CHAPTER I

INTRODUCTION

The ERICACEAE is a large plant family consisting of more than 125 genera and 4000 spicies. The genus *Agapetes* belonging to this family comprises 80 species distributed in east Himalayas through southwest China and Indochina to southeast Asia.[1] In Thailand, 10 species have been recorded including *A. hosseana* (Saphao lom),[2,3] *A. lobbii* (Sa mek),[2] *A. parishii* (Khaao yen),[2] *A. saxicola* (Ngao nam thip),[2,3] *A. megacarpa* (Prathat doi),[3] *A. bracteata* (Sa mek khao),[3] *A. macrostemon* (Prathat Kra reang),[4] *A. mannii* (Prathat kheaw),[4] *A. variegata* (Prathat yai)[4] and *A.thailandica* (Prathat suthep).[4] Some species have been used as traditional medicine for example, *A. saxicola , A.thailandica, A. lobbii, A. hosseana* and *A. megacarpa.* In china, *A.neriifolia* has been used in Chinese medicinal paste for treating fracture.[5]

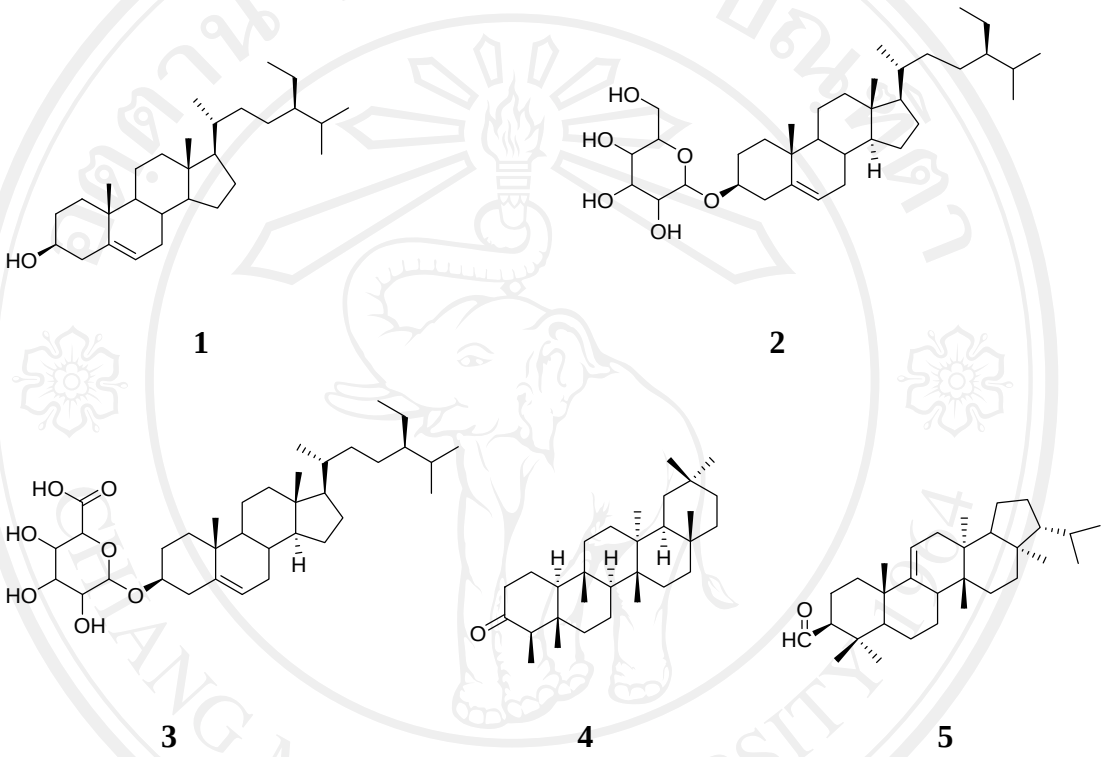
Agapetes megacarpa W.W. Smith (in Thailand commonly known as Prathat doi) in the ERICACEAE family. It has a restricted distribution with a few records from Yunan. In Thailand, it is widely distributed in the North especially, Doi Tung (Chiang Rai), Doi Phukha (Nan), Doi Angkang (Chiang Mai) and Doi Inthanon (Chiang Mai). Prathat doi is a tree, shrub or epiphytic shrub, up to 7 m in height (Figure 1). The leaves have long apex acuminate, base rounded, margin entire, mid rib evident on both sides, petiole very short. Its flowers are reddish and the umbella-like racemens with 3-9 flowers and peduncle of 2-10 mm long, receptacle turbinate, 5-6 mm in diameter, pedicels 2-2.5 cm long, fruit a burry, ca. 10 x 8 mm, greenish.[6]

(Photo: R. Pooma, Doi Tung, Chiang Rai)

Figure 1. Morphological illustration of *Agapetes megacarpa*

A. megacarpa has not been reported on the chemical constituents and biological activities. However, phytochemical investigations on *Agapetes* genus have been reported as below.

In 1990, Huong and co-workers reported the isolation of three sterols, β-sitosterol (**1**), daucosterol (**2**), β-sitosterol glucutonide (**3**) and two pentacyclic triterpenes, friedelin (**4)** and fernenal (**5**) from *Agapetes obuata*.[7] The structures of which are shown in Figure 2.



1 **2**

3 **4** **5**

Figure 2. The structure of isolated compounds from *Agapetes obuata*

In 1991, Deng and Chen reported the chemical constituents of *Agapetes hosseana* Diels.[8] They found seven triterpenes and sterols, identified as fernenol (**6**), 3β-friedelanol (**7**), urs-12-en- 2β-ol-28-oic acid (**8**), 6β-hydroxyursolic acid (**9**), β-sitosterol(**1**), daucosterol (**2**) and β-sitosterol glucutonide (**3**) as shown in Figure 3.

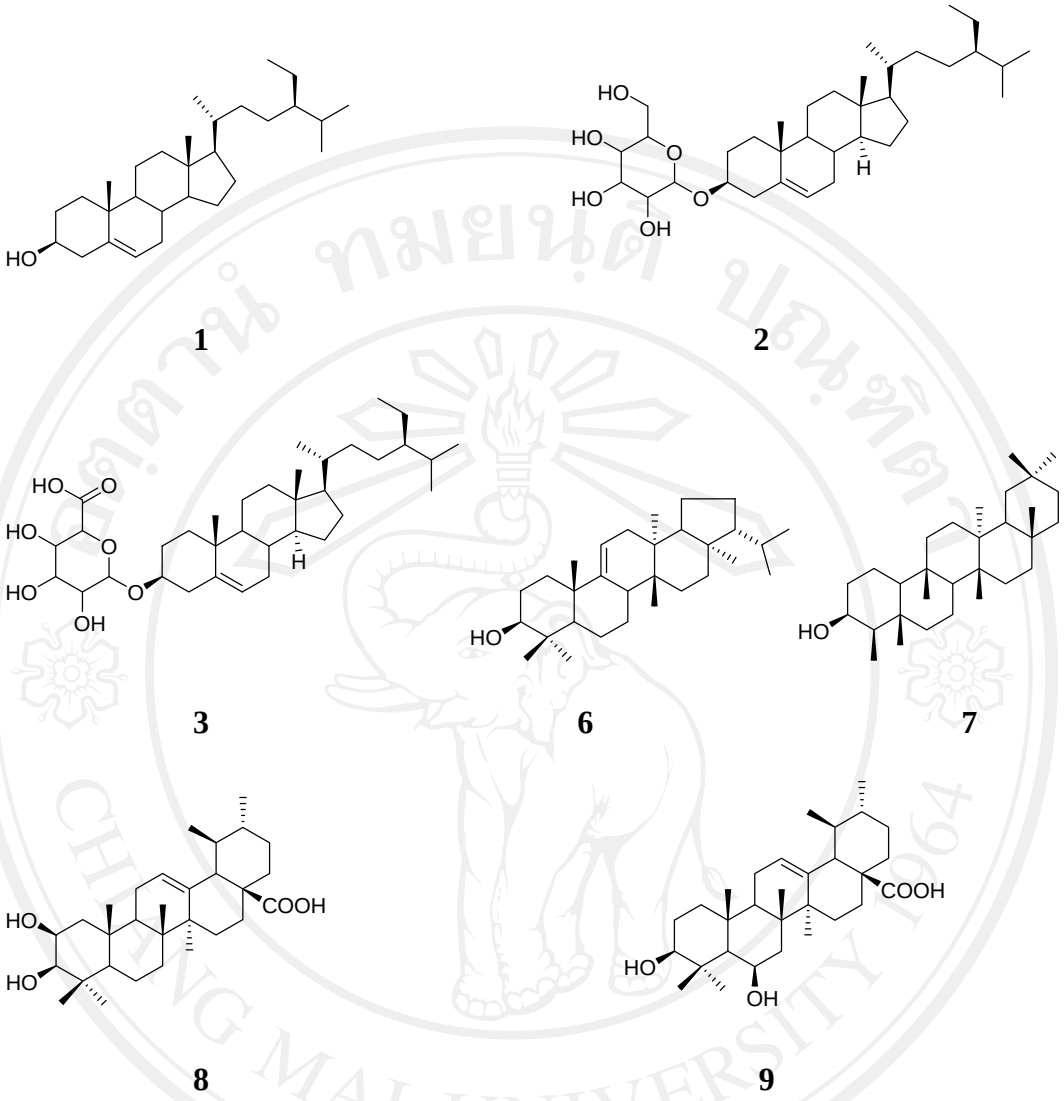


Figure 3. The structure of isolated compounds from *Agapetes hosseana* Diels

Literature search has been carried out and focused on *A. megacarpa* constituents and their biological activities obtained from ERICACEAE family as well as other families. The information are shown in Table 1.

Our preliminary study on *A. megacarpa* revealed the presence of pentacyclic triterpenoids, sterols, xanthone and cinnamic ester derivatives.

Since, 2,7-dihydroxyxanthone constituted in *A. megacarpa*. Although, there has been no report on biological activity of this compound, but biological activity of the related xanthones have been investigated and the literature searchs are summaried in Table 2.

Table 1. Review of *A. megacarpa* constituents and their biological activities

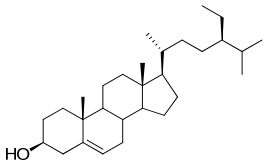
Compounds	Sources	Family	Part of plant	Biological activities	References
Sterols  β-sitosterol (**1**)	*Vaccinium ashei*	ERICACEAE	Fruit	Inactive against lung (PC-12) and colon (HCT116) cancer cell lines	Ono *et al*., 2004[9]
	Euphorbia segetalis	EUPHORBIACEAE	Whole plant	Inactive against Herpes simplex virus (HSV-2) and African swine fever virus (ASFV)	Madureira *et al*., 2003[10]
	Plumbago zeylanica	PLUMBAGINACEAE	Aerial parts	Cytotoxicity against Bowes cancer cell lines (IC_{50} = 36.5 μM)	Nguyen *et al*., 2004[11]
	Cyrtandra cupulata	GESNERIACEAE	Leaves	Induces apoptosis in human breast (MCF-7) cancer cell lines	Chai *et al*., 2008[12]
	Rhus sylvestris	ANACARDIACEAE	Stems and leaves	- Cytotoxicities against Hela and MCF-7 cancer cell lines (IC_{50} = 46.22 and 42.10 μM) - Anti-inflammatory activity for the IL-6 inflammatory cytokine secretion	Ding *et al*., 2009[13]
	Pereskia bleo	CACTACEAE	Leaves	Cytotoxicities against CasKi, MCF-7 and A549 carcinoma cell lines (IC_{50} = 72, 62 and 78 μg /ml, respectively)	Malek *et al*., 2009[14]

Table 1. (Continued)

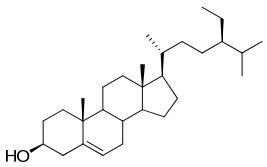
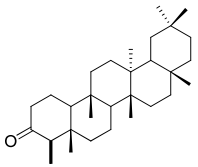
Compounds	Sources	Family	Part of plant	Biological activities	References
 β-sitosterol (**1**)	-	-	-	Induces apoptosis in SGC-7901 human stomach cancer cell	Zhao *et al*., 2009[15]
Triterpenes  Friedelin (**4**)	*Alchornea latifolia*	EUPHORBIACEAE	Leaves	-	Setzer *et al*., 2000[16]
	Mallotus apelta	EUPHORBIACEAE	Leaves	-	Kiem *et al*., 2004[17]
	Celastrus punctatus	CELASTRACEAE	Stems	Inactive against KB, COLO-205, Hepa-3B and Hela cancer cell lines (ED_{50} = > 20 μg /ml)	Kuo *et al*., 2001[18]
	Allanblackia monticola	GUTTIFERAE	Leaves	Inactive against *Plasmodium falciparum* (no antimalarial activity)	Azebaze *et al*., 2007[19]

Table 1. (Continued)

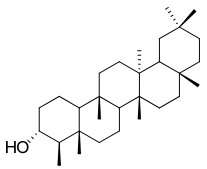
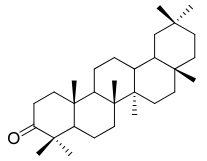
Compounds	Sources	Family	Part of plant	Biological activities	References
 3α-friedelanol (**10**)	*Alchornea latifolia*	EUPHORBIACEAE	Leaves	-	Setzer *et al*., 2000[16]
	Mallotus laapelta	EUPHORBIACEAE	Leaves	-	Kiem *et al*., 2004[17]
	Drynaria quercifolia	DRYNARIACEAE	Rhizomes	-	Ramesh *et al*., 2001[20]
	Vitis trifolia	VITACEAE	Whole plant	Antitumor activity in a potato discs bioassay	Kundu *et al*., 2000[21]
	Dichapetalum barteri	DICHAPETALACEAE	Stem bark	-	Ivan *et al*., 2007[22]
 3-ketooleanane (**11**)	*Pterocarpus santalinus*	FABACEAE	Stem	-	Krishnaveni and Rao, 2000[23]

Table 1. (Continued)

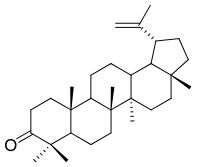
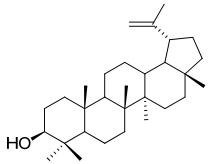
Compounds	Sources	Family	Part of plant	Biological activities	References
 Lupenone (**12**)	*Celastrus punctatus*	CELASTRACEAE	Stems	Inactive against KB, COLO-205, Hepa-3B and Hela cancer cell lines (ED_{50} = > 20 μg /ml)	Kuo *et al*., 2001[18]
	Euphorbia segetalis	EUPHORBIACEAE	Whole plant	Antiviral activities against Herpes simplex virus (HSV-1 and HSV-2 with plaque reduction = 5.2 and 5.3)	Madureira *et al*., 2003[10]
	Acacia mellifera	LEGUMINOSAE	Stem bark	Inactive against NSCLC-N6 cancer cell line	Mutai *et al*., 2004[24]
	Diospyros mespilifomis	EBENACEAE	Stem bark	α-glucosidase enzyme inhibitory activity	Mohamed *et al*., 2009[25]
 Lupeol (**13**)	*Vaccinium ashei*	ERICACEAE	Fruit	Inactive against lung (PC-12) and colon (HCT116) cancer cell lines	Ono *et al*., 2004[9]
	Calendula officinalis	CALENDULEAE	Flowers	Anti-inflammatory activity against TPA induced inflammation in mice (ID_{50} = 0.6 mg per ear)	Akihisa *et al*., 1996[26]

Table 1. (Continued)

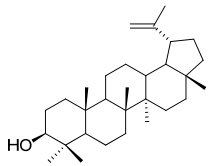
Compounds	Sources	Family	Part of plant	Biological activities	References
 Lupeol (**13**)	*Celastrus punctatus*	CELASTRACEAE	Stems	Inactive against KB, COLO-205, Hepa-3B and Hela cancer cell lines (ED_{50} = >20 μg /ml)	Kuo *et al*., 2001[18]
	-	-	-	human Topoisomerase II inhibitory activity (IC_{50} = 10.4 μM)	Wada *et al*., 2001[27]
	Acacia mellifera	LEGUMINOSAE	Stem bark	Cytotoxicity against NSCLC-N6 cancer cell line (IC_{50} = >30 μg/ml)	Mutai *et al*., 2004[24]
	Bursera graveolens	BURSERACEAE	Stems	Cytotoxicity against human HT1080 fibrosarcoma cells (ED_{50} = 16.7 μg/ml)	Nakanishi *et al*., 2005[28]
	Crataeva nuruala	-	Stem bark	Protective effect against cyclophosphamide-induced cardiac mithocondria toxicity	Sudharson *et al*., 2006[29]

Table 1. (Continued)

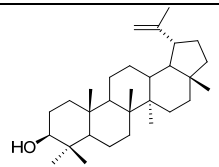
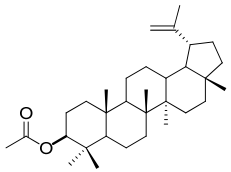
Compounds	Sources	Family	Part of plant	Biological activities	References
 Lupeol (**13**)	*Celastrus punctatus*	CELASTRACEAE	Stems	Inactive against KB, COLO-205, Hepa-3B and Hela cells	Kuo *et al*., 2001[18]
	Diospyros mespilifomis	EBENACEAE	Stem bark	α-glucosidase enzyme inhibitory activity	Mohamed *et al*., 2009[25]
 Lupeol acetate (**14**)	*Himatanthus articulata*	APOCYNACEAE	Bark	-	Sabarreto *et al*., 1998[30]
	Himatanthus sucuuba	APOCYNACEAE	Bark	-	Wood *et al*., 2001[31]
	Plumbago zeylanica	PLUMBAGINACEAE	Aerial parts	-	Nguyen *et al*., 2004[11]
	Alstonia scholaris	APOCYNACEAE	Stem bark	Inhibited spermatogenesis and ultimately induced infertility in male rats	Gupta *et al*., 2005[32]
	Eupatorium macrocephalum	ASTERACEAE	Aerial part	-	Vega *et al*., 2008[33]
	Acetylation of lupeol	-	-	α-glucosidase enzyme inhibitory activity	Mohamed *et al*., 2009[25]

Table 1. (Continued)

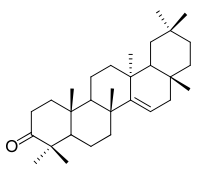
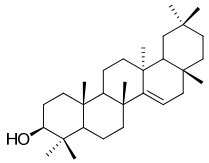
Compounds	Sources	Family	Part of plant	Biological activities	References
 Taraxerone (**15**)	*Craibiodendron henryi*	ERICACEAE	Roots	-	Huang *et al*., 2007[34]
	Alchornea latifolia	EUPHORBIACEAE	Leaves	-	Setzer *et al*., 2000[16]
	Mallotus apelta	EUPHORBIACEAE	Leaves	-	Kiem *et al*., 2004[17]
	Sebastiania adenophora	EUPHORBIACEAE	Leaves	Phytotoxic effects on barn yard grass	Rubalcava *et al*., 2007[35]
	Rhus alata	ANACARDIACEAE	Leaves	-	Parveena *et al*., 2008[36]
 Taraxerol (**16**)	*Craibiodendron henryi*	ERICACEAE	Roots	-	Huang *et al*., 2007[34]
	Cosmos bipinnatus	HELIANTHEAE	Flowers	Anti-inflammatory activity against TPA induced inflammation in mice (ID_{50} = 0.4 mg per ear)	Akihisa *et al*., 1996[26]
	Rhizophora mangle	RHIZOPHORACEAE	Leaves and stems	Insecticidal activity against adult *Cylas formicaruis* (LC_{50} = 1.82 mg/g)	William., 1999[37]
	Alchornea latifolia	EUPHORBIACEAE	Leaves	-	Setzer *et al*., 2000[16]

Table 1. (Continued)

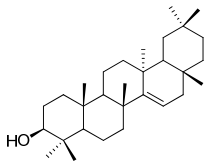
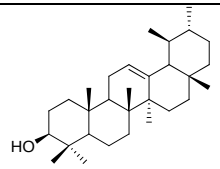
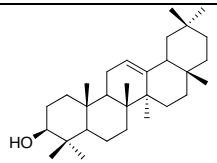
Compounds	Sources	Family	Part of plant	Biological activities	References
 Taraxerol (**16**)	*Sebastiania adenophora*	EUPHORBIACEAE	Leaves	Phytotoxic effects on barn yard grass	Rubalcava *et al*., 2007[35]
	Alnus hirsuta	BETULACEAE	Stem bark	Inactive against HIF-1 in AGS cells	Jin *et al*., 2007[38]
 α-amyrin (**17**)	*Vaccinium ashei*	ERICACEAE	Fruit	Inactive against lung (PC-12) and colon (HCT116) cancer cell lines	Ono *et al*., 2004[9]
	Cosmos bipinnatus	HELIANTHEAE	Flowers	Anti-inflammatory activity against TPA induced inflammation in mice (ID_{50} = 0.2 mg per ear)	Akihisa *et al*., 1996[26]
 *β*-amyrin (**18**)	*Vaccinium ashei*	ERICACEAE	Fruit	Cytotoxicity against lung (PC-12) and colon (HCT116) cancer cell lines (IC_{50} = 1.0 and 3.1μg/ml)	Ono *et al*., 2004[9]

Table 1. (Continued)

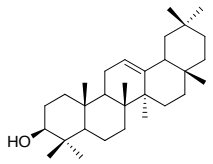
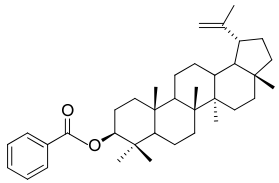
Compounds	Sources	Family	Part of plant	Biological activities	References
 *β*-amyrin (**18**)	*Craibiodendron henryi*	ERICACEAE	Roots	-	Huang *et al*., 2007[34]
	Helianthus annuus	HELIANTHEAE	Flowers	Anti-inflammatory activity against TPA induced inflammation in mice (ID_{50} = 0. 4 mg per ear)	Akihisa *et al*., 1996[26]
	Drynaria quercifolia	DRYNARIACEAE	Rhizomes	-	Ramesh *et al*., 2001[21]
	Rhus alata	ANACARDIACEAE	Leaves	-	Parveena *et al*., 2008[36]
 Lupeol benzoate (**19**)	*Befaria racemosa*	ERICACEAE	Leaves (benzylation of fraction)	-	Ueda *et al*., 1961[39]

Table 1. (Continued)

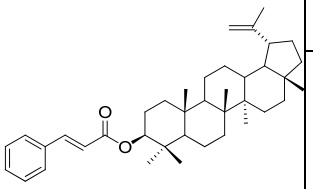
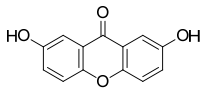
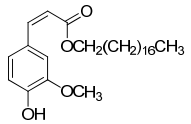
Compounds	Sources	Family	Part of plant	Biological activities	References
 Lupeol cinnamate (**20**)	*Himatanthus articulata*	APOCYNACEAE	Bark	-	Sabarreto *et al*., 1998[30]
	Rhizophora mangle	RHIZOPHORACEAE	Leaves and stems	Insecticidal activity against adult *Cylas formicaruis* (LC_{50} = 1.37 mg/g)	William., 1999[37]
	Himatanthus sucuuba	APOCYNACEAE	Bark	-	Wood *et al*., 2001[31]
Xanthone  2,7dihydroxyxanthone(**21**)	*Mammea acuminata*	GUTTIFERAE	Stems and bark	-	Tosa *et al*., 1997[40]
	Araucaria angustifolia	ARAUCARIACEAE	Cells cultures	-	Fonseca *et al*., 2000[41]
Cinnamate derivatives  *cis*-octadecyl ferulate (**22**)	Esterification of ferulic acid and octadecanol	-	-	Antioxidant activity (DPPH Radical Scavenging, IC_{50} = 24.97 μM)	Anselmi *et al*., 2004[42]

Table 2. The biological activities of xanthones

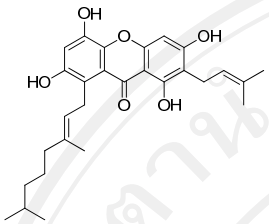
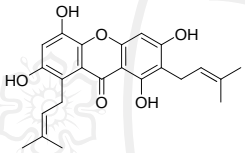
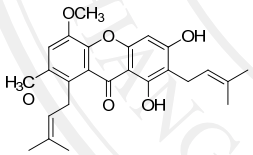
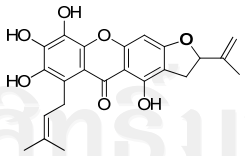
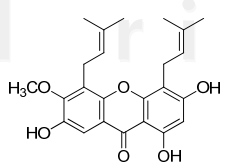
Xanthones	Sources	Biological activities	References
 Cratoxyarborenone A (**23**)	*Cratoxylum sumatranum* (GUTTIFERAE)	Cytotoxicity against KB cancer cell line (ED_{50} = 4.3 μg/ml)	Seo *et al.,* 2002[43]
 Cratoxyarborenone B (**24**)	*Cratoxylum sumatranum* (GUTTIFERAE)	Cytotoxicity against KB cancer cell line (ED_{50} = 1.0 μg/ml)	Seo *et al.,* 2002[43]
 Cratoxyarborenone C (**25**)	*Cratoxylum sumatranum* (GUTTIFERAE)	Cytotoxicity against KB cancer cell line (ED_{50} = 1.5 μg/ml)	Seo *et al.,* 2002[43]
 Cratoxyarborenone D (**26**)	*Cratoxylum sumatranum* (GUTTIFERAE)	Cytotoxicity against KB cancer cell line (ED_{50} = 1.7 μg/ml)	Seo *et al.,* 2002[43]
 Cratoxyarborenone E (**27**)	*Cratoxylum sumatranum* (GUTTIFERAE)	Cytotoxicity against KB cancer cell line (ED_{50} = 4.3 μg/ml)	Seo *et al.,* 2002[43]

Table 2. (Continued)

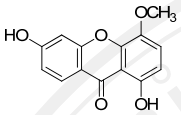
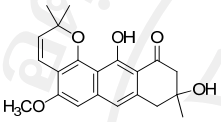
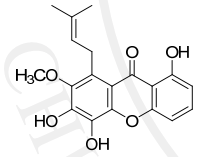
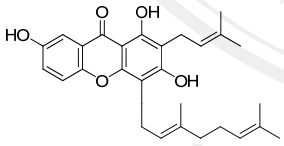
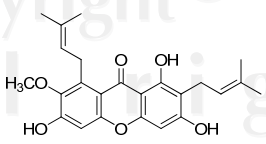
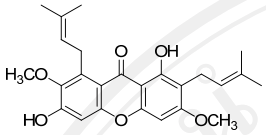
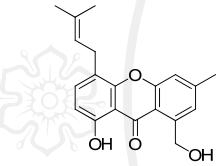
Xanthones	Sources	Biological activities	References
 Cratoxyarborenone F (**28**)	*Cratoxylum sumatranum* (GUTTIFERAE)	Cytotoxicity against KB cancer cell line (ED_{50} = 4.1 μg/ml)	Seo *et al.,* 2002[43]
 Vismione (**29**)	*Cratoxylum sumatranum* (GUTTIFERAE)	Cytotoxicity against KB cancer cell line (ED_{50} = 1.3 μg/ml)	Seo *et al.,* 2002[43]
 Celebixanthone (**30**)	*Cratoxylum cochinchinense* (GUTTIFERAE)	Cytotoxicity against NCI-H187 lung cancer cell line (IC_{50} = 5.2 μg/ml) -Antimalarial activity against *Plasmodium falciparum* (IC_{50} = 4.9 μg/ml)	Laphookhieo *et al.,* 2006[44]
 Cochinchinone A (**31**)	*Cratoxylum cochinchinense* (GUTTIFERAE)	Cytotoxicity against NCI-H187 lung cancer cell line (IC_{50} = 0.65 μg/ml)	Laphookhieo *et al.,* 2006[44]
 α-Mangostin (**32**)	*Cratoxylum cochinchinense* (GUTTIFERAE)	Cytotoxicity against NCI-H187 lung cancer cell line (IC_{50} = 2.4 μg/ml)	Laphookhieo *et al.,* 2006[44]

Table 2. (Continued)

Xanthones	Sources	Biological activities	References
 *β*- Mangostin (**33**)	*Cratoxylum cochinchinense* (GUTTIFERAE)	- Cytotoxicity against NCI-H187 lung cancer cell line (IC_{50} = 1.7 μg/ml) - Antimalarial activity against *Plasmodium falciparum* (IC_{50} = 7.2 μg/ml)	Laphookhieo *et al.,* 2006[44]
 Paeciloxanthone (**34**)	*Paecilomyces* sp.(fungus)	Cytotoxicity against hepG2 (IC_{50} = 1.08 μg/ml)	Wen *et al.,* 2008[45]

Due to the uses of *A. megacarpa* in Thailand in Thai traditional medicine, especially the study on the chemical constituents and biological activities of *A. megacarpa* has not been carried out. So, it is interesting to explore every part of the plant. Whole parts of this plant were collected from Doi Phahompok, Chiang Mai, Thailand for study. The extracts of the rhizomes, stems and leaves of *A. megacarpa* were preriminary subjected to some biological assays, such as antimycobacterial, antifungal, antioxidant and cytotoxicities against cancer cell lines. The results are summarized in Table 3-4.

Table 3. The results of antimycobacterial, antifungal and antioxidant activities of crude extracts of *A. megacarpa*

Part of plant	Crude extracts	Anti-TB	Antifungal	Antioxidant (IC_{50}, μg/ml)
Rhizomes	*n*-Hexane	Inactive	Inactive	Inactive
	Ethyl acetate	Inactive	Inactive	6.01
	n-Butanol	Inactive	Inactive	5.16
Twigs	*n*-Hexane	Inactive	Inactive	Inactive
	Ethyl acetate	Inactive	Inactive	14.78
	n-Butanol	Inactive	Inactive	1.97
Leaves	*n*-Hexane	-	-	Inactive
	Ethyl acetate	-	-	12.14
	n-Butanol	-	-	10.55

$IC_{50} > 50$ μg/ml is considered inactive.

Table 4. The results of cytotoxicities of crude extracts of *A. megacarpa*

Part of plant	Crude extracts	Cytotoxicities (IC_{50}, μg/ml)				Vero cell
		NCI-H1299	A549	MDA-MB-231	MCF-7	
Rhizomes	*n*-Hexane	Inactive	Inactive	Inactive	4.1	Inactive
	Ethyl acetate	39.9	29.8	Inactive	3.2	Inactive
	n-Butanol	13.0	24.3	Inactive	0.9	Inactive
Twigs	*n*-Hexane	41.95	Inactive	Inactive	Inactive	Inactive
	Ethyl acetate	Inactive	Inactive	Inactive	Inactive	Inactive
	n-Butanol	33.9	Inactive	Inactive	Inactive	Inactive
Leaves	Methanol	-	5.9	12.1	-	-

IC_{50} > 50 μg/ml is inactive.

MCF-7 and MDA-MB-231 = human breast cancer cell lines

NCI-H1299 and A549 = human lung cancer cell lines

Vero cell = African green monkey kidney

According to biological activities screening of crude extracts from all parts of *A. megacarpa*, we found that the crude extracts from the rhizomes showed strong cytotoxicity against MCF-7, breast cancer cell line and strong antioxidant activity by DPPH assay. Therefore, the objective of this research is to extract and isolate of active constituents with anticancer and antioxidant activities from the rhizomes of *A. megacarpa.*