P., 1999)	82	71	8	12	10	45
Crotohalimaneic acid (Singtothong, P., 1999)	64	7	3	82	6	11
Crotohalimoneic acid (Singtothong, P., 1999)	91	86	0	0	70	0

Table 3 continued

Compounds	% survival					
	L929 fibroblast	HepG2 hepatoma	SW620 colon	Chago lung	Kato gastric	BT474 breast
Crovatin (Singtothong, P., 1999)	18	29	8	0	30	16
Isokolavenol (Singtothong, P., 1999)	36	93	97	18	94	89
Nidorellol (Singtothong, P., 1999)	9	21	12	27	30	16
(-)-Hardwickiic acid (Bunyamanee, P., 2000)	104	79	112	104	67	115
Kaur-16-en-19-oic acid (Sirimongkhon, S., 2000)	108	77	42	52	73	80
(-)-Pimara-9(11),15-diene-19-ol (Tanwattanakun, T., 1999)	89	14	62	66	16	43