ความเป็นพิษของสมุนไพรไทยในกลุ่มยาอายุวัฒนะต่อเซลล์มะเร็งเต้านม (MCF-7) และเซลล์มะเร็งลำไส้ใหญ่ (SW-620)

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CYTOTOXICITY OF THAI REJUVENATING HERBS ON MAMMARY CANCER CELLS (MCF-7) AND COLON CANCER CELLS (SW-620)

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อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.วิชัย เชิดชีวศาสตร์, 118 หน้า.

การศึกษาความเป็นพิษต่อเซลล์มะเร็งของสารสกัดหยาบเอทานอลจากสมุนไพรซึ่งเป็นส่วนประกอบในตำรับยาอายุวัฒนะ 22 ชนิด โดยทำการทดสอบความเป็นพิษต่อเซลล์มะเร็งเพาะเลี้ยง 2 ชนิด ได้แก่ เซลล์มะเร็งเต้านม (MCF-7) และ เซลล์มะเร็งลำไส้ (SW-620) โดยใช้ 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) หลังจากการบ่มสารสกัดหยาบที่ระดับความเข้มข้น 0.1, 1, 10, 25, 50, 75, 100 , 500 และ 1,000 ไมโครกรัม/มิลลิลิตร ในเซลล์เพาะเลี้ยงเป็นเวลานาน 72 ชั่วโมง การวิเคราะห์ความเป็นพิษต่อเซลล์ได้เปรียบเทียบกับชุดควบคุม และหาค่าร้อยละการเติบโตของเซลล์เพื่อหาค่า IC_{50} จากการศึกษาสารสกัดสมุนไพรที่มีฤทธิ์ในการยับยั้งการเติบโตของเซลล์มะเร็งที่ค่า IC_{50} ต่ำกว่า 30 ไมโครกรัม/มิลลิลิตร พบว่ามีเพียงสารสกัดจากขันทองพยาบาท (ใบ) ชนิดเดียวที่มีฤทธิ์ต้านเซลล์มะเร็งเต้านม โดยมีค่า IC_{50} เท่ากับ 23.80 ไมโครกรัม/มิลลิลิตร สำหรับเซลล์มะเร็งลำไส้มีทั้งหมด 9 ชนิด ได้แก่ บัวบกป่า (หัว) ข่อย (เมล็ด) ขันทองพยาบาท (ใบ) พริกไทย (ผล) พริกไทย (เมล็ด) บอระเพ็ดพุงช้าง (หัว) กำลังเสือโคร่ง (ต้น) ตะโกนา (เปลือกต้น) และ แห้วหมู (หัว) โดยมีค่า IC_{50} เท่ากับ 5.08 5.53 5.90 14.60 14.67 15.73 23.83 29.34 และ 29.47 ไมโครกรัม/มิลลิลิตร ตามลำดับ อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ซึ่งสารสกัดที่มีฤทธิ์จำเพาะ (Selectivity index) กับเซลล์มะเร็งลำไส้ ได้แก่ ตะโกนา ข่อย ขันทองพยาบาท บัวบกป่า และ บอระเพ็ดพุงช้าง ซึ่งเป็นที่น่าสนใจจากการศึกษานี้ได้พบสมุนไพรหลายชนิดซึ่งมีฤทธิ์ที่ดีในการยับยั้งการเติบโตของเซลล์มะเร็ง

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JIRAPHAT CHAROENKUPT : CYTOTOXICITY OF THAI REJUVENATING HERBS ON MAMMARY CANCER CELLS (MCF-7) AND COLON CANCER CELLS (SW-620). THESIS ADVISOR : ASSOC. PROF. WICHAI CHERDSHEWASART, D.Sc., 118 pp.

The present study evaluated the cytotoxic activity potential of 24 crude extracts obtained from 22 plant species used in the Thai traditional medicine against MCF-7 (human breast cancer cells) and SW-620 (human colon cancer cells). The cytotoxic activity was analyzed by incubating the cancer cells with the plant ethanol extracts at the doses of 0.1, 1, 10, 25, 50, 75, 100, 500 and 1,000 µg/ml, respectively for 72 hours. The 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was used to determine cytotoxicity of the harvested cells in comparison with the DMSO control group. The plant extracts that showed a significantly cytotoxicity was/were verified from the IC_{50} value of less than 30 µg/ml. The leaf extract of *Suregada multiflorum* showed the highest cytotoxicity against MCF-7 cells with IC_{50} value of 23.80 µg/ml. The ethanolic extract of *Stephania erecta* (tuberous root), *Streblus asper* (seed), *Suregada multiflorum* (leaf), *Piper nigrum* (seed), *Piper nigrum* (fruit), *Stephania venosa* (tuberous root), *Betula alnoides* (whole stem), *Diospyros rhodocalyx* (stem bark) and *Cyperus rotundus* (rhizome), exhibited cytotoxicity against SW-620 cells with IC_{50} values of 5.08, 5.55, 5.90, 14.60, 14.78, 15.73, 23.83, 29.34, and 29.47 µg/ml, respectively. The ethanolic extracts of *Betula alnoides*, *Streblus asper*, *Stephania erecta*, *Stephania venosa* and *Suregada multiflorum* extract showed the positive selectivity (Selectivity index) against SW-620 cells. The study provided information on some of the Thai rejuvenating herbs with potential anti-proliferation effect against the studied cancer cell lines.

Field of Study : Biotechnology Student's Signature Jiraphat Charoenkupt

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
FBS	fetal bovine serum
RPMI	Rosewell Park Memorial Institute
PBS	phosphate-buffered saline
DMSO	dimethylsulfoxide
O.D.	optical density
IC_{50}	the 50% reduction of absorbency in MTT assay
CO_2	carbon dioxide
°C	degree Celsius
%	percentage
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide
rpm	revolution per minute
rcf	relative centrifugal force
h	hour
min	minute
l	liter
ml	milliliter
µl	microliter
g	gram
mg	milligram
µg	microgram
etc.	et ectera (Latin), other things
e.g.	for example
et al.	et alli (Latin), and other people
N/A	not applicable or not available

CHAPTER I

INTRODUCTION

Nowadays, cancer is the first common leading cause of death in Thailand and death from cancer is still increasing. The present drugs use in cancer therapy is mostly direct against all rapid proliferative cells which affect not only cancer cells, but also to normal cells. Alternatively, the medicinal plants for treatment cancer patients have been developed as alternative of drugs or substances that could candidate with the standard methods, i.e., surgery, chemotherapy, radiation, biological products.

The use of natural products as anticancer agents has a long history including traditional medicines and several drugs currently used in chemotherapy which were isolated from plant. Over 50 % of the drugs in clinical trials for anticancer activity were isolated from natural sources (Costa-Lotufo *et al.,* 2005). In Asian countries, herbal formulations from a mixture of plants are often used by traditional medical practitioners for the treatment of cancer. Ayurveda, the traditional Indian medicine has been consumed for various tumor prevention or suppression (Abu-Dahab and Afifi, 2007). Phytotherapy is considered as an alternative to reduce the side effect on the indiscriminate use of synthetic drug. Moreover, the advancement in biochemical and pharmacological studies of plant-derived drugs has opened a chance for new drug development (Balick *et al.,* 1996).

The plant derived anticancer agents in clinical use, for examples of the vinca alkaloids, vinblastine and vincristine were isolated from the *Catharanthus roseus* (Cragg and Newman, 2005). The etoposide and teniposide which are the semi-synthetic derivatives of epipodophyllotoxin, isolated from *Podophyllum spp.*, the naturally derived taxanes isolated from *Taxus spp.*. In recent study of Thai medicinal plants, *Stephania venosa* has a lot of isoquinoline alkaloids (Charles *et al.,* 1987) and *Dioscorea membranacea* has dioscorealide B and dioscoreanone, which were potent antiproliferation against MCF-7 (breast adenocarcinoma cell line) (Itharat *et al.,* 2004, Tewtrakul and Itharat, 2006). Manosroi and co-worker (Manosroi *et al.,* 2006) studied the essential oil from Thai medicinal plants, *Psidium guajava* L. leaf and *Ocimum basilicum* L. which showed anti-proliferative activity against KB cells (human mouth epidermal carcinoma) and P388 cells (murine leukemia).

The present study, the screening for the cytotoxic potential of the 24 ethanolic extracts from 22 Thai rejuvenating plants belonging to different plant families against MCF-7 (human breast cancer cell line) and SW-620 (human colon cancer cell line) were investigated with the aid of MTT assay. The plants with anti-proliferative effects are interesting as they might be potential sources that could be developed into anticancer drugs.

Purpose of the study is as follows:

To evaluate the cytotoxicity of 22 Thai rejuvenating herbal extracts against the human breast adenocarcinoma (MCF-7) and human colorectal adenocarcinoma (SW-620) cell lines.

CHAPTER II

REVIEW OF THE LITERATURE

1. Rejuvenating herbal plants

Medicinal plants have long term been traditionally used for treatment of various diseases including infection, immunological disorders and cancers (Farnsworth and Bunyapraphatsara, 1992). The rejuvenating herbal plants with the efficacy of reversal of aging and repair of the damage associated with aging (Govindarajan, *et,al.*, 2005). Thailand locates in a tropical area with abundance of diverse herbal plants (Suna, 2005). Thai rejuvenating herbs are widely and legally used in traditional Thai medicine. Twenty-two plants used in Thai traditional rejuvenating remedies were investigated for cytotoxicity in this study.

1.1 *Dracaena conferta* Ridl.

D. conferta (Family Agavaceae) locally known as "Kamlang Hanuman" is a shrub, (2-3 m height) widespread in the tropical, subtropical and warm temperate regions (Bogler and Simpson, 1995). The plant leaves are parallel-veined and usually appear long and pointed, often with a hardened spine on the end, and sometimes with additional spines along the margins. Flowers are clustered and sometimes solitary. Stamens have six inside a perianth and anthers versatile. Ovary has 3-lobules; each lobule has one ovule (ราชบัณฑิตยสถาน, 2538). *D. conferta* has been used by Thai traditional medicines for the treatment of bile disorder, analeptic muscle and sinew (วุฒิ, 2540).

Figure 1 *D.* species; **Source of photos:** http://www.arbolesornamentales.com/Dracenas.htm

1.1.1 Known chemical constituents

There was no report on the chemical constituents of *D. conferta*

1.1.2 Pharmacological activity and clinical trial

There was no report on biological activities of *D. conferta*

1.2 *Anaxagorea luzonensis* Gray

A. luzonensis (Family Annonaceae) locally known as "Kamlang wua thaloeng" is an indigenous shrub to Thailand. The leaves are oblong (7 to 15 cm long and 3 to 5 cm wide) with smooth and obtuse at the ends. The flower is solitary with leaf-opposed (about 2 cm in diameter). The sepals are ovate and the petals are ovate or oblong. The fruit is clavate (3 to 4 cm long). The black seeds are shining and obovoid (Hooker, 1875). The plant is traditional used as a blood tonic, stomachic, antipyretic and for treatment of muscular pain. The fresh leaves are used as topicals for articular rheumatism (Gonda *et al.*, 2000). *A. luzonensis* contains different classes of chemical constituents listed in Table 1.

Figure 2 *A. luzonensis* Gray

Source of photos: http://www.dnp.go.th
http://www.bloggang.com/viewdiary.php?id=endless9&group=11

1.2.1 Known chemical constituents

Table 1 The classes of chemical constituents reported in *A. luzonensis*

Class	Chemical constituents	Reference
Phenolic (xanthone)	1,3,6-trihydroxy-5-methoxy-4-prenylxanthone, 1,3,6-trihydroxy-4-prenylxanthone,	Gonda *et al.* 2000

Table 1 The classes of chemical constituents reported in *A. luzonensis* (Continued)

Class	Chemical constituents	Reference
	1,3,5-trihydroxy-6-methoxy-2-prenylxanthone,	Gonda *et al.* 2000
	3,6-dihydroxy-1,5-dimethoxyxanthone	
	Methylbutyl)xanthone,	Sabphon, 2008
	6-deoxy-isojacareubin	
Flavonoid	Flavone, biochanin A, chrysin, 39-methylorobol, orobol, taxifolin, kaempferol, quercetin, naringenin, aromadendrin	Gonda *et al.* 2000 Sabphon, 2008
Isoflavones	Daidzein, genistein	Sabphon, 2008
Sterols	Stigmasterol	Sabphon, 2008

1.2.2 Pharmacological activity and clinical trial

Antioxidative activity:

The flavonoids and xanthone isolated from the bark of *A. luzonensis* showed antioxidant activity (Gonda *et al.* 2000).

Estrogenic activity:

The prenylflavonoids obtained from this plant had estrogenic activity (Kitaoka *et al.* 1988).

Acetylcholinesterase inhibitory activity:

The bioactivity test revealed that 1,3,5-trihydroxy-4-(3-hydroxy-3-metylbutyl) xanthone and orobol obtained from this plant had strong activity with IC_{50} 2.36, and 2.57 μM against acetylcholinesterase (Sabphon, 2008).

1.3 *Betula alnoides* Buch.-Ham.

B. alnoides (Family Betulaceae) locally known as "Kamlang sueakhrong" is a tree of medium to large (about 20 to 35 m) with gray bark. The feathery leaves are alternate with a doubly serrate and stipulate. The flowers are monoecious with individual flowers are either male or female. Female inflorescences are 3-5 in a raceme with pendulous, narrowly cylindric and densely yellow villous. The obovate seeds (1.5-2 mm in diameter) are sparsely pubescent at apex with membranous wings. The plant has been traditional used as an antidote in the treatment of snake bites (Chopra, *et al.*, 1986). A decoction of

the bark is used to treat dislocated bones (Manandhar, 2002)

Figure 3 *B. alnoides* Buch.-Ham.

Source of photos: http://www.geocities.com/sawasdeenan/aboutnan.htm
http://herba.msu.ru/shipunov/w-album/20080427_boston/thumb.html

1.3.1 Chemical constituents

The known chemical compounds are terpene group (Kamperdick, *et al.* 1995)

1.3.2 Pharmacological activity: and clinical trial

Anti-inflammatory activity:

B. alnoides bark extract had been reported to contain anti-inflammatory (Kumar *et al.*, 2002)

Acetylcholinesterase inhibitory activity:

Acetylcholinesterase (AChE) inhibitor has been used as a drug for the symptomatic treatment of Alzheimer's disease.

1.4 *Cyperus rotundus* Linn.

C. rotundus (Family Cyperaceae) locally known as "Yha haewmoo" is a perennial herb that may reach a height of up to 40 cm. The plant is widely distributed in the tropical, sub-tropical and temperate regions (Santisuk and Larsen, 1999). The leaves sprout in ranks of three from the base of the plant. The stems have a triangular cross-section. The flower is bisexual and has a three-stigma carpel. The fruit is a three-angled achene. The root system of a young plant initially forms white and fleshy rhizomes (Anderson and Dunford, 1970). Some rhizomes grow upward in the soil with a bulb-like structure from which new shoots and roots grow. Other rhizomes grow horizontally or

downward and form dark reddish-brown tubers or chains of tubers (Harborne *et al.*, 1982, Uddin *et al.*, 2005).

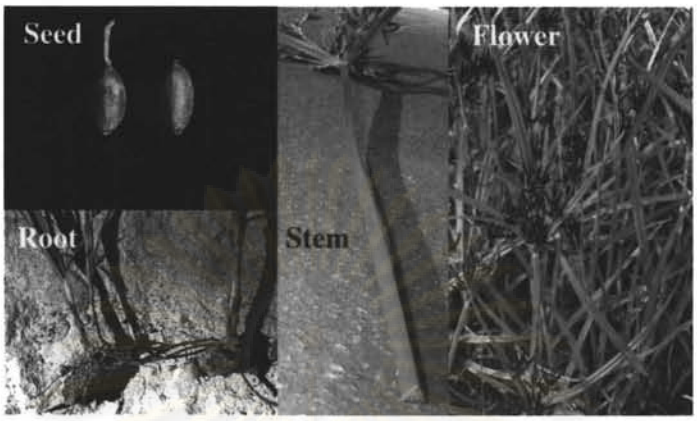

Figure 4 *C. rotundus* Linn.

Source of photos: http://en.wikipedia.org/wiki/Cyperus_rotundus
http://luirig.altervista.org/photos-int/cyperus-rotundus-tiririca.htm
http://www.cdfa.ca.gov/PHPPS/IPC/weedinfo/cyperus-rotundus.htm

C. rotundus is a traditional medicinal plant used in Indian, Chinese and Japanese natural drugs. *C. rotundus* rhizomes is used to treat inflammatory bowel disease, fever, pain, and various blood disorders (Raut and Gaikwad 2006, Soumaya *et, al.* 2009). The plant has been reported to contain oils, alkaloids, glycosides, tannins, starch and carbohydrates. It also contains proteins and traces of Mg, V, Cr, Mn and Co (Raut and Gaikwad, 2006). *C. rotundus* contains different classes of chemical constituents listed in Table 2.

1.4.1 Known chemical constituents

Table 2 The classes of chemical constituents reported in *C. rotundus*

Class	Chemical constituents	Reference
Flavonoid	Quercetin, luteolin, afzelechin, catechin and auresidin	Cai *et al.*, 1991 Masahiko and Kimiye, 1996 Tamura, *et al.*, 2002. Kiendrebeogo, *et al.*, 2005
Coumarin	6,7-dimethoxycoumarin	Uzi, *et al.*, 1986
Phenolic acids	Galloyl quinic acid, ferulic acid and 3-hydroxy- 4-methoxy-benzoic acid	Chen *et al.*, 2002 Romani *et al.*, 2002 Kuete, *et al.*, 2007

Table 2 The classes of chemical constituents reported in *C. rotundus* (Continued)

Class	Chemical constituents	Reference
Sterols	α-cyperone, sitosterol	Thebtaranonth, *et al.*, 1995 Kapadia, *et al.*, 1967
Saponin	Saponon-olenolic acid	Hikino, *et al.*, 1967
Sesquiterpenes	Cyperene, cyperenone, rotunol and selinene	Soumaya *et al.*, 2009

1.4.2 Pharmacological activity and clinical trial

Antiproliferation activity:

Luteolin exhibited anti-proliferation to K-562 (chronic myelogenous leukemia) cells at IC_{50} of 25 μ/ml (Soumaya *et al.*, 2009).

Antimicrobial activity:

The ethanolic extracts of *C. rotundus* were active against bacterial (Parekh and Chanda 2006).

Antimalarial activity:

α-cyperone, endoperoxide, sesquiterpene, 10,12-peroxycalamenene isolated from tubers of *C. rotundus* exhibited antimalarial activity (Soumaya *et al.*, 2009).

Anti-inflammatory activity:

The methanolic extract from this plant exhibited anti-inflammatory diseases mediated by overproduction of nitric oxide and superoxide (Gupta, *et al.*, 1980).

1.5 *Diospyros rhodocalyx* Kurz

D. rhodocalyx (Family Ebenaceae) locally known as "Takona", is a shrub, found in the central and northeast regions. (Thanakijcharoenpath and Theanphong, 2007). *Diospyros* is the largest genus of the family Ebenaceae comprises approximately 400 species widespread mainly in the tropics (Phengklai, 1981). In Thailand, sixty species of *Diospyros* have been described (Phengklai, 1978). Their fine and hard wood is of high value and the leaves, bark and fruit have been used as traditional medicine (Utsunomiya, *et al.*, 1998). The fruit has a small volume of flesh when fruit mature is sweet edible. The fruits are used for treatments of diarrhea, bleeding, abdominal discomfort, parasitic infestation abscess and renal disease. The bark is used for symptomatic relief of leucorrhea and as antidiuretic (Sutthivaiyakit et al., 1995).

1.5.1 Chemical constituents

Phytochemical investigation of more than 130 *Diospyros* species led to the isolation of a variety of compounds, the majority of which is triterpenoids and naphthoquinones (Mallavadhani *et al.*, 1998). Lupeol, β-sitosterol, stigmasterol, diospyrin and betulinaldehyde were isolated from the woods of *D. rhodocalyx* (Theerachayanan *et al.*, 2007).

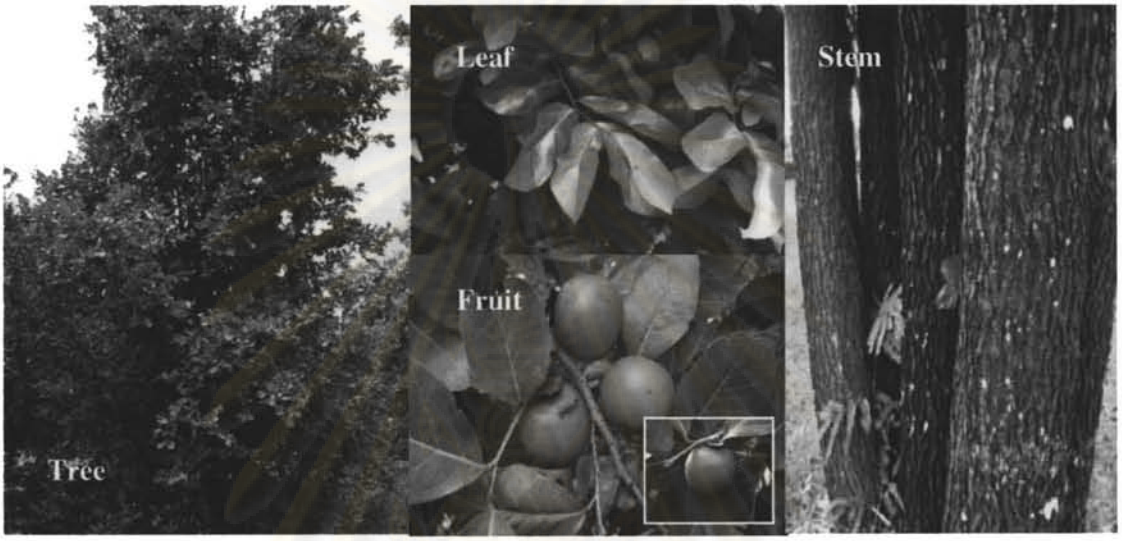

Figure 5 *Diospyros rhodocalyx* Kurz

Source of photos: http://thaiherb.most.go.th/plantdetail.php?id=223

1.5.2 Pharmacological activity and clinical trial

Antimalarial activity:

Diospyrin and betulinaldehyde showed *in vitro* antimalarial activity against *Plasmodium falciparum* with IC_{50} value of 6.25 μg/ml (Theerachayanan *et al.*, 2007).

Antimycobacterial activity:

Diospyrin and betulinaldehyde showed antimycobacterial activity with MIC values 25 μg/ml (Theerachayanan *et al.*, 2007).

1.6 *Phyllanthus emblica* Linn.

P. emblica (syn. *Emblica officinalis*, Family Euphorbiaceae) locally known as "Makham pom" is a tree of small or moderate size (8 to 18 m in height) with a greenish-grey bark. The greenish-yellow flowers formed auxiliary clusters. The feathery leaves are linear-oblong with a rounded base and obtuse or acute apex (7-10cm long). The tender fruits are green, fleshy, globosely and shining, and when mature change to light yellow or brick-red (Summanen, 1999). This plant grows in tropical and subtropical countries such as China, India, Indonesia, and on the Malay Peninsula. *P. emblica* have been widely

traditional used for the treatment of different types of diseases. The fresh (or) the dry fruit is used in traditional medicines for the treatment of diarrhea, jaundice and inflammations (Deokar, 1998). The pulp of the fruit is smeared on the head to dispel headache and dizziness (Perry, 1980). *P. emblica* leaves and fruit have been used for fever and inflammatory effects in Chinese herbal medicine (Perianayagama *et al.*, 2004). *P. emblica* contains different classes of chemical constituents listed in Table 3.

Figure 6 *P. emblica* Linn.

Source of photos: http://www.agronavigator.cz
http://www.nationaalherbarium.nl/thaieuph/ThPspecies/ThPhyllanthus.htm

1.6.1 Known chemical constituents

Table 3 The classes of chemical constituents reported in *P. emblica*.
(Modified from Jari Olavi Summanen, 1999)

Class	Chemical constituents	Reference
Alkaloid	Phyllantine, phyllantidine, zeatin, zeatin, nucleotide, zeatin and riboside	Khanna and Bansal, 1975 Ram and Rao, 1976
Benzenoid	Chebulic acid, chebulinic acid and chebulagic acid	Theresa *et al.* 1965, 1967
	gallic acid	Theresa *et al.* 1965, 1967 Basa and Srinivasulu, 1987
	ellagic acid	Theresa *et al.* 1965, Hui and Sung 1968, Subramanian *et al.*,1971 Desai *et al.* 1977

Table 3 The classes of chemical constituents reported in *P. emblica*.
(Modified from Jari Olavi Summanen, 1999) (Continued)

Class	Chemical constituents	Reference
	amlaic acid, corilagin,	Theresa *et al.* 1967
	3-6-di-O-galloyl-glucose and	Srivastava and Ranjan, 1967
	ethyl gallate	
	ß-glucogallin	Theresa *et al.* 1967
		Srivastava and Ranjan, 1967
	1,6-di-O-galloyl-ß-D-glucose,	El-Mekkawy *et al.*1995
	1-di-O-galloyl-ß-D-glucose,	
	putranjivain A and digallic acid	
	phyllemblic acid, emblicol and	Pillay and Iyer, 1958
	music (=galactaric) acid	Basa and Srinivasulu, 1987
Triterpene	Lupeol	Desai *et al.* 1977
		Hui and Sung 1968
Furanolactone	Ascorbic acid	Damoradan and Srinivasan, 1935
		Quadry *et al.*, 1962
		Shah and Hamid, 1968
		Basa and Srinivasulu, 1987
Diterpene	Gibberellin	Ram and Raja, 1978
Flavonoid	Leucodelphinidin,	Laumas and Seshardi, 1958
	kaempherol, quercetin and	Subramanian *et al.*,1971
	kaempherol-3-glucoside rutin	Yrjönen *et al.*,
		unpublished results
	kaempherol-3-O-ß-D- glucoside	El-Mekkawy *et al.*1995
	and quercetin-3-O-ß-D-glucoside	
Sterol	ß-sitosterol	Hui and Sung 1968
Carbohydrate	Acidic, neutral polysaccharides	Nizzamuddin *et al.* 1982
	and glucose	Theresa *et al.* 1967

1.6.2 Pharmacological activity and clinical trial

Antisnake venom activity

The methanolic root extracts of *P. emblica* showed potent antisnake (*Vipera russellii* and *Naja kaouthia*) venom activity (Alama and Gomesb, 2003).

Antioxidant activity

The fruit of *P. emblica* has more potent antioxidant than vitamin C (Khopde, *et al.*, 2001).

Antiproliferative activity

Eighteen main compounds, including phenolic compounds, proanthocyanidin polymers and norsesquiterpenoids isolated from *P. emblica* roots were estimated for their antiproliferative activities against MK-1 (human gastric adenocarcinoma), HeLa (human uterine carcinoma), and B16F10 (murine melanoma) cells (Zhang, *et al.*, 2004).

Anti-inflammatory activity

The leaf extracts of *P. emblica* have been shown to possess anti-inflammatory activity (Gupta *et al.*, 1994, Ihantola-Vormisto *et al.*, 1997).

1.7 *Suregada multiflorum* Baill.

S. multiflorum (Family Euphorbiaceae) locally known as "Khunthong phayabat" presents in the tropical and the subtropical areas of Asia and Africa (Choudhary *et al.*, 2004). The plant is a shrubs or shrubby trees (3-7 m high). Hairless branches are gray-yellow to gray-brown. Leaves are obovate-elliptic (5-16 cm long, 3-8 cm wide). Male and female flowers separate on the same tree. Male flowers have circular sepals with 30-60 stamens. Female flowers have an annular disk and a spherical ovary. Fruits are subglobular with shallowly 3 lobed and mostly smooth red-orange when ripe (พะยอม, 2521 and Cheenpracha et al., 2006).

The plant is traditional consumed for treatment of hepatic and gum diseases, inflammation and skin diseases (Choudhary *et al.*, 2004). Leaves, roots, and seeds of *S. multiflorum* contain several diterpene lactones, kaurane-type diterpenes, and flavonoids (Choudhary *et al.*, 2004). Triterpenoids isolated from bark contains GAP31, a protein which inhibits HIV-1 (Bourinbaiar and Lee-Huang, 1996). *S. multiflorum* contains different classes of chemical constituents listed in Table 4.

1.7.1 Known chemical constituents

Table 4 The classes of chemical constituents reported in *S. multiflorum*

Class	Chemical constituents	Reference
Terpene	Gelomulide, jolkinolide B, multiflorenol, gelomusid A and gelomusid B	Talapatra *et al.*, 1989 Das and Chakravarty, 1993 Choudhary *et al.*, 2004
Sterol	β-sitosterol	Talapatra *et al.*, 1989

Table 4 The classes of chemical constituents reported in *S. multiflorum* (Continued)

Class	Chemical constituents	Reference
Flavonoid	Luteolin and scutellarein	Parveen and Khan, 1987 Das and Chakravarty, 1993

Figure 7 *S. multiflorum* Baill.

Source of photos: http://thaiherb.most.go.th/plantdetail.php?id=265
http://www.rspg.or.th/plants_data/kp_bot_garden/kpb_10-10.htm
http://www.nationaalherbarium.nl/ThaiEuph/ThSspecies/ThSuregada.htm

1.7.2 Pharmacological activity and clinical trial

Antifungus activity:

Suregadolides A extract of *S. multiflora* bark showed moderate inhibitory activity in a mutant yeast strain bioassay (Jahan *et al.,* 2002).

Antibacterial activity:

The potential of chemical constituents from *S. multiflorum* showed inhibitory activity to *Xanthomonas campestris* (plant bacterial diseases) (Khuntong and Sudprasert, 2008).

Anti-human immunodeficiency virus activity:

S. multiflora extract contained anti-human immunodeficiency virus type 1 (HIV-1) protein, GAP31, and also exhibited the inhibitory effect on the infection and replication of herpes simplex virus (HSV) (Bourinbaiar and Lee-Huang, 1996).

1.8 *Melia azedarach* Linn.

M. azedarach (Family Meliaceae) is native to tropical Asia. It is widespread and naturalized in most of the tropics and subtropical countries such as Persia, India and China (Nakatani et al., 1998). *M. azedarach* locally known as "Lian" is a medium-sized tree (up 10-15 m tall). The plant is dense and dark green crown. Its bark is dark brown with grey lenticels. The leaves are alternate, leaflets are short stalked and thin, hairless, dark green (ventral) and relatively pale (dorsal). Flowers are white with purple stripes. Fruits or berries are yellow, round, smooth, and fleshy. Dried fruits are hard with 4 to 5 seeds (Ramya et al., 2009).

The plant has long been recognized for traditional medicinal and insecticidal properties (Cabral et al., 1995 and Bohnenstengel *et al.*, 1999). *M. azedarach* extracts inhibited vesicular stomatitis (VSV), polio and herpes simplex (HSV) viruses in cell cultures (Wachsman *et al.*, 1982). Fruits are poisonous, have been used traditionally for the treatment of a variety of diseases, especially dermatitis and rubella (Alche, et al., 2003). The fruits extracts possess ovicidal and larvicidal activity (Wandscheer *et al.*, 2004 and Corpinella *et al.*, 2007). The leaf extracts possess antiviral and antifertility activity (Choudhary *et al.*, 1990)

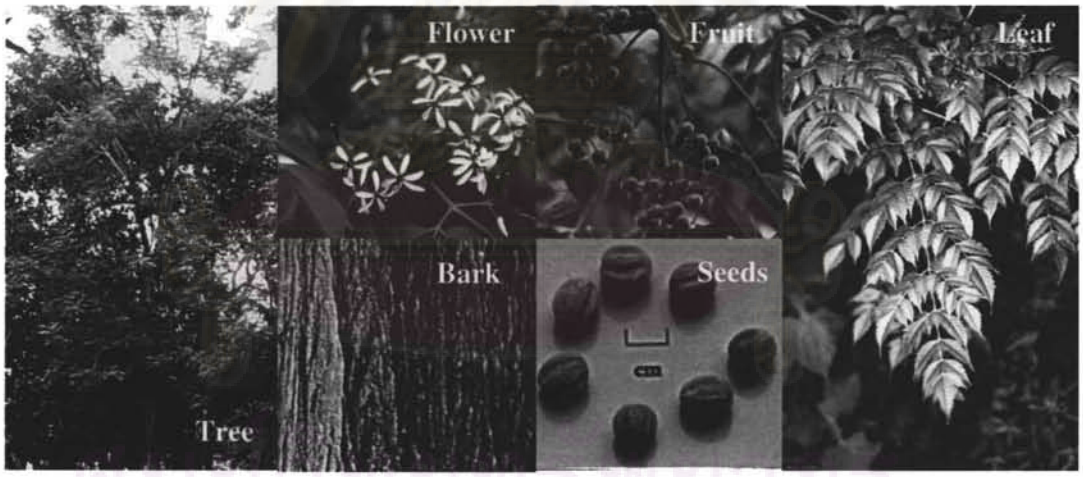

Figure 8 *M. azedarach* Linn.

Source of photos: http://www.swsbm.com/Images/New2-2001/Melia_azedarach.jpg
http://www.dnp.go.th/MFCD1/saraburisite/webpage/tree19.htm

1.8.1 Known chemical constituents

Different phytochemicals have been isolated from fruits including melianoninol, melianol, melianone, meliandiol, vanillin and vanillic acid (Han *et al.*, 1991). In addition, limonoids and triterpenoids have been isolated from fruits and bark

(Lee *et al.*, 1999). The euphane triterpene (Kelecom *et al.*, 1996) and four lignans (Cabral *et al.*, 1995) were isolated from the methanol extract of the seeds of *M. azedarach*.

1.8.2 Pharmacological activity and clinical trial

Antiparasitic activity:

M. azedarach fruit extracts showed anti-tapeworm and an earthworm activity (Szewczuk et al., 2003).

Antiviral activity:

M. azedarach leaves extracts has limonoid 1-innamoyl-3, 11-dihydroxymeliacarpin with antiviral activity , and with IC_{50} values of 6 μm and 20 μm for vesicular stomatitis (VSV) and herpes simplex (HSV-1) viruses (Andrei *et al.*, 1988 and Alche *et al.*, 2003).

Antioxidative activity:

M. azedarach leaf extract exhibits antioxidant activity (Fazil Ahmed *et al.*, 2008).

1.9 *Stephania erecta* Craib

S. erecta (Family Menispermaceae), known in Thai name as "Buabokpa" is a slander herbaceous climber. The plant is found in the tropical rain forest in Southeast Asia, especially in Malaysia and Thailand. It has a large tuber and round membranous leaves. Flowers are very small and yellow (Smitinand and Larsen, 1991). *S. erecta* is used in Thai folk remedies as a skeletal muscle relaxant and other diseases, as well as an analgesic and tonic (นันทวัน บุณยะประภัศร และอรนุช โชคชัยเจริญพร, 2541 เล่ม 2). The plant produces a large number of alkaloids, including at least 17 bisbenzylisoquinolines (Lin *et al.*, 1993). Several bisbenzylisoquinoline alkaloids have been shown to possess cytotoxicity against a number of human cancer cell lines (Buck, 1987), with antimalarial activity of some members of this group of alkaloids (Lin *et al.*, 1993).

1.9.1 Known chemical constituents:

Cepharanthine and homoaromoline have been isolated from the tubers of *S. erecta* (Prawat, *et al.* 1982). Obaberine, stephibaberin, telobine, tetradrine, thalrugosin, thalrugosine (Likhiwitayawuid, *et al.* 1993), methyltelobine, dehydrotelobine, isotetrandrine, thalrugosine, stephibaberine, and dephnandrine (Saxena *et al.*, 2003) are shown to be present in this plant.

1.9.2 Pharmacological activity and clinical trial

Antiplasmodial activity:

The alkaloids isolated from *S. erecta* have potent inhibitor for *Plasmodium falciparum* (Likhiwitayawuid *et al.*, 1993).

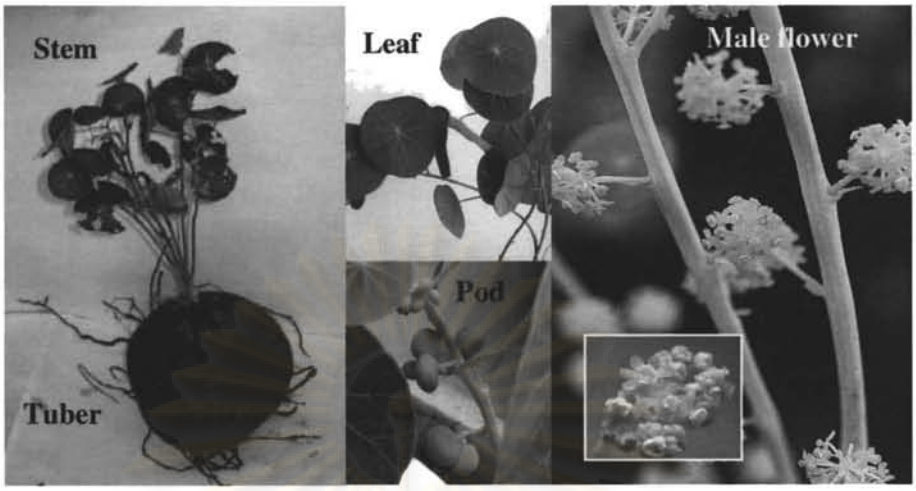

Figure 9 *S. erecta* Craib

Source of photos: http://public.fotki.com/plumo/stephania/
http://www.toptropicals.com

1.10 *Stephania venosa* (Blume) Spreng

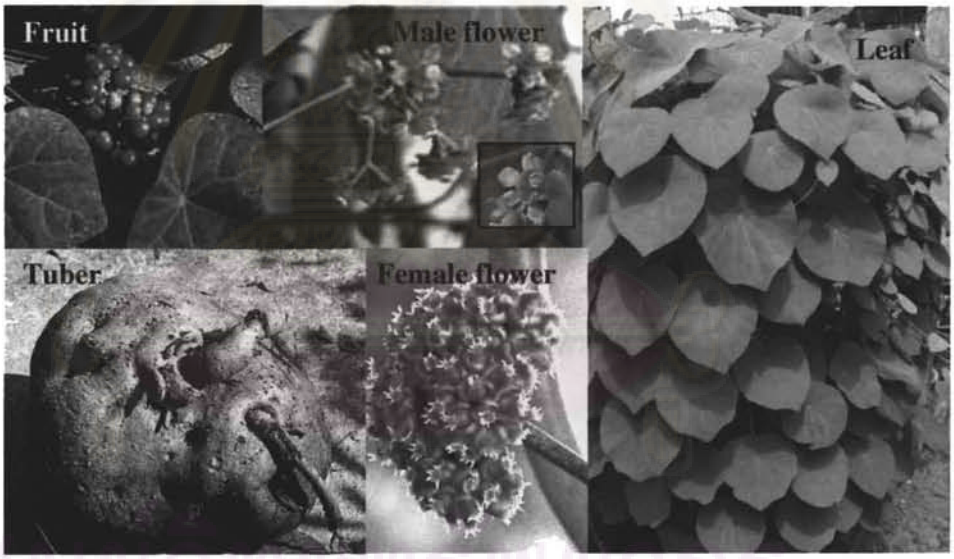

Figure 10 *S. venosa* (Blume) Spreng

Source of photos: http://www.herblpg.com/thai/herb55.html
http://www.tropicaflore.com

S. venosa (Family Menispermaceae) locally known as "Saboo luad" or "Boraphet plungchang", is a climber (around 4 m tall) with a large woody caudex. The leaves are arranged spirally on the stem. The plant has a large exposed tuber (up 20-40 cm in diameter) with bitter taste. The genus *Stephania* of the family Menispermaceae comprises of approximately 45 species and 15 of which have been reported in Thailand (Forman, 1991). Several *Stephania* species have been used in traditional medicine to treat a variety

of disease (Perry and Metzger, 1980). The genus *Stephania* is also well known as an important source of isoquinoline alkaloids, one of the largest groups of natural products which display interesting pharmacological activity. The genus *Stephania* could be a potential source of biologically active compounds which might be used as lead molecules for the development of new drugs (Suna Jongsomboonkusol, 2001).

1.10.1 Known chemical constituents

The most plant chemical compounds are alkaloid and alkaloid derivative, i.e. anonaine, asimilobine, glaziovine, kikemanine, reticuline, stepharine, stesakine. sukhodianine, ushinsunine, thalirugosamine, tudoranine (Charles, *et al.*, 1987) liriodenine, mecambroline, nuciferoline, stepharine, stephanosine, ushinsuninine (Pharadai, *et al.*, 1985) crebanine, sukhodianine, thailandine, uthongine (Guinaudeau, *et al.*, 1981), kamaline (Banerji, *et al.*, 1994) and protoberberines (Ingkaninan *et al.*, 2001).

1.10.2 Pharmacological activity and clinical trial

Anticancer activity:

Berberine substance in berberine sulfate in this plant has anticancer activity (Keawpradub *et al.*, 2001 and Simon *et al.*, 1989). Coralyne chloride has anti-leukemic activity on P-388 and L-1210 strain in mice (Pharadai *et al.*, 1985 and Simon *et al.*, 1989).

Antimicrobial activity:

Extracts from *S. venosa* has protoberberine alkaloids with antimicrobial activity and berberine in this plant has cytotoxicity on fungi, protozoa and bacteria (Simon *et al.*, 1989).

Antiplasmodial activity:

Dehydrostephanine and dehydrocrebanine shows potent antiplasmodial activity (Ingkaninan *et al.*, 2001).

1.11 *Tinospora crispa* Miers ex Hook. f. & Thoms.

T. crispa (Family Menispermaceae) locally known as "Boraphet" is a climber found in tropical and subtropical, also widely distributed in Indonesia, Malaysia (and Borneo), Thailand and Vietnam (Chavalittumrong *et al*, 1997). *T. crispa* is one ingredient in Thai folk remedies for maintaining good health, as a hepatoprotectant (Adhvaryu *et al*, 2008). In general folklore, a decoction of the stems, leaves and roots is used to treat fever, cholera, diabetes, rheumatism and snake-bites (Sinsh *et al*, 2003). A decoction of the stem is used for washing sore eyes, reduces thirst, internal inflammation, increases appetite

(Sartori and Swift, 2003), anti-malarial and a wash for skin ulcers, treatment of fever due to malaria, treatment of jaundice and against intestinal worms. The antimalarial effect was confirmed (Rahman *et al*, 1999). *T. crispa* contains different classes of chemical constituents listed in Table 5.

1.11.1 Chemical constituents

Table 5 The classes of chemical constituents reported in *T. crispa*

Class	Chemical constituents	Reference
Terpenes	Cycloeucalenol and cycloeucalenone	Kongkathip *et al*, 2002
Terpenoid	β-carotene, *N*-formylanondine, *N*-formylnornuciferine, *N*-acetyl nornuciferine, picrotein, tinotubride	Pathak *et al.*, 1995
Flavonoid	flavone O-glycosides (apigenin), picroretoside, berberine, palmatine and picroretine	Umi and Noor, 1995 Cavin *et al*, 1998
Terpenes glucoside	Borapetoside, tinotureride, tinotufolin, borapetol and tinocrisposide,	Fukuda, *et al*., 1983 Pachaly, *et al.*, 1992
Sterol	γ-sitosterol	Pathak *et al.*, 1995
Alkaloid	Colombine; glucoside, picroretine, berberine, palmatine, jatrorrhizine, tembetarine, choline and feruloyltyamine	Bisset and Nwaiwu, 1983 Cavin *et al*, 1998

Figure 11 *T. crispa* Miers ex Hook. f. & Thoms.

Source of photos: http://www.sunglass.diaryis.com/
http://home.hiroshima-u.ac.jp/~shoyaku/photo/Malaysia/1023Tino.jpg

1.11.2 Pharmacological activity and clinical trial

Antioxidative activity:

The *N*-cis-feruloyltyramine, *N*-trans-feruloyltyramine and secoisolariciresinol exhibited antioxidant and radical scavenging properties (Cavin *et al*, 1998).

Antimalarial activity:

T. crispa stem extract had been reported to have antimalarial (Nguyen-Pouplin *et al*, 2007)

Hypoglycaemic and insulinotropic activity:

T. crispa stem shows the hypoglycaemic effect which is associated with a potent *in vitro* insulinotropic activity in the human and rat islets and insulinoma cell line (HIT-T15) (Noor, *et al*, 1989).

Cardiac contractility:

Cycloeucalenol and cycloeucalenone present in the stems produced mild cardiotonic effects (Kongkathip *et al*, 2002).

1.12 *Acacia farnesiana* Willd.

A. farnesiana (Family Mimosaceae, subfamily of Leguminosae) locally known as "Krathin thet", is native to throughout Mexico, tropical America, and other subtropical tropical climates countries (García *et al.,* 2006). The exotic plant is a medium sized shrub with many spreading branches and basal stems. The alternate leaves are bi-pinnate compound with two to six pairs of pinnate each with 10 to 25 pairs of narrow leaflets. The twigs are dark brown with light-colored dots (lenticels) (Siegler *et al.* 1986). Flowers are bright yellow or orange flowers, very fragrant with a smell of violet. The older bark is

also dark brown and smooth. (Little and Wadsworth, 1964) The flowers are the source of "cassia oil". The bark is rich in tannin (Siegler *et al.* 1986).

Various parts of the plant are used in traditional medicine (Liogier 1990, and Parrotta 2001). In Mexico, the flowers are used to treat headache and indigestion, whereas a decoction of the green pods is used to treat dysentery and skin inflammations. In India, the bark, heartwood, and leaves are all used medicinally to treat a variety of ailments (Parrotta, 2001), the dried grated gum (2-3 g) mixed with water is considered to be effective against diarrhoea. In Malaya, the pulp surrounding the seeds is used as a plaster for treating tumors and furuncle (Watt and Breyer-Brandwijk, 1962). *A. farnesiana* contains different classes of chemical constituents listed in Table 6.

1.12.1 Chemical constituents

Table 6 The classes of chemical constituents reported in *A. farnesiana*

Class	Chemical constituents	Reference
Cyanogenic glycosides	Linamarin and lotaustralin	Seigler and Ebinger, 1987
Benzenoid	Salicylic acid, palmitic acid gallic acid, m-digallic acid, ellagic acid and methyl gallate	El Sissi, *et al.* 1973 Duke, 1981
Phenylpropanoids	Methyl-eugenol and eugenol	Duke, 1981
Ketones	Coumarin and hydroxyacetophenone	Duke, 1981
Flavonoids	Kaempferol, atomadendrin, and farnesol,	El Sissi, *et al.* 1973 Siegler *et al.* 1986
Flavonoids glycosides	Naringin	El Sissi, *et al.* 1973
Terpenoid	Terpineol, nerolidol and geraniol	Siegler *et al.* 1986

1.12.2 Pharmacological activity and clinical trial

Antimicrobial activity:

The ethanolic extracts of *A. farnesiana* barks efficiently inhibited bacterial growth (García *et al.*, 2006).

Antimalarial activity:

The ethanolic extracts of *A. farnesiana* barks exhibited cytotoxicity against

Plasmodium falciparum, with IC_{50} values 1.3 μg/ml (Garavito *et al.*, 2006).

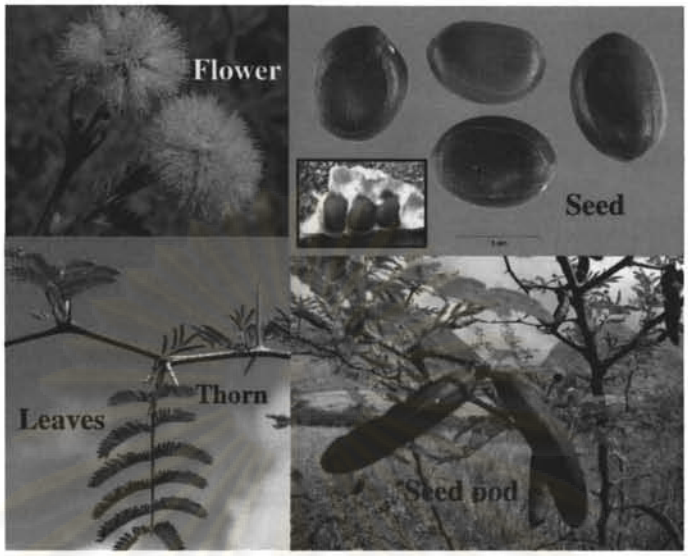

Figure 12 *A. farnesiana* Willd.

Source of photos: http://www.hear.org/starr/plants/images/species/?q=acacia+farnesiana

1.13 *Albizia procera* Benth.

A. procera (Family Mimosaceae) native to tropical Asia and Australia, locally known as "Thing thon" is a large and fast-growing tree (25 m in height). The bark is nearly smooth and whitish to light-greenish gray or light-brown. The bi-pinnate leaves are reddish when mature to a length of 12-25 cm and leaflets are 2-4 cm long and 8-16 mm wide. Flowers are borne on racemes 8-25 cm long (Little and Wadsworth 1964). The fruits are flattened pods 10 to 20 cm long and 1.8 to 2.5 cm broad, changing from green to deep red or reddish brown on maturity; each contains 6 to 12 seeds (Troup 1921, Little and Wadsworth 1964).

A. procera is commonly used in traditional medicines (Venkalarammany 1968). The bark contains tannins and a reddish gum, can be used to make a poison, useful in pregnancy and stomachache (Melek, *et al.*, 2007). The leaves are used to treat ulcers and have insecticidal properties (Parrotta 1987). *A. procera* contains different classes of chemical constituents listed in Table 7.

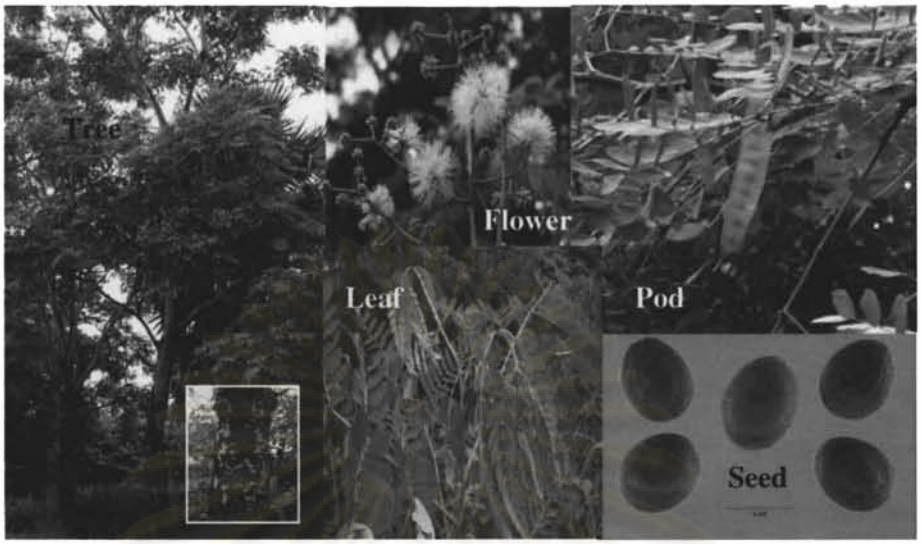

Figure 13 *A. procera* Benth.

Source of photos: http://thaiherb.most.go.th/plantdetail.php?id=350
http://commons.wikimedia.org/wiki/File:Albizia_procera_seeds.jpg
http://www.tistr.or.th

1.13.1 Known chemical constituents

Table 7 The classes of chemical constituents reported in *A. procera*

Class	Chemical constituents	Reference
Triterpenoid saponin	oleanoliic acid,	Varshney and Badhwar,
	echinocystic acid,	1972a
	proceranin A	Banerji, *et al.*, 1979
	and proceric acid	Melek, *et al.*, 2007
Amino acid	albizzin	Gmelin, *et al.* 1958
Flavonoid	biochanin A, daidzein,	Deshpande and Shastri, 1977
	genistein, formononetin	
Sterol	β-sitosterol and	Varshney, *et al.*, 1965
	α-spinasterol	Banerji, *et al.*, 1979

1.13.2 Pharmacological activity and clinical trial

Anticancer activity:

Saponins isolated from bark of *A. procera* exhibited cytotoxicity against HepG2 cell line with IC_{50} value 9.13 μg/ml (Melek, *et al.*, 2007).

1.14 *Leucaena leucocephala* de Wit

Figure 14 *L. leucocephala* de Wit

Source of photos: http://www.tropicalforages.info/key/Forages/Media/Html/Leucaena_leucocephala.htm

L. leucocephala (Family Mimosaceae, subfamily of Leguminosae) locally known as "Krathin thai" is a tropical plant. The plant is shrub or tree up to 18 m tall with gray bark and prominent lenticels. Leaves are bi-pinnate with 4-9 pairs of pinnae. Flowers numerous are globose heads with a diameter of 2-5 cm. Pod is pendant and brown at maturity with seeds 18-22 per pod (พะยอม, 2527). *L. leucocephala* was reported to have few medicinal properties ranging from controlling of stomach diseases to contraception (Jagan Mohan and Azeemoddin, 1988). Galactomannans are the most abundant storage polysaccharide in *Leucaena* sp., found in the endosperm cell wall of seeds from the Leguminosae family (Reid, 1985). They have biological activities of including cancer chemopreventive, anti-cancer (Ingolfsdottir *et al.,* 1994) immunostimmulation (Ingolfsdottir *et al.,* 1994 and Ramesh *et al.,* 2002), anti-viral (Herold *et al.,* 1995), anticoagulant and anti-thrombotic (Martinichen-Herrero *et al.,* 1995) activities. *L. leucocephala* contains different classes of chemical constituents listed in Table 8.

1.14.1 Chemical constituents

Table 8 The classes of chemical constituents reported in *L. leucocephala*

Class	Chemical constituents	Reference
Diterpenoid acids	Gibberellin (phytohormone)	Arigayo, *et al.* 1983
Sterol	ß-sitosterol	Verma and Chandra, 1979
Flovanoid	Guaijaverin, kaempferol, hyperoside quercetagetin and quercetin	Morita, *et al.* 1977 Ranganathan and Nagarajan, 1980

1.14.2 Pharmacological activity and clinical trial

Antibacterial activity:

Extracts of *L. leucocephala* had been reported to have antibacterial (Avirutnant and Pongpan, 1983).

1.15 *Streblus asper* Lour.

S. asper (Family Moraceae) locally known as "Khoi" is a tree with rigid and dense branch. The oblong-ovate leaves (4 to 12 cm in long) are sub-rhomboid with very rough on both sides and finely toothed margin. The male flowers (4 to 7 mm in diameter) are in rounded heads with greenish-yellow or nearly white. The female flowers usually in pairs are green peduncle with the ac-crescent sepals and nearly enclosing the fruit. The ovoid fruit (8 to 10 mm in long) is yellow pericarp with soft and fleshy (Hooker, 1886). Various parts of this plant are used in Ayurveda and other folk medicines for the treatment of different ailments such as filariasis, leprosy, toothache, diarrhea, dysentery and cancer (Rastogil, *et al.*, 1964). *S. asper* contains different classes of chemical constituents listed in Table 9.

1.15.1 Chemical constituents

Table 9 The classes of chemical constituents reported in *S. asper*

Class	Chemical constituents	Reference
Cardiac glycoside	Kamloside, asperoside, indroside, cannodimemoside, glucokamloside, glucogitodimethoside, glucostrebloside, strebloside, strophanolloside, sarmethoside, strophalloside, and 16-O-acetyl-glucogitomethoside	Khare, *et al.*, 1962 Manzetti and Reichstein, 1964
Pregnane glycoside	Sioraside, N-Triacontane, tetraiacontan-3-one, β-sitosterol, stigmasterol, betulin and oleanolic	Chawla, *et al.*, 1990 Prakash, *et al.*, 1992
Triterpene	α-amyrin acetate, lupeol acetate, lupeol, diol, strebloside and mansonin	Barua, *et al.*, 1968 Fiebig, *et al.*, 1985

The other constituents were α-copaene, β-elemene, caryophyllene, geranyl acetone, germacrene, α-cadinene, caryophyllene oxide and 8-heptadecene (Phutdhawong, *et al.*, 2004). In addition, the major constituents of the volatile oil from fresh leaves of *S. asper* were phytol, α-farnesene, trans-farnesyl acetate, caryophyllene and trans-trans-α-farnesene (Phutdhawong, *et al.*, 2004).

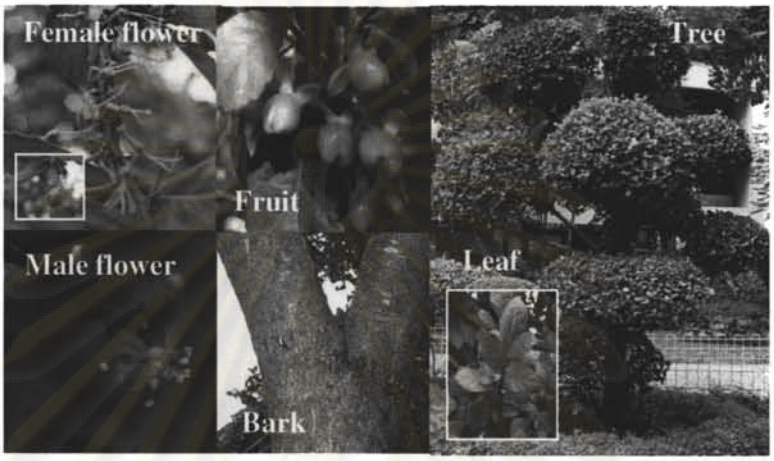

Figure 15 *S. asper* Lour.

Source of photos: http://en.wikipedia.org/wiki/Streblus_asper
http://thaiherb.most.go.th/plantdetail.php?id=366

1.15.2 Pharmacological activity and clinical trial

Cardiotonic activity:

Ethanolic extract of root bark of *S. asper* exhibited cardiotonic activity (Rastogil, *et al.*, 1964).). Pharmacological studies indicated that the drug has definite action on myocardium (Gaitonde *et al.*, 1964).

Antifilarial activity:

The crude aqueous extract of the stem bark of *S. asper* revealed significant macrofilaricidal activity (Rastogil, *et al.*, 1964).

Anticancer activity:

Cardiac glycosides: strebloside and mansonin in this plant have significant anticancer activity in KB cell (human carcinoma of the nasopharynx) culture system (Fiebig, *et al.*, 1985). The volatile oil from fresh leaves of *S. asper* showed significant anticancer activity in P388 (mouse lymphocytic leukemia) cells (Phutdhawong, *et al.*, 2004).

Antimicrobial activity:

Ethanol extracts from the sticks and leaves of *S. asper* have been shown to inhibit the growth of *Streptococcus mutans* (Triratana, *et al.*, 1987).

Anti-allergic activity:

S. asper showed promising anti-allergic activity in experimental models, such as anti-PCA (passive cutaneous anaphylaxis) and mast cell stabilizing activity (Amarnath, 2002).

1.16 *Butea superba* Roxb.

B. superba (Family Papilionaceae-Leguminosae) locally known as "red Kwao Krua", is a large climber. This plant can be found growing in forests in Thailand's northern and eastern regions (Roengsumran *et al.*, 2000). Leaves are pinnately trifoliate, acuminate leaflet and long leafstalk. Flowers are of a yellowish orange color. Pods are 3-4 inches long, oblong shaped with silvery silky short hair (Kruz, 1877 and Brandis, 1990).

The tuber and stem of *B. superba* are used as Thai traditional medicine for tonic and rejuvenate, believed to give strength and power and increase male sexual performance (Suntara, 1931). The plant is enriched with flavonoid and flavonoid glycoside. The bioactivity of each constituent was tested for an inhibitory effect towards cAMP phosphodiesterase, which has been shown to be important in controlling bodily function and involved a wide number of diseases (Roengsumran *et al.*, 2000). *B. superba* contains the different classes of chemical constituents listed in Table 10.

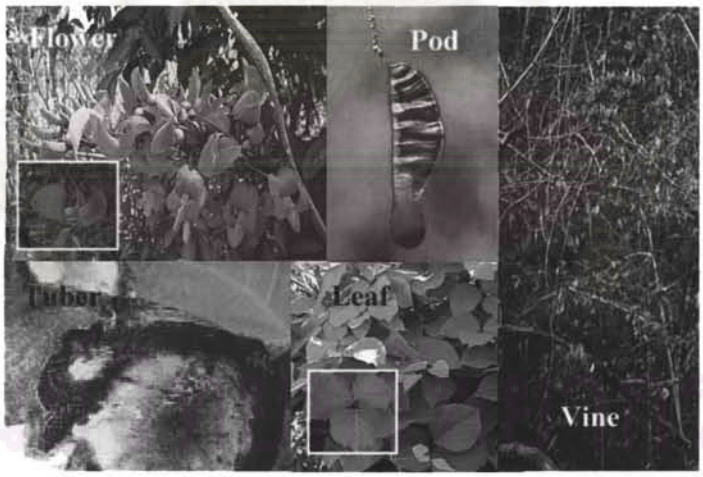

Figure 16 *B. superba* Roxb.

Source of photos: http://thongthailand.igetweb.com/index.php?mo=3&art=274294
http://www.pantown.com/board.php?id=5050&area=4&name=board13&topic=335&action=

1.16.1 Known chemical constituents

Table 10 The classes of chemical constituents reported in *B. superba* (Sangkapong, 2005)

Class	Chemical constituents	Reference
Carboxylic acid	Straight acid carboxylic acid (C_{22}-C_{26}), 3-hexacosanoloxy-propane-1,2-diol	Rakslip, 1995
Steroid	Campesterol	Rakslip, 1995
Steroid glycoside	β-sitosteryl 1-3-O-β-D-glucopyranside, Stigmasteryl 1-3-O-β-D-glucopyranside	Rakslip, 1995
Flavonoid	3-hydroxy-9-methoxypterocarpan (Medicarpin), 7-hydroxy-4_-methoxy-isoflavone (Formononetin), 5,4'-dihydroxy-7-methoxy-isoflavone (Prunetin)	Ngamrojanavanich, *et al.*, 2007
Flavonoid glycoside	7-hydroxy-6,4'-dimethoxyisoflavone	Roengsamran *et al.*, 2000 Ngamrojanavanich, *et al.*, 2007
Isoflavone	7,4'-dimethoxyisoflavone	Ngamrojanavanich, *et al.*, 2007

1.16.2 Pharmacological activity and clinical trial

Antiproliferation activity:

Formononetin and prunetin showed moderate cytotoxic activity on KB (human epidermoid carcinoma of cavity) cell lines with IC_{50} values 37.3 and 71.1 μM and on BC (breast cancer) cell lines with IC_{50} values 32.7 and 47.3 μM, respectively (Ngamrojanavanich *et al.*, 2007)

Acetylcholinesterase inhibitory activity:

The root bark of *B. superba* extracts showed inhibitory activity on AChE (Ingkaninan *et al.*, 2003)

Androgenic activity:

B. superba tubers have an androgenic effect on the reproductive organs of intact

and ovariectomized rats, and exhibit anti-estrogenic activity on serum luteinizing hormone (LH) secretion in ovariectomized rats (Malaivijitnond *et al.*, 2009). *B. superba* tuberous powder showed effective treatment of erectile dysfunction in mature Thai males (Cherdshewasart and Nimsakul, 2003)

1.17 *Mucuna collettii* Lace

M. collettii (Family Papilionaceae-Leguminosae) locally known as "black Kwao Krua", is a large soft woody climber. The leaves are trifoliate; leaflet 4-8 by 2-4 inches sparsely hairy, entire margin; petiole 5-10 cm long. Flowers are hanging on the stem up to 12 inches long with 5 sepals covered with brown rough hair unite into a bell-shaped tube. Petals are blackish-purple pea-like shaped. Stamens are two bundles. Pods are linear-oblong shaped up to 16 inches long. Seed are hard and flat (Pengklai, 1977). The sap is black when all parts of this plant were cut and exposed to the air. The roots of *M. collettii* have long been used as a folk medicine for invigorating males (หลวงอนุสารสุนทร, 2474).

Figure 17 *M. collettii* Lace

Source of photos: http://thaiherb.most.go.th/plantdetail.php?id=407

1.17.1 Chemical constituents

The whole stem of *M. collettii* contains the flavonoids namely; kaempferrol, quercetin, and hopeaphenol (Roengsamran, *et al.* 2001,)

1.17.2 Pharmacological activity and clinical trial

Antiproliferation activity:

Hopeaphenol isolated from whole stem of *M. collettii* exhibited cytotoxicity against KB cell line (Ohyama, *et al.* 1999). The plant crude extract exhibited cytotoxicity to HeLa (Cherdshewasart *et al.*, 2004a) and MCF-7 (Cherdshewasart *et al.*, 2004b)

Antioxidant activity:

Quercetin in this plant has been reported to provide antioxidant activity (Lamson and Brignall, 2000)

Antimutagenic activity:

The extract of *M. collettii* has been reported to have antimutagenic potential (Cherdshewasart *et al*., 2008).

1.18 *Pueraria mirifica* Airy Shaw et Suvatabhandu

P. mirifica (Family Papilionaceae-Leguminosae) locally known as "white Kwao Krua" is a climber, found in the deciduous forest of the northern, western and northeastern and southern region of Thailand (Lakshnakara and Suvatabandhu, 1952 and Cherdshewasart *et al*., 2007). The plant is a long-live twin wood. Leaves are pinnately three foliate stipulate; terminal leaflet. Tuberous roots are varied in sizes and shape. Flowers are bluish-purple legume shape. The inflorescence of certain flowers is approximately 15-40 cm long. Pods are slender typically ahort elongate, smooth or hairy, including 1-10 single seeds when fully matured and dried which turned into various color (Smitasiri and Wungjai, 1986 and Cherdshewasart and Sriwatcharakul, 2007).

Figure 18 *P. mirifica* Airy Shaw & Suvatabhandu

In Thailand, the tuberous roots have been consumed effectively as "rejuvenating" folk medicine for both aged men and women for its efficacy to grow hair, strengthen and darken existing ones, help improve domplexion and remove wrinkles, improve eyesight, increase energy and vigor leading to more reflexive body movements. *P. mirifica* contains a high phytoestrogen content and concentrated isoflavones, which support several health functions; prostate, cardiovascular, bone structure, breast and skin appearance, and

menopausal or post-menopausal symptoms. *P. mirifica* contains different classes of chemical constituents listed in Table 11.

1.18.1 Known chemical constituents

Table 11 The classes of chemical constituents reported in *P. mirifica* (Modifiled from Cheewasopit, 2001)

Class	Chemical constituents	Reference
Isoflavonoids	Daidzein, genistein, kwakhurin and kwakhurin hydrate	Ingham *et al.*, 1986a Ingham *et al.*, 1986b Ingham *et al.*, 1989
Isoflavone glycoside	Daidzin, genistin, mirificin, puerain 6'-monoacetate and puerarin	Ingham *et al.* 1986b Ingham *et al.* 1989
Chromene	Miroestrol, deoxymiroestrol and isomiroestrol	Schoeller *et al.*, 1940 Chansakaew *et al.*, 2000a
Coumestans	Coumestrol, mirificoumestan, miricoumestan glycol and miricoumestan hydrate	Ingham *et al.*,1986 Ingham *et al.*,1988
Sterol	β-sitosterol and stigmasterol	Hoyodom, 1971
Pterocapans	Pueeriicapene and tuberosin	Chansakaew *et al.*, 2000a
Acid	Tetracosanoic acid	Chansakaew *et al.*, 2000b

1.18.2 Pharmacological activity and clinical trial

Antioxidative activity:

Pueraria glycoside, mangiferin and major isoflavonoid showed potent antioxidant activity (Takashi *et al.*, 1992 and Cherdshewasart and Sutjit, 2008)

Estrogenic activity:

Two isoflavonoids, genistein and daidzein of the dichloromethane extract from *P. mirifica* (Sookvanichsilp *et al.*, 2008) have potent estrogenic activity (Cherdshewasart and Sriwatcharakul, 2008 and Sookvanichsilp *et al.*, 2008).

Preventive bone loss:

P. mirifica, the phytoestrogen-rich has been reported to prevent bone loss in orchidectomized rats (Urasopon *et al.*, 2007 and Urasopon *et al.*, 2008).

1.19 *Piper nigrum* Linn.

P. nigrum (Family Piperaceae) locally known as "Phrik thai" or black pepper, is a climber. The plant is native of Southern India and Sri Lanka. The blackish-green leaves are elliptical ovate. The fruits first turn green, then red and finally turning black. The plant is widely cultivated in the tropics as the source of black and white pepper. Black pepper is obtained from the dried unripe fruit; white pepper is obtained when the pericarp is removed (Craib, 1992). *P. nigrum* (black pepper) finds extensive use in Ayurvedic system of medicine (Kirtikar and Basu, 1981). A number of piperidine and pyrrolidine alkamides are known to occur in plant (Parmar *et al.,* 1997), the most important being piperine, known to possess a variety of biological properties like CNS stimulant, analgesic, antipyretic and antifeedant activities (Miyakado *et al.,* 1979). In Iranian traditional medicine, black pepper is used to relieve menorrhagia in women (Craib, 1992).

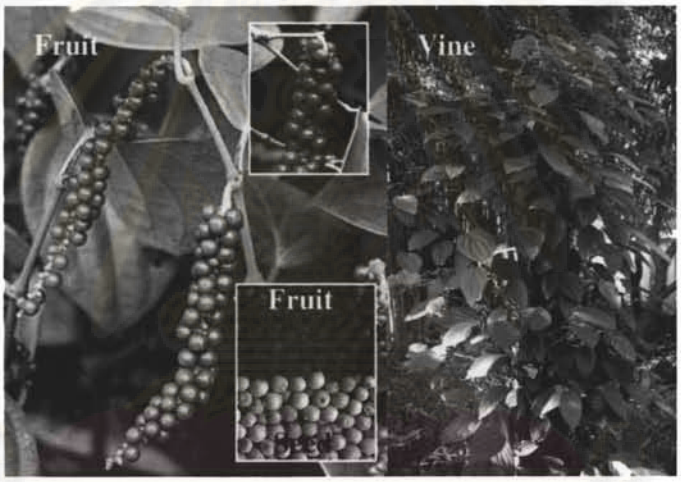

Figure 19 *Piper nigrum* Linn.

Source of photos: http://www.manager.co.th
http://www.bloggang.com/viewdiary.php?id=nukoy-handmade&month=09-2008&date=21&group=5&gblog=1
http://th.wikipedia.org/wiki.
http://www.nipahutgardens.com/products.asp?cat=23

1.19.1 Known chemical constituents

The major constituent isolated from *P. nigrum* fruits is piperine, 8Z-N-isobutyleicosatrienamide, pellitorine, trachyone, pergumidiene, isopiperolein B (Venkat, *et al.*, 2004), piperidine, pyrrolidine amides (Parmar *et al.,* 1997). The compounds 3, 4-dihydroxyphenyl ethanol glucoside and 3, 4-dihydroxy-6-(N -ethylamino) benzamide reported to be present in green pepper but absent in black pepper (Pradhan, *et al.,* 1999).

1.19.2 Pharmacological activity and clinical trial

Acetylcholinesterase inhibitory activity:

Acetylcholinesterase (AChE) inhibitor has been used as a drug for the symptomatic treatment of Alzheimer's disease. The seeds of *P. nigrum* extract showed inhibitory activity on AChE (Ingkaninan *et al.*, 2003).

Antioxidant activity:

Both water extract and ethanol extract of black pepper showed exhibit strong antioxidant activity (Gülçin, 2005)

Antibacterial activity:

The ethanol extracts of *P. nigrum* were active against both gram-positive and gram-negative bacteria (Venkat, et al., 2004). The compounds 3, 4-dihydroxyphenyl ethanol glucoside and 3, 4-dihydroxy-6-(N -ethylamino) benzamide were found to inhibit the food borne pathogens (Pradhan, *et al.*, 1999).

Anti-allergic activity:

P. nigrum EtOH extract exhibited potent antigen-induced β-hexosaminidase release from RBL-2H3 cells (rat-basophilic leukemia cell line), a tumor analog of mast cell, with an IC_{50} value of 14.0 μg/ml, which was higher than that of ketotifen fumarate, a positive control (IC_{50} = 20.2 μg/ml) (Kraithep, *et al.*, 2008).

1.20 *Fagraea fragrans* Roxb.

F. fragrans (Family Potaliaceae) locally known as "Kan krao", is native to South-east Asia. The plant is a large evergreen tree (about 10 to 25 m tall) with a dark brown trunk and deeply fissured bark. Branches arise from the main trunk and grow upwards. The leaves are simple with elliptical and narrowing towards the tip. Its yellowish flowers have a distinct fragrance and the fruits of the tree are bitter tasting red berries (พร้อมจิต ศรลัมพ์ และคณะ. 2535). The plant has been used in ancient medicine as a blood tonic, fever, treating hemorrhoid. A decoction of the stem is used to treat dislocated bones (นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร. 2541 เล่ม 1).

The research identified potential activities *in vitro* tests in *F. fragrans*: anti-aging and anti-skin roughness effects, reported by the National Center for Genetic Engineering and Biotechnology (BIOTEC) and Shiseido. The findings will act as a catalyst for further research development of cosmetic ingredients and skin care (www.sciencepark.or.th).

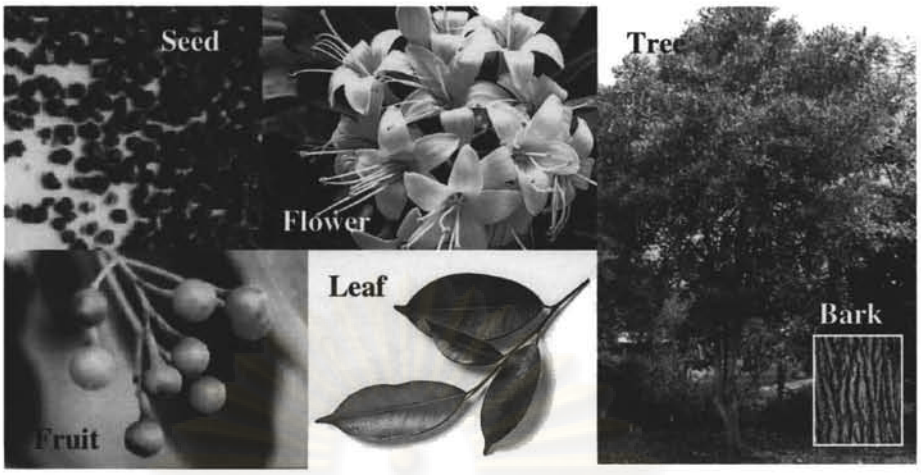

Figure 20 *F. fragrans* Roxb.

Source of photos: http://www.rspg.thaigov.net/plants_data/plantdat/loganiac/ffragr_1.htm
http://www.agri.ubu.ac.th/publish/Movie1.html

1.20.1 Chemical constituents

The plant known chemical compounds are gentianine (Natarajan, *et al.* 1974), secoiridoid glycoside namely, swertiamarin (Kun-Anake and Rajvatin, 1976). A secoiridoid, named fagraldehyde, gentiopicroside, sweroside and swertiamarin were isolated from the bark and leaves of *F. fragrans*. (Marie, *et al.* 2008)

1.20.2 Pharmacological activity and clinical trial

Antibacterial activity:

Extracts of *Fagraea fragrans* had been reported to have antibacterial (Kun-Anake and Rajvatin, 1976).

Antiplasmodial activity:

Fagraldehyde isolated from *F. fragrans* was potent inhibitors for *P. falciparum* (Marie, *et al.* 2008).

1.21 *Vitex trifolia* Linn.

V. trifolia (Family Verbenaceae) locally known as "Khon thiso" that is a shrub (up to 8 m tall) within tropical and sub-tropical regions. It has a smooth light grey to brown bark. The leaves consist of 3 or 5 smaller leaflets which are all connected at one point and are elliptic. The upper of the leaves surface are green and the lower surface grayish green that covered with white hairs. The purple to blue flowers consist of a tube with five lobes and the central lobe is bigger than the others. The fleshy globose fruits are black when mature (up to 7 mm in diameter) and contain 4 small black seeds (Herna´ndez *et al.,* 1999).

V. trifolia has been reported to have both medicinal and insecticidal properties. In traditional medicines, this plant is the most important in the field of medicine. Leave are commonly used as poultice for rheumatic pains, inflammation, sprains and fever. Roots are used to treat febrifuge, painful inflammations, cough and fever. Flowers are used in treating fever and fruit in amenorrhoea (Ramesh *et al.*, 1986 and Herna´ndez *et al.*, 1999). Seed are used an insect antifeeding activity (Hosozawa *et al.*, 1974). There have been various studies on the chemical structures of compounds isolated from both *V. trifolia* leaves and fruit (Pan et al., 1989). The *V. trifolia* contains the different classes of chemical constituents listed in Table 12.

1.21.1 Chemical constituents

Table 12 The classes of chemical constituents reported in *V. trifolia*

Class	Chemical constituents	Reference
Terpenoid	Caryophyllene, friedelin, sabinene and α-pinene,	Suksamrarn *et al.*, 1991
Sterol	Daucosterol, β-sitosterol and β-sitosterol-β-D-glucoside	Vedantham and Subramanian, 1976 Zeng *et al.*, 1996
Flavonoid	Casticin, agnuside, luteolin, isoorientin, persicogenin, penduletin, artemetin, chrysosplenol-D, vitexin, vitexicarpin 5-methyl artemetin, 7-desmethyl artemetin, and 3, 6, 7-trimethyl quercetagetin	Nair *et al.*, 1975 Ramesh *et al.*, 1986 Zeng *et al.*, 1996 Li *et al.*, 2005
Flavonoid glycoside	Luteolin-7-*O*-β-D-glucuronide, luteolin-3-*O*-β-D-glucuronide	Ramesh *et al.*, 1986
Terpenoid	Caryophyllene, friedelin, sabinene and α-pinene,	Suksamrarn *et al.*, 1991
Sterol	Daucosterol, β-sitosterol and β-sitosterol-β-D-glucoside	Vedantham and Subramanian, 1976 Zeng *et al.*, 1996
Fatty acid	Linoleeic acid, myristic acid, palmitic acid, palmitoleic acid, γ-tocopherol and steric acid	Prasad and Nigam, 1982

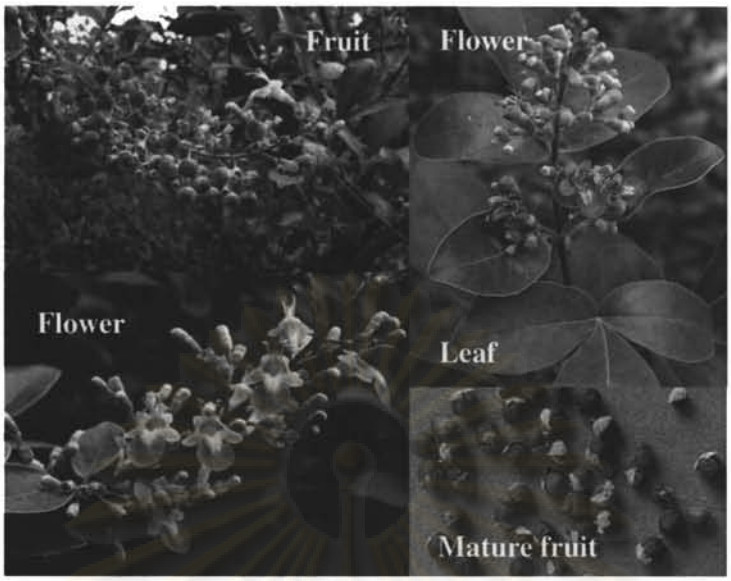

Figure 21 *V. trifolia* Linn.

Source of photos: http://bot.swu.ac.th/upload/meattree_document/1229050283.pdf
http://thaiherb.most.go.th/plantdetail.php?id=517

1.21.2 Pharmacological activity and clinical trial

Antibacterial activity:

The ethanol extracts of *V. trifolia* leaves exhibited moderate inhibiting activity against both gram-positive and gram-negative bacteria (Hossain, et al., 2001).

Hepatoprotective activity:

Leaf extracts of *V. trifolia* have hepatoprotective activity against carbon tetrachloride (CCl_4) induced hepatocellular injury. (Manjunatha and Vidya, 2008)

Anticancer activity:

Extracts from dried fruit of *V. trifolia* contains persicogenin and penduletin with anticancer activity (Li et al., 2005), inducing apoptosis and inhibiting cell cycle at theG0/G1 and G2/M phases in mammalian cancer (tsFT210 cells) (Li et al., 2005).

Antimalarial activity:

The cyclohexane extracts of *V. negundo* inhibits the activity of *P. falciparum* at IC_{50} 17.3 μg/ml (Nguyen-Pouplin, et al., 2007).

1.22 *Kaempferia parviflora* Wall. Ex. Baker

K. parviflora (Family Zingiberaceae) locally known as "black Krachai" is perennial herb. The black rhizome has been considered to be of highest quality, compared to other different types: yellow, white, and red ones.

The fresh or dried rhizome has been used in Thai folk medicine as an aphrodisiac and for the treatment of colic disorder and hypertension (Patanasethanont *et al.*, 2007). The plant has been known as Thai ginseng, is believed to have sexual enhancing activities (Churdboonchart, 2000; Wutythamawech, 2000) The laboratory results support its potential with positive effects on the seminal vesicle, spermatogenesis and health safety at doses of 60 and 120 mg/kg for 30 days (Jitjaingam *et al,* 2005).

Figure 22 *K. parviflora* Wall. Ex. Baker

Source of photos: http://gotoknow.org/file/jannoniramai/view/79836
http://www.gingersrus.com/DataSheet.php?PID=4747

From *in vitro* study, the nine flavonoids isolated from the rhizomes of *K. parviflora* exhibited antiplasmodial, antifungal, and antimycobacterial activities, but no cytotoxicity against KB, (oral human epidermoid carcinoma), BC (breast cancer), and NCI-H187 (human, small cell lung cancer) cell lines (Yenjai *et al.*, 2004), anti-peptic ulcer (Rujjanawate et al., 2005), anti-viral protease effects (Sookkongwaree et al., 2006) and anti-allergic (Tewtrakul and Subhadhirasakul, 2007) as well as modulators of multidrug resistance in cancer cells. The flavone derivatives from the rhizome of *K. parviflora* can inhibit P-gp function, which may be useful for overcoming P-gp-mediated multidrug resistance and improving the oral bioavailability of anticancer agents (Patanasethanont et al., 2007). *K. parviflora* contains different classes of chemical constituents listed in Table 13.

1.22.1 Known chemical constituents

Table 13 The classes of chemical constituents reported in *K. parviflora*

Class	Chemical constituents	Reference
Flavonoid	5-hydroxy-3, 7-dimethoxyflavone, 5-hydroxy-7-methoxyflavone, 5-hydroxy-7,49-dimethoxyflavone, 5-hydroxy-3,7,49-trimethoxyflavone, 5-hydroxy-3,7,39,49-tetramethoxyflavone, 3,5,7-trimethoxyflavone, 3,5,7,49-tetramethoxyflavone, 5,7,49-trimethoxyflavone and 5,7,39,49-tetramethoxyflavone	Yenjai *et al.*, 2004 วงศ์วิวัฒน์ ทัศนียกุล และ อำไพ ปิ้นทอง 2528
Chalcone	Hydroxypanduratin A and panduratin A	Tuchinda *et al.*, 2002

1.22.2 Pharmacological activity and clinical trial

Anti-allergic activity:

Active constituents of *K. parviflora* rhizomes, 5-hydroxy-7,4'-dimethoxyflavone, 5-hydroxy-3,7,3',4'-tetramethoxyflavone and 5-hydroxy-7-methoxyflavone were responsible for anti-allergic effect of the plant (Tewtrakul *et al.*, 2008).

Antiplasmodial activity:

The 5,7,4′-trimethoxyflavone and 5,7,3′,4′-tetramethoxyflavone showed potent antiplasmodial activity (Yenjai *et al.*, 2004).

Anti-gastric ulcer activity:

The ethanolic extract of *K. parviflora* has been reported to provide anti-gastric ulcer activity which is preserve of gastric mucus secretion (Rujjanawate *et al.*, 2005).

Anti-inflammatory:

Two chalcone derivatives, (–)-hydroxypanduratin A and (–)-panduratin A, exhibited anti-inflammatory activity (Tuchinda *et al.*, 2002).

2. The knowledge of cancer

Cancer is a group of disease with uncontrolled growth and spread of cells that may affect almost any tissue of the body. Cancer cell is generally the results of certain

genetic changes, involving activation of oncogenes or inactivation of tumor suppressor genes. These changes allow the cell to escape normal control mechanisms in cell proliferation, differentiation, migration and death which collectively maintain the normal cellular architecture and functions in an organized tissue. The frequency of somatic mutations leading to cancer beings is dictated largely by chemicals in the cellular microenvironment and to a small extent by heritable genetic predisposition (Suna, 2005).

There are more than 200 different kinds of cancer. Lung, stomach, liver, colon and breast cancer cause the most cancer deaths each year in the worldwide. More than 10 million people are diagnosed with cancer every year. Cancer causes 7.9 million deaths or 13% of deaths worldwide in 2007. It is estimated that there will be 12 million deaths in 2030 (WHO).

In Thailand, the statistical report of cancer from Siriraj Cancer Center in the year 2007 is shown in Table 14. Prostate cancer is the most common cancer in males, follows by, liver, lung, colon and lymphoma cancer. In women, breast cancer is the most important, followed by, cervix, colon, lung and thyroid cancer.

Table 14 The ten leading sites of cancer in Thailand (2007) from Siriraj Cancer Center (Ratanawichitrasin, 2007)

Sites	Male	Female	Population	Percentage
Breast	6	1,004	1,010	14.04
Liver	477	178	655	9.10
Colon and rectum	304	290	594	8.26
Lung	395	189	584	8.12
Cervix	0	546	546	7.59
Prostate	525	0	525	7.30
Lymphoma	178	124	302	4.20
Thyroid	60	196	256	3.56
Leukaemia	151	104	255	3.54
Oral cavity	143	100	243	3.38
All Sites	**3,370**	**3,825**	**7,195**	**100.00**

2.1 Types of cancer

Cancer cells within a tumor are the descendents of a single cell, even after it has metastasized. Hence a cancer can be classified into 5 types by the type of cell in which it originated and by the location of the cell (Phonnok, 2008).

2.1.1 Carcinoma

The majority of cancers (about 85%) are carcinomas. They start in the epithelium, which is the covering of organs and of the body. Carcinomas are named after the type of epithelial cell that they started in and the part of the body that is affected. There are four different types of epithelial cells. Cancer that starts in squamous cells is called a squamous cell carcinoma. Cancer that starts in glandular cells is called an adenocarcinoma. Cancers that start in transitional cells are transitional cell carcinomas, and those that start in basal cells are basal cell carcinomas. This group is including i.e., breast, prostate, lung and colon cancer.

2.1.2 Sarcoma

They are malignant tumors derived from connective tissue, or mesenchymal cells. They are a group of cancers that form in the supporting tissues of the body such as the bone, cartilage, fat, connective tissue and muscle.

2.1.3 Lymphoma

The cancer is a general for malignancies derived from hematopoetic (blood-forming) cells, develop in the lymph node and the tissues of the immune system.

2.1.4 Leukemia

The cancer of the blood or bone marrow is characterized by an abnormal proliferation of blood cells.

2.1.5 Melanoma

The cancer is a malignant tumor of melanocytes. Melanocytes predominantly occur in the skin but can be found elsewhere, especially the eye. The vast majority of melanomas originate in the skin.

2.2 Breast cancer

Breast cancer is the leading cause of death as the first leading cause of Thai female cancer (Table 14). Breast cancer occurs also in men but in less frequent than women. Most cases occur during age 45 to 55 year old (Cooper; 1992).

There are 2 types of breast carcinoma.

(1) Ductal carcinoma is a noninvasive in which abnormal cells are found in the lining of a breast duct. The abnormal cells do not spread outside the duct to other tissues in the breast. Approximately 90% of breast cancer is this type. The most common is called "invasive ductal", NOS (for "not otherwise specified").

(2) Lobular carcinoma which about 5% of breast cancer occurs in the lobules of the breast. This condition seldom becomes invasive cancer; however, having lobular carcinoma in one breast increases the risk of developing breast cancer in either breast.

Risk factors for breast cancer relate principally to the effect of hormones on the breast tissue. Breast cancer risk is increased about three fold in women who never had children or who had their first child after age 35. The women who use the birth control pill for several years prior to first pregnancy may result in modest increase in breast cancer risk (Kelsey, 1979). Long-term post menopause estrogen replacement therapy may also be associated with a modest increase in breast cancer risk (Miller, 1973). Environmental influences, such as diet, have been proposed to play a major role in mammary gland carcinogenesis (Forbes, 1997 and Catteau 2002), and most individuals are exposed to carcinogens in their daily diet through the eating of cooked meats and fish (Wakabayashi *et al.,* 1992). Cooking protein-rich foods at high temperatures forms heterocyclic amines. Several heterocyclic amines induce mammary gland carcinomas in rodent models (Nagao *et al.,* 1994), and evidence suggests that human breast cancer risk may be to diets high in cooked meats

2.3 Colon cancer

Colon cancer continued to be the leading cause of death as the third leading cause of Thai patient cancer (Table 14), the second leading cause of cancer deaths in the U.S., accounts for 50,000 deaths annually. Colon cancer is cancer of the large intestine (colon), the lower part of digestive system. Rectal cancer is cancer of the last 6 inches of the colon. Together, they're often referred to as colorectal cancers. About 112,000 people are diagnosed with colon cancer annually, and about 41,000 new cases of rectal cancer are diagnosed each year, according to the American Cancer Society. Most cases of colon cancer begin as small, noncancerous (benign) clumps of cells called adenomatous polyps. Over time some of these polyps become colon cancers. Polyps may be small and produce few, if any, symptoms. Regular screening tests can help prevent colon cancer by identifying polyps before they become cancerous. If signs and symptoms of colon cancer do appear, they may include changes in bowel habits, blood in stool, persistent cramping, gas or abdominal pain.

The risk of developing colorectal cancer increases as age. The disease is more common in people over 50, and the chance of getting colorectal cancer increases with each decade. The risk overall are equal, but women have a higher risk for colon cancer, while men are more likely to develop rectal cancer. Diet high in fat and calories and low

in fiber may be linked to a greater risk of developing colorectal cancer. A new risk study for developing colon cancer is genetic mutations reported by Markowitz-Ingalls and Kishore Guda (Markowitz-Ingalls, 2009). The presence of mutations in a group of enzymes called GALNTs, which are required for normal glycoslylation, used to synthesize mucus and is involved in many cellular activities. These mutations contribute to alterations in the glycosylation process, and in turn, to the development of colon cancer.

3. Cytotoxicity

The cytotoxicity test method is provided which measures the effect of test substances or physical stimulation on living cells. The method is both simple and sensitive and is useful for a wide variety of substances and stimulations. There are many applications in cancer research of assays, which quantitative numbers of viable cells present following therapeutic procedures. A number of cytotoxicity assays, each of them using specific approach to detect different aspects of cell viability, such as cell integrity, proliferation and metabolic functions. The potential comparison of commonly employed cytotoxicity assays (WST-1, XTT, MTT, Brilliant blue and Neutral red assay) to detect antiproliferative effects of selenium compounds on three colorectal cancer cell lines *in vitro* as reported by Schröterová and co-worker (Schröterová *et al.*, 2009). The metabolic activity of selenium treated cells measured by MTT, Neutral red and Brilliant blue assays were more sensitive and yielded mutually comparable results. Among the most often used assays are those measuring the metabolic activity of viable cells using colorimetric changes based on tetrazolium salt reduction.

3.1 MTT assay

This assay presented the changing of colorimetric formation in wells and measuring by automatic microplate reader as described by Mosmann (Mosmann, 1983). The only living cells could reduce the soluble yellow tetrazolium salt, 3-4,5 dimethylthiazol-2,5 diphenyl tetrazolium bromide (MTT), into an insoluble blue formazan crystal by intracellular succinate dehydrogenase. With some modification, this assay can be applied clinically to select the effective drugs because the assay can be done in a 96-well plate, and MTT formazan production can be analyzed using a scanning multi-well spectrophotometer. Thus, many samples and anti-cancer drugs can be analyzed simply and rapidly (Rocha D and Lopes, 2001).

Table 15 Summary of some study of the anticancer potential of the plant extracts

Plant used in study	Cancercell lines	Method	Results	Reference
Hemidesmus indicus *Polyalthia longifolia* *Aphanamixis polystachya* *Oroxylum indicum* *Tribulus terrestris* *Nigella sativa* *Cuscuta reflexa* *Paederia foetida* *Emblica officinalis* *Moringa oleifera* *Aegle marmelos*	B-16 (murine melanoma) HCT-8 (human colon carcinoma) CEM and HL-60 (leukemia)	Brine shrimp lethality assay Sea urchin eggs assay MTT assay Hemolysis assay	- Brine shrimp lethality assay *A. marmelos* showed a positive result (LD_{50} = 17.5 µg/ml.) - Sea urchin eggs assay *O. indicum* (IC_{50} was in the range of 10µg/ml in all tested phases of the sea urchin) *A. marmelos* was strongly active (IC_{50} = 50.5 µg/ml, inhibiting for the first cleavage), (IC_{50} = 22.7 µg/ml, for third cleavage), (IC_{50} = 13.2 µg/ml, for blastulae) - MTT assay *O. indicum* was the most active IC_{50} = 14.2 µg/ml for HL-60 IC_{50} = 19.6 µg/ml for CEM IC_{50} = 17.2 µg/ml for B-16 IC_{50} = 32.5 µg/ml for HCT-8.	Studies of the anticancer potential of plants used in Bangladeshi folk medicine (Costa-Lotufo *et al*,. 2005)

Table 15 Summary of some study of the anticancer potential of the plant extracts (Continued)

Plant used in study	Cancer cell lines	Method	Results	Reference
			- Hemolysis assay The plant extracts were tested for the ability to induce lysis of mouse erythrocytes. *N. sativa* was the most active (EC_{50} = 116.8µg/ml)	
Bidens pilosa *Centella asiatica* *Cnicus benedictus* *Dicoma capensis* *Hypoxis hemerocallidea* *Sutherlandia frutescens*	DU-145 (prostate cancer) MDA-MB-231 and MCF-7 (breast cancer cells) MCF-12A. (nonmalignant breast cell)	XTT Assay	*D. capensis* has cytotoxic effects on MCF-7 and MCF-12A. (IC_{50} = 30 µg/ml and 31 µg/ml, respectively)	Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer. (Steenkamp and Gouws, 2006)
Seventeen Thai medicinal plants	KB (human mouth epidermal carcinoma) P388 (murine leukemia)	MTT assay	Guava (*Psidium guajava*) leaf oil showed the highest anti-proliferative activity (IC_{50} = 37.9 µg/ml for KB) Sweet Basil (*Ocimum basilicum*) oil showed the highest antiproliferative activity (IC_{50} = 36.2 µg/ml forP388)	Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines (Manosroi *et al.*, 2006)

Table 15 Summary of some study of the anticancer potential of the plant extracts (Continued)

Plant used in study	Cancer cell lines	Method	Results	Reference
Eleven Thai medicinal plants	COR-L23 (human large cell lung carcinoma) MCF-7 (human breast Adenocarcinoma) LS-174T (human colon adenocarcinoma) SVK-14 (normal human keratinocytes)	SRB assay	The results showed that three plants; *Dioscorea membranacea*, *Dioscorea birmanica* and *Siphonodon celastrineus*, exhibited high cytotoxic activity *D. membranacea* against cancer cells was selectively toxic that showed the most activity for breast cancer (IC_{50} = 7.7 µg/ml) for lung and colon cancer cell lines (37.6 and 23.2µg/ml, respectively).	In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer (Itharat *et al.*, 2004)
Fourteen Thai medicinal plants	HepG2 (malignant human hepatoma) Vero (normal African green monkey kidney)	MTT assay	*P. evecta* (IC_{50} = 70 µg/ml) possessed the highest selectivity (SI>14.3) to HepG2	Cytotoxic activity screening of some indigenous Thai plants (Prayong *et al.*,2003)

Table 15 Summary of some study of the anticancer potential of the plant extracts (Continued)

Plant used in study	Cancer cell lines	Method	Results	Reference
Five Thai medicinal plants in the family Meliaceae: *Azedirachta indica* *Azedirachta indica* var. *siamensis*, *Melia azedarach*, *Sandoricum indicum* *Swietenia macrophylla.*	-	Brine shrimp lethality assay	The leaf and seed of *A. squamosa*, and the stem bark of *M. azedarach* had potential for cytotoxic compounds.	Brine Shrimp lethality activity of Thai medicinal plants in the family Meliaceae (Pisutthanana *et al.*,2004)
Fourteen Yemen medicinal plants	ECV-304 (human bladder carcinoma)	MTT assay	Seven plants,*Dracaena cinnabari, Eucalyptus camaldulensis, Pulicaria crispa, Euclea divinorum, Euphorbia cactus and Withania somnifera* marked toxicity (IC_{50} < 30 μg/ml) against ECV-304 cells	Cytotoxicity of plants used in traditional medicine in Yemen (Al-Fatimia *et al.*, 2005)

IC_{50} = The inhibitory concentration; concentration of an inhibitor at which 50% inhibition of the response (i.e. an enzyme, cell or cell receptor).

EC_{50} = The effective concentration; concentration at which the substance concerned produces a specified effect in 50% of the organisms treated.

LD_{50} = The lethal dose; dose that can be expected to cause mortality in 50% of dosed animals (http://www.encyclo.co.uk/search.php).

4. Mode of cell death

Cell death is part of normal development and maturation cycle, and is the component of many response patterns of living tissues to xenobiotic agents (i.e. micro organisms and chemicals) and to endogenous modulations, such as inflammation and disturbed blood supply (Clavien, *et al.*, 2000, Vaupel and Hockel, 2001). Cell death is an important variable in cancer development, cancer prevention and cancer therapy (Schulte-Hermann *et al.*, 1997). There are two forms of cell death: apoptosis and necrosis (Figure 24) that have been defined on the basis of morphological criteria (Kanduc *et al.*, 2002).

4.1 Necrosis

Necrosis occurs when cells are exposed to extreme variance from physical and chemical trauma which may result in damage to the cell. It is begins with an impairment of the cell's ability to maintain homeostasis, leading to an influx of water and extracellular ions. Intracellular organelles, most not ably the mitochondria, and the entire cell swell and rupture (cell lysis). Due to the ultimate breakdown of the plasma membrane, the cytoplasmic contents including lysosomal enzymes are released into the extracellular fluid. Therefore, necrotic cell death is often associated with extensive tissue damage resulting in an intense inflammatory response (Van Furth and Van Zwet, 1988).

4.2 Apoptosis

Apoptosis is a mode of cell death that occurs under normal physiological conditions and the cell is an active participant in its own demise (cellular suicide). It is most often found during normal cell turnover and tissue homeostasis, embryogenesis, induction and maintenance of immune tolerance, development of the nervous system and endocrine-dependent tissue atrophy. The cell is includes chromatin aggregation, nuclear and cytoplasmic condensation, partition of cytoplasm and nucleus into membrane bound-vesicles (apoptotic bodies) which contain ribosomes, morphologically intact mitochondria and nuclear material. The apoptotic bodies are rapidly recognized and phagocytized by either macrophages or adjacent epithelial cells (Savill et al., 1989). Due to this efficient mechanism for the removal of apoptotic cells no inflammatory response is elicited. The apoptotic bodies as well as the remaining cell fragments ultimately swell and finally lyse. The terminal phase of cell death is secondary necrosis (Krähenbühl and Tschopp, 1991)

A characteristic biochemical feature of the process is double-strand cleavage of nuclear DNA at the linker regions between nucleosomes leading to the production of

oligonucleosomal fragments. In many, although not all of the circumstances in which apoptosis occurs, it is suppressed by inhibitors of messenger RNA and protein synthesis.

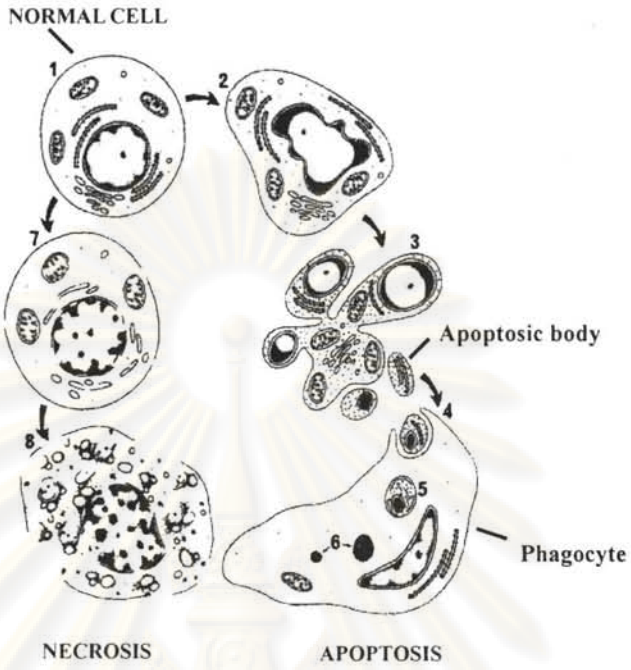

Figure 23 The different cell death pathway between necrosis and apoptosis
(Kerr *et al.*, 1944)

In the diagram (Figure 24) shows the sequence of ultra structural changes in apoptosis and necrosis: (2-6 show pathways of apoptosis, 7 and 8 show pathway of necrosis)

(1) Normal cell (early apoptosis)

(2) The cell is characterized by compaction and margination of nuclear chromatin, condensation of cytoplasm, and convolution of nuclear and cell outlines.

(3) At a later stage, the nucleus fragments, and protuberances that form on the cell surface separate to produce apoptotic bodies.

(4) Apoptotic bodies were phagocytosed by nearby cells.

(5 and 6) Apoptotic bodies were degraded within lysosomes.

(7) The development of necrosis is associated with irregular clumping of chromatin, marked swelling of organelles and focal disruption of membranes.

(8) Membranes subsequently disintegrate, but the cell usually retains its overall shape until removed by mononuclear phagocytes.

Apoptosis occurs spontaneously in malignant tumors, often markedly retarding their growth, and it is increased in tumors responding to irradiation, cytotoxic chemotherapy, heating and hormone ablation. It has been suggested that apoptotic cells

utilize the same proto-oncogene products and regulators of the cell cycle in a unique manner to induce a tightly controlled cell death. Several typical events of early cell cycle traverse are associated with apoptosis, e.g. unregulation of proto-oncogenes such as *c-myc*, *ras*, *cfos*, *c-jun*, *cdc-2*, and phosphorylation of the protein product of the tumor suppressor retinoblastoma gene. Some of the molecules that induce apoptosis are also involved in the regulation of proliferation and differentiation. For example, the nuclear transcription factor *c-myc* which is classically associated with the promotion of cell growth has also been demonstrated to be a central mediator of apoptosis. Ceramide, a hydrophilic component of sphingolipids (especially spingomyelin) which induces differentiation, growth suppression and cell cycle progression, also induces apoptosis. Finally, the demonstration that antibodies against a cell-surface protein designated APO-1 or Fas can enhance apoptosis in some human lymphoid cell lines may have therapeutic implications (Kerr *et al.*, 1994).

Table 16 Gene products influencing apoptosis (Modified from Sangkapong, 2005)

Enhancers of apoptosis	Inhibitors of apoptosis
Bcl-x	*Bcl*-2
Bax	*Bcl*-x_L
Bak	*Bcl*-w
Bad	*Mcl*-1
Nbk	*p53*
Bik 1	Colony-stimulating factors
TNF-α	
Fas/Apo1/CD95	
Interleukin-1β-converting enzyme (ICE)	
c-myc	

4.3 Apoptosis as a predictive factor for cancer therapy

The investigation of the expression of protein regulating apoptosis as a predictive factor for cancer treatment is at its beginning. There are three main points are already clear (Weichselbaum *et al.*, 1997).

1. The expression of each of the protein regulating apoptosis is likely to have a different significance in different at organs, as recently pointed out for *bcl-2*. Indeed it has already been demonstrated that this protein has a predictive meaning in myeloid leukaemias and breast cancer.

2. It will be necessary to investigate not only the primary tumor, that also the post-treatment residual tumor and the metastasis. These approach logical markers respond to treatment. This type of information could possibly lead to choose both the best treatment for resistant clones and to identify in the primary tumors potentially resistant cells already present and treatment them immediately in an appropriate way.

3. It is likely that, in most type of tumor, to predict the sensitivity to treatment it will be necessary to assess the expression of more than one protein. The possibly belonging to different pathways involved in both suppression and induction of apoptosis such as the study of *bcl-2* and *p53* system that is the model available.

4.4 The differences of necrosis and apoptosis

They have been defined on the basis of distinguishable, as differences morphological characteristic, physiological impact, biochemical features (Table 17-19) (Modified from Rode *et al.*, 2008).

Table17 The differences morphological characteristic between apoptosis and necrosis

Charecteristic	**Necrosis**	**Apoptosis**
Outset	Swelling of cytoplasm and mitochondria.	Shrinking of cytoplasm, condensation of nucleus.
Plasma membrane	Loss of membrane integrity	Blebbing of plasma membrane without loss of integrity
Chromatin	-	Aggregation of chromatin at the nuclear membrane.
Organelles	Disintegration (swelling) of organelles	Mitochondria become leaky due to pore formation involving proteins of the *bcl-2* family.
Vesicles	No vesicle formation, complete lysis	Formation of membrane bound vesicles (apoptotic bodies)
Terminal	Total cell lysis	Fragmentation of cell into smaller bodies

Table 18 The differences physiological impact between apoptosis and necrosis

Charecteristic	**Necrosis**	**Apoptosis**
Extent	Affects groups of contiguous cells.	Localized effect that destroys individual cells.
Phagocytosis	Phagocytosis by macrophages	Phagocytosis by adjacent cells

Table 18 The differences physiological impact between apoptosis and necrosis (Continued)

Charecteristic	Necrosis	Apoptosis
Immune system	Significant inflammatory response.	No inflammatory response.
Induction	Appeared by non-physiological disturbances (complement attack, lytic viruses, hypothermia, hypoxia, ischemica, metabolic poisons)	Induced by physiological stimuli (lack of growth factors, changes in hormonal environment).

Table 19 The differences biochemical features between apoptosis and necrosis

Features	Necrosis	Apoptosis
Regulation	Loss of regulation of ion homeostasis.	Tightly regulated process involving activation and enzymatic steps.
Energy input	No energy requirement (passive process, also occurs at 4°C)	Energy (ATP)-dependent (active process, does not occur at 4°C)
DNA	Random digestion of DNA (smear of DNA after agarose gel electrophoresis)	Non-random mono- and oligonucleosomal length fragmentation of DNA (Ladder pattern after agarose gel electrophoresis)
Biochemical events	-	• Release of various factorsrious factors (cytochrome C, AIF) into cytoplasm by mitochondria. • Activation of caspase cascade. • Alterations in membrane asymmetry (translocation of phosphatidylserine from the cytoplasmic to the extracellular side of the membrane)

CHAPTER III

MATERIALS AND METHODS

1. Materials

1.1 Chemical reagent for preparation and dissolving of crude plant extracts

Chemicals and Reagents	Catalog No.	Company
95% Ethyl alcohol		Alcohol Refinery
Dimethyl Sulfoxide (DMSO)	317275	Merck

1.2 Thai rejuvenating plants

The plants were identified and authenticated by traditional medicinal experts in the collecting places.

	Plants in this study	Family	Part used	Source
1.	*Acacia farnesiana*	Mimosaceae	Root	Khon Kaen
2.	*Albizia procera*	Mimosaceae	Stem bark	Khon Kaen
3.	*Anaxagorea luzonensis*	Annonaceae	Whole stem	Chiang Mai
4.	*Betula alnoides*	Betulaceae	Whole stem	Chiang Mai
5.	*Butea superba*	Papilionaceae	Tuberous root	Chiang Mai
6.	*Cyperus rotundus*	Cyperaceae	Rhizome	Bangkok
7.	*Diospyros rhodocalyx*	Ebenaceae	Stem bark	Khon Kaen
8.	*Dracaena conferta*	Agavaceae	Whole stem	Chiang Mai
9.	*Fagraea fragrans*	Potaliaceae	Whole stem	Khon Kaen
10.	*Kaempferia parviflora*	Zingiberaceae	Rhizome	Bangkok
11.	*Leucaena leucocephala*	Mimosaceae	Root	Bangkok
12.	*Melia azedarach*	Meliaceae	Whole stem	Khon Kaen
13.	*Mucuna collettii*	Papilionaceae	Whole stem	Chiang Mai
14.	*Phyllanthus emblica*	Euphorbiaceae	Fruit	Sa Kaeo
15.	*Piper nigrum*	Piperaceae	Seed and fruit	Bangkok
16.	*Pueraria mirifica*	Papilionaceae	Tuberous root	Chiang Mai
17.	*Stephania erecta*	Menispermaceae	Tuberous root	Chaiyaphum
18.	*Stephania venosa*	Menispermaceae	Tuberous root	Sakon Nakhon
19.	*Streblus asper*	Moraceae	Seed	Bangkok

20.	*Suregada multiflorum*	Euphorbiaceae	Stem and leaves	Khon Kaen
21.	*Tinospora crispa*	Menispermaceae	Whole stem	Khon Kaen
22.	*Vitex trifolia*	Verbenaceae	Stem bark	Khon Kaen

1.3 Culture media

Chemicals and Reagents	Catalog No.	Company
RPMI 1640 medium	T121-01	Biochrom
DMEM medium	D-2902	Sigma
EMEM medium	15-611D	BioWhittaker
Fetal bovine serum	S0215	Biochrom
Trypsinase	25300	Gibco
Penicillin-Streptomycin Solution	SV30010	HyClone
HEPES, free acid	0511	Amresco
Sodium bicarbonate	478537	Carlo Erba
Sterile water		

1.4 Cell lines

Human mammary cancer cells (MCF-7) and human colon cancer cells (SW620) were continuously cultured and grown as monolayer used in this study.

1.4.1 Human mammary cancer cell line (MCF-7)

MCF-7 (ATCC HTB-22), ERα positive human mammary cancer cell line which was isolated from pleural effusion of a Caucasian female 69 years old patient with a breast adenocarcinoma tumor. Cell line was obtained from the Section of Experimental Oncotherapy, Research Division, National Cancer Institute, Ministry of Public Health, Thailand.

1.4.2 Human colon cancer cell line (SW-620)

SW-620 (ATCC CCL-227), human colon cell line which was isolated from lymph node of a Caucasian male 51 years old patient with a Dukes' type C of colorectal adenocarcinoma tumor. The cell line was obtained from The Institute of Biotechnology and Genetic Engineering, Chulalongkorn University.

1.5 Chemical reagents for cell viability evaluation by MTT test

Chemicals and Reagents	Catalog No.	Company
MTT	M5655	Sigma
Glycine	453804	Carlo Erba

Normal saline	1580	GPO,Thailand
Trypan blue	17-942E	BioWhittaker

1.6 Equipments and instruments used in this study

Name	Company
Air pump	Emerson
Autoclave	Consolidated
Biohazard laminar air flow	Faster
Centrifuge	Kokusan
Heamacytometer	Bright-Line
Hot-air oven	Contherm
Microplate reader	Tecan
Microtiter plate 96 wells	Nunc
Inverted microscope	Olympus
Plate shaker	IKA
Rotary evaporator	EYELA
Tissue culture flask (25 cm^2)	Nunc
Vortex mixer	Scientific Industries
Water bath	Forma Scientific
Water jacketed CO_2 incubator	Thermo Forma

2. Methods

2.1 Preparation of plant powder

The plant materials were sliced into pieces and dried in hot air oven at 70°C. The dried materials were ground into powder.

2.2 Preparation of plant crude extracts

Fifty grams of plant powders were extracted with 500 ml 95% ethanol for 7 days in dark place. The solutions of plant extraction were filtered through No.1 filter paper. The precipitate was percolated two times with 500 ml 95% ethanol for 3 days. The total supernatants were evaporated in the rotary evaporator until completely dried. The crude extracts were stored in light-protect bottle at 4°C until used.

2.3 Preparation of stock solution of plant crude extracts

Each plant crude extracts were dissolved in 100% dimethyl sulfoxide (DMSO) as 100 mg/ml stock solution and stored at 4°C until further experiments within 1 month. Stock solutions were diluted to test concentration (0, 0.1, 1, 10, 25, 50, 75, 100, 500 and 1000 μg/ml) with 100% DMSO which did not exceed 2% of the total volume that should not effect against cell growth and viability.

2.4 Cell Culture

2.4.1 Culture of MCF-7 cell lines

MCF-7 cells were continuously grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% (v/v) fetal bovine serum (FBS), 1 ml of 10,000 units/ml penicillin and 10,000 μg/ml streptomycin for antibiotic reagent and 1 g sodium bicarbonate ($NaHCO_3$) were added in each liter of DMEM medium. The culture cells were maintained at 37°C with 95% humidity atmosphere in 5% CO_2 incubator.

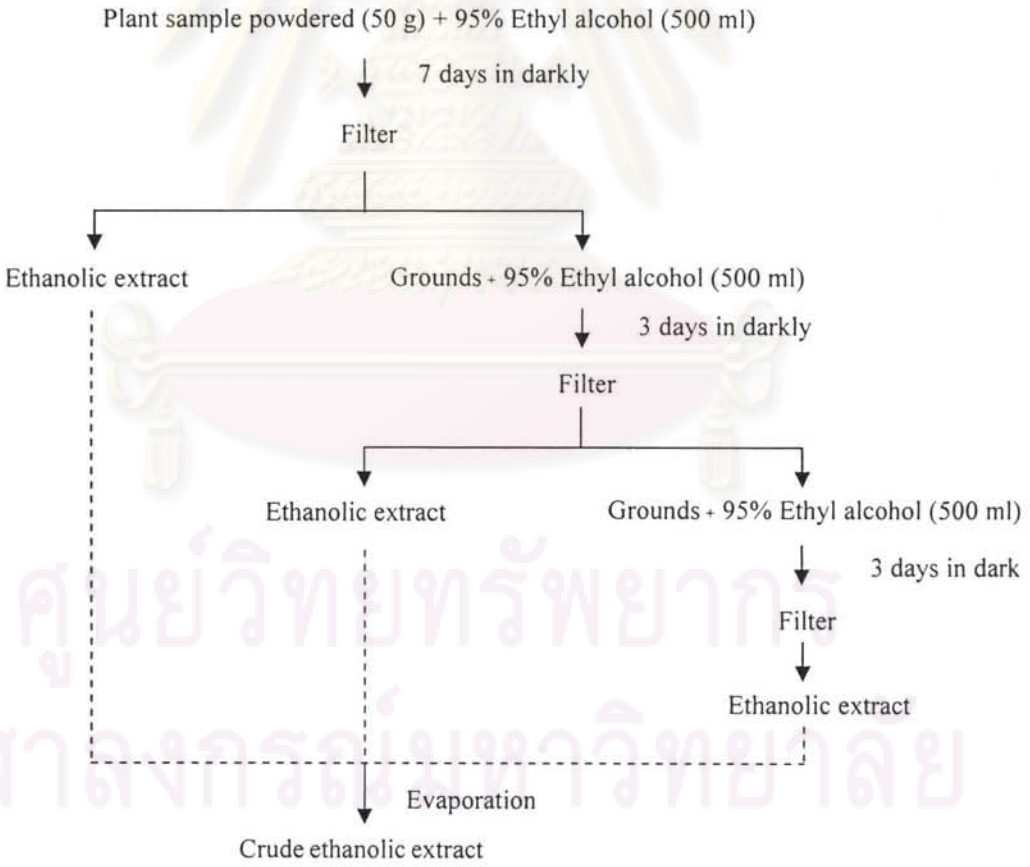

Figure 24 The preparation of plant crude ethanolic extract

2.4.2 Culture of SW620 cell lines

SW620 cells were continuously grown in RPMI-1640 medium supplemented with 5%(v/v) FBS, 1.5 mM L-glutamine, 1 ml of 10,000 units/ml penicillin and 10,000 μg/ml streptomycin for antibiotic reagent and 2 g $NaHCO_3$ were added in each liter of RPMI 1640 medium. The culture cells were maintained at 37°C with 95% humidity atmosphere of 5% CO_2 incubator.

2.5 Subculture of MCF7 and SW620 cells

Both MCF-7 and SW620 cells were refreshed once every 3-4 days to maintain the optimum conditions for exponential growth. The culture medium was removed from the flask and washed with 3 ml of phosphate buffer saline (PBS). These cells were detached from surface of the 25 cm^2 T-flask with 2 ml of 0.05% trypsin in 0.01% EDTA for 2-3 min then the solution was removed. After trypsinization, cells were washed with culture medium without FBS. The cells were resuspended with culture medium with FBS until became single cells, dispense into the new culture flasks. DMEM and RPMI-1640 medium were used for MCF-7 and SW-620, respectively. Culture medium were added to the final volume at 8 ml and stored in 37°C, 5% CO_2 incubator.

2.6 Cell suspension preparation for assay

MCF-7 and SW-620 cells were propagated 3-4 days before the experiment. The cells were rinsed with 3 ml of PBS followed by removal of the solution. Cells were detached from the surface by trypsinization. For MCF-7 and SW-620 cells were cultured with Eagle's modified Eagle's medium (EMEM), and RPMI-1640 medium, respectively. The fresh culture medium was added and aspirated gently with the aid of a pipette in order to dissociate into single cells. Cell suspensions were transferred to 15 ml conical tube and centrifuged at 100 x g for 10 minutes. The supernatant was discarded and resuspended with fresh culture medium until became single cells. The cell suspensions were counted and diluted as described in 2.7.

2.7 Cell count and dilution

In each experiment, cells were counted on hemocytometer under inverted microscope. The 0.4% Trypan blue dye solution and hemacytometer were applied to determine the viable cell number. Trypan blue will only enter across the membranes of non-viable cells. For MCF-7 and SW-620 cells were cultured in EMEM and RPMI-1640 medium, respectively. The cell suspensions were diluted with 1:10 culture medium and

mixed 20 µl of cells with 20 µl of 0.4% trypan blue suspension, by Pasture pipette and allowed to stand for 10 minutes. Ten microliters of stained cells were placed in a hemocytometer and counted for the number of viable (unstained). The viable cells were counted in the 1 mm middle square and 1 mm four corner squares of the hemacytometer. (Figure 25)

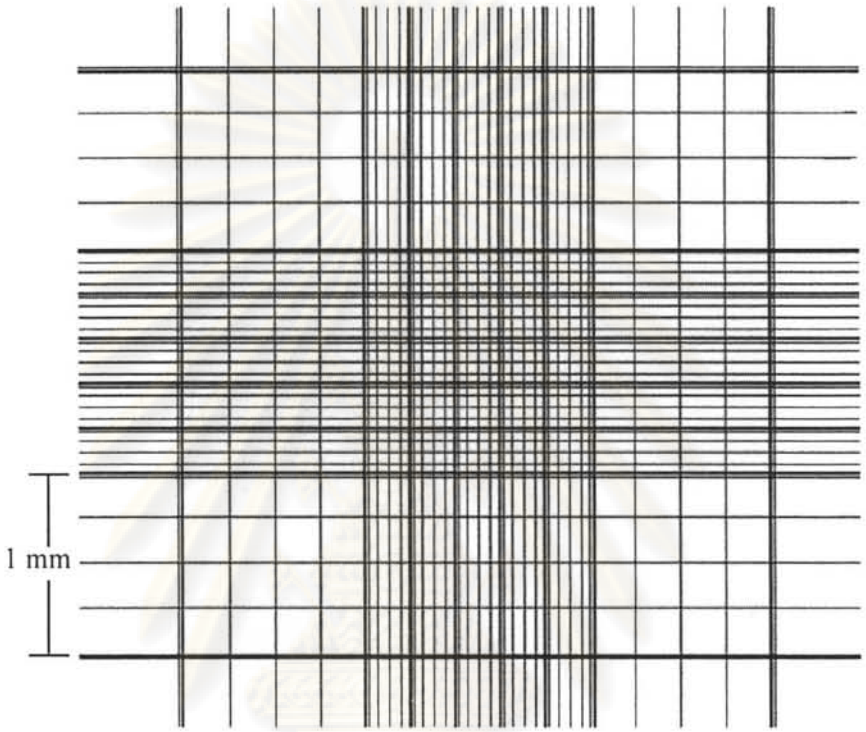

Figure 25 Magnified view of the cell counting chamber grid. The central 1 mm^2 area is divided into 25 smaller squares, each 1/25 mm^2. There are enclosed by triple rule line and are further subdivided into 16 squares, each 1/400 mm^2

The cells in each square of the hemacytometer were equivalent to approximately 1 mm, represent a total volume of 0.1 mm^3 and the subsequent cell density per ml was calculated using the following equation:

Cell density = average cell count per square x dilution factor x 10^4

Cell per ml = (total cell count /5) x (10x2) x 10^4

Then, calculate for dilution (desired cell density = 2.5 x 10^4 cells/ml)

Dilution factor (x) = cell per ml/2.5 x 10^4

Diluted cell suspension with culture medium to desirable volume (y)

Media x-1 ml : Cell 1 ml

Media y ml : Cell z ml

(z = cell volume for dilution)

2.8 Antiproliferative assay

2.8.1 Cell viability evaluation by MTT test

Cytotoxicity assay was a conventional method to assess cellular damage. The 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) assay presented the changing of colorimetric formation for measuring the activity of mitochondrial enzyme in living cells. The intracellular succinate dehydrogenase could reduce the yellow tetrazolium salt MTT to an insoluble purple formazan crystal. The amount of formazan product is directly proportional to the number of viable cells (Carmichael *et al.*, 1987 and Twentyman, 1987). Therefore, this procedure could be determined the inhibitory dose of plant crude extracts on cancer cells.

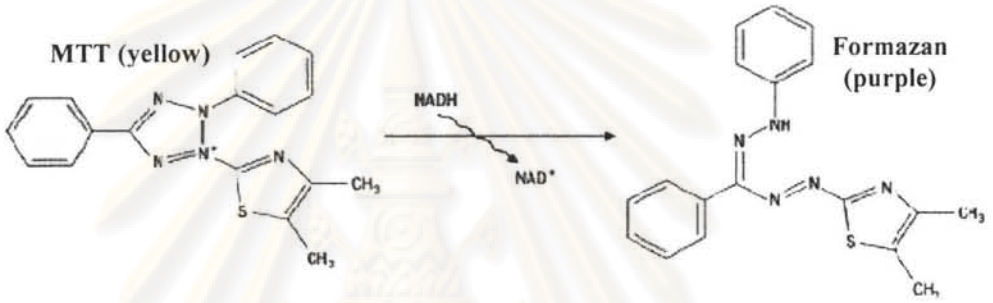

Figure 26 Molecular structure of MTT and its corresponding reaction product

Two hundred microliters of 5 x 10^3 cells in culture medium were plated into each well of 96-well plate and incubate for 24 h until cell attachment. The cells were treated with the plant crude extracts, 2µl/well of various concentrations of samples ranging from 0 to 1000 µg/ml and incubated at 37°C, 5% CO_2, for 72 h. Plates were added with 10 µl of 5 mg/ml of MTT in normal saline to each well and then incubated under darkness at 37°C for 4 h. MTT solutions were discarded and 150 µl of DMSO were added into each well. Plates were then gently shaken to dissolve formazan crystal by plate shaker (IKA, Werk Janke & Kunkel) at 25°C for 5 min. Finally, 25 µl Sorensen's glycine buffer was added to each well, then the plate was shaken for at 25°C 5 min for complete solubilization. The solutions were determined by a microplate reader (Tecan, Sunrise) at 540 nm. The results were shown in line graph between the percentage of cell viability (Y-axis) and the concentrations of each sample (X-axis) and calculated the concentration of 50% cytotoxicity (IC_{50}).

Calculation of the percentage of cell viability

$$\text{The percentage of cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of vehicle cells}} \times 100$$

The IC_{50} value could calculate from this curve. It was defined as the 50% reduction of the absorbance or 50% of the percentage of cell viability compared with cells that were treated by DMSO as a negative control in MTT assay.

2.10 Statistical analysis

The results were shown as mean ± standard error mean (S.E.M.) of five replication experiments (n = 5). Statistical analysis was performed using a one-way ANOVA for the analysis of the test results and Duncan analysis of variance at the significance levels of $P < 0.05$ were considered significantly.

CHAPTER IV

RESULTS

1. Characteristics of the plant crude extracts

The plant use, percentage yields and characteristics of the 24 plant ethanolic crude extracts are shown in Table 20.

Table 20 The percentage yields and characteristics of the plant extracts

Plants	Part used	Yield of extract (%)	Crude extract characteristic
Acacia farnesiana	Root	12.36	Light brown sticky
Albizia procera	Stem bark	18.2	Red-brown crystal
Anaxagorea luzonensis	Whole stem	4.72	Yellow viscous liquid
Betula alnoides	Whole stem	23.22	Red-brown solid
Butea superba	Tuberous root	15.14	Brown sticky
Cyperus rotundus	Rhizome	4.9	Red-brown viscous liquid
Diospyros rhodocalyx	Stem bark	4.74	Black sticky
Dracaena conferta	Whole stem	4.52	Red powder
Fagraea fragrans	Whole stem	11.64	Brown sticky
Kaempferia parviflora	Rhizome	2.64	Green-yellow viscous liquid
Leucaena leucocephala	Root	4.9	Red-brown sticky
Melia azedarach	Whole stem	18.84	Black-brown powder
Mucuna collettii	Whole stem	4.76	Red-brown viscous liquid
Phyllanthus emblica	Fruit	9.96	Brown viscous liquid
Piper nigrum	Fruit	11.08	Black-brown viscous liquid
Piper nigrum	Seed	9.63	Yellow viscous liquid
Pueraria mirifica	Tuberous root	14.72	Light yellow solid
Stephania erecta	Tuberous root	10.82	Red-brown powder
Stephania venosa	Tuberous root	5.34	Red-brown solid
Streblus asper	Seed	4.18	Green-brown viscous liquid
Suregada multiflorum	Leaves	10.56	Black sticky
Suregada multiflorum	Stem	12.42	Yellow viscous liquid
Tinospora crispa	Whole stem	4.52	Brown viscous liquid
Vitex trifolia	Stem bark	5.26	Yellow-brown solid

2. Anti-proliferative assay

2.1 Cell viability evaluation by MTT test

MTT assay, a simple and reliable technigue, which performed to demonstrate the percentage of cell viability that can be used for screening of anti-proliferation agents. In order to evaluate the cytotoxic effect of 24 extracts from rejuvenating herbs against MCF-7 and SW-620 cells after treated with various concentrations (0, 0.1, 1, 10, 25, 50, 75, 100, 500, 1000 μg/ml) for the exposure time 72 h. The effects were obtained in five replicate (*n=5*) from each experiment (Table 21-44, Figure 28-51).

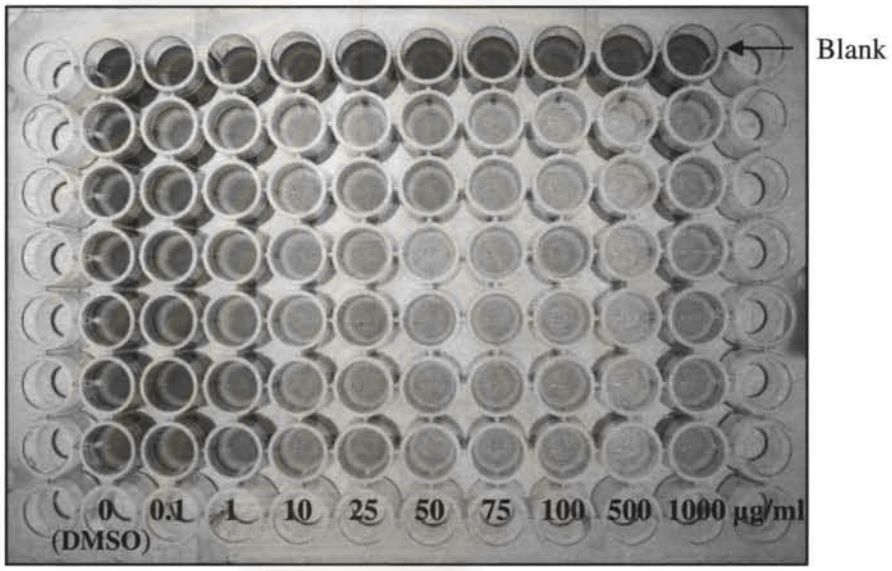

Figure 27 Cell viability was quantified by conventional MTT colorimetric assay.

2.1.1 Human mammary cancer cell line (MCF-7)

The results of cytotoxicity of the extracts from Thai rejuvenating herbs on MCF-7 cell lines are presented as the ability of the antiproliferative in Table 45.

MTT test indicated that 8 out of 21 plant extracts, *Suregada multiflorum* (leaf), *Diospyros rhodocalyx*, *Stephania erecta*, *Piper nigrum* (seed), *Piper nigrum* (fruit), *Streblus asper*, *Cyperus rotundus* and *Stephania venosa* extracts exhibited a cytotoxicity on cell proliferation between 0-100 μg/ml. At the IC_{50} of them which are 23.79, 33.91, 35.34, 37.02, 39.61, 46.28, 53.46 and 76.15 μg/ml, respectively.

The 6 out of 21 plant extracts, *Tinospora crispa*, *Suregada multiflorum* (stem), *Anaxagorea luzonensis*, *Acacia farnesiana*, *Kaempferia parviflora* and *Albizia procera* exhibited cytotoxicity on cell proliferation between 100-1,000 μg/ml, showed the IC_{50} were 279.03, 299.64, 439.97, 652.39, 694.92, and 765.23 μg/ml, respectively.

The 7 out of 21 plant extracts, *Betula alnoides*, *Dracaena conferta*, *Fagraea fragrans*, *Leucaena leucocephala*, *Melia azedarach*, *Phyllanthus emblica*, and *Vitex trifolia* showed no proliferative effect on MCF-7 cell. The IC_{50} values were greater than 1,000 μg/ml.

For ethanolic extracts of *Butea superba*, *Mucuna collettii* and *Pueraria mirifica* were not analyzed in this study because there were reports on the anti-proliferation effect of the 3 plant extracts on the growth of MCF-7 cells by Cherdshewasart and colleague in 2004. The report showed that the ethanolic extracts of *Butea superba* and *Pueraria mirifica* exhibited no significant anti-proliferation activity against MCF-7 cells with an ED_{50} value of 370.91 and 642.83 μg/ml, respectively. While the ethanolic extracts of *Mucuna colletti* had anti-proliferation effect on the growth of MCF-7 cells with an ED_{50} value of 85.36 μg/ml (Cherdshewasart *et,al* 2004).

2.1.2 Human colon cancer cell line (SW-620)

The results in Table 46 indicated that 11 out of 24 plant extracts, *Stephania erecta*, *Streblus asper*, *Suregada multiflorum* (leaf), *Piper nigrum* (seed), *Piper nigrum* (fruit), *Stephania venosa*, *Betula alnoides*, *Diospyros rhodocalyx*, *Cyperus rotundus*, *Phyllanthus emblica* and *Anaxagorea luzonensis* exhibited cytotoxicity on cell proliferation between 0-100 μg/ml. The IC_{50} values were 5.08, 5.55, 5.90, 14.60, 14.78, 15.73, 23.83, 29.34, 29.47, 33.06 and 36.14 μg/ml, respectively.

The 6 out of 24 plant extracts, *Kaempferia parviflora*, *Tinospora crispa*, *Mucuna collettii*, *Acacia farnesiana*, *Vitex trifolia*, *Suregada multiflorum* (stem), *Dracaena conferta*, *Fagraea fragrans*, *Leucaena leucocephala*, *Albizia procera* and *Butea superba* exhibited a cytotoxicity on cell proliferation between 100-1,000 μg/ml. The IC_{50} values were 282.29, 290.86, 332.86, 345.49, 362.25, 377.05, 407.37, 468.18, 482.51, 529.03 and 689.59 μg/ml respectively.

The 2 out of 24 plant extracts, *Melia azedarach* and *Pueraria mirifica* showed no proliferative effect on SW-620 cell. The IC_{50} values were greater than 1,000 μg/ml.

According to the American National Cancer Institute (NCI) guidelines set the limit of activity for crude extracts at a 50% inhibition (IC_{50}) of proliferation of less than 30 μg/ml after an exposure time of 72 h (Suffness and Pezzuto, 1990). Thus, the result obtained that only the ethanolic extract of *Suregada multiflorum* (leaves) showed the most activity against MCF-7 cells (IC_{50} = 23.79 μg/ml). As for, the ethanolic extracts exhibited

a pronounced cytotoxic effect on the SW-620 cells such as *Stephania erecta*, *Streblus asper*, *Suregada multiflorum*, *Piper nigrum* (seed), *Piper nigrum* (fruit), *Stephania venosa*, *Betula alnoides*, *Diospyros rhodocalyx* and *Cyperus rotundus* which IC_{50} of them are 5.08, 5.55, 5.90, 14.60, 14.78, 15.73, 23.83, 29.34 and 29.47μg/ml respectively. These data indicate that the extract of *Suregada multiflorum* leaves is highest cytotoxic activity, against both cell lines.

Table 21 The percentage of cell viability after treatment with *Acacia farnesiana* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 1.50	100.00 ± 7.29
0.1	99.47 ± 1.78	105.32 ± 7.14
1	108.20 ± 5.78	100.70 ± 8.19
10	98.08 ± 2.31	83.23 ± 2.53
25	120.15 ± 4.23	89.33 ± 5.51
50	108.4 ± 2.48	79.43 ± 8.51
75	103.54 ± 3.41	77.94 ± 8.85
100	97.64 ± 3.78	71.91 ± 8.68
500	48.45 ± 2.74	35.52 ± 0.83
1000	26.14 ± 1.38	48.37 ± 3.09
IC_{50} (µg/ml)	**652.39 ± 18.99**	**345.49 ± 23.63**

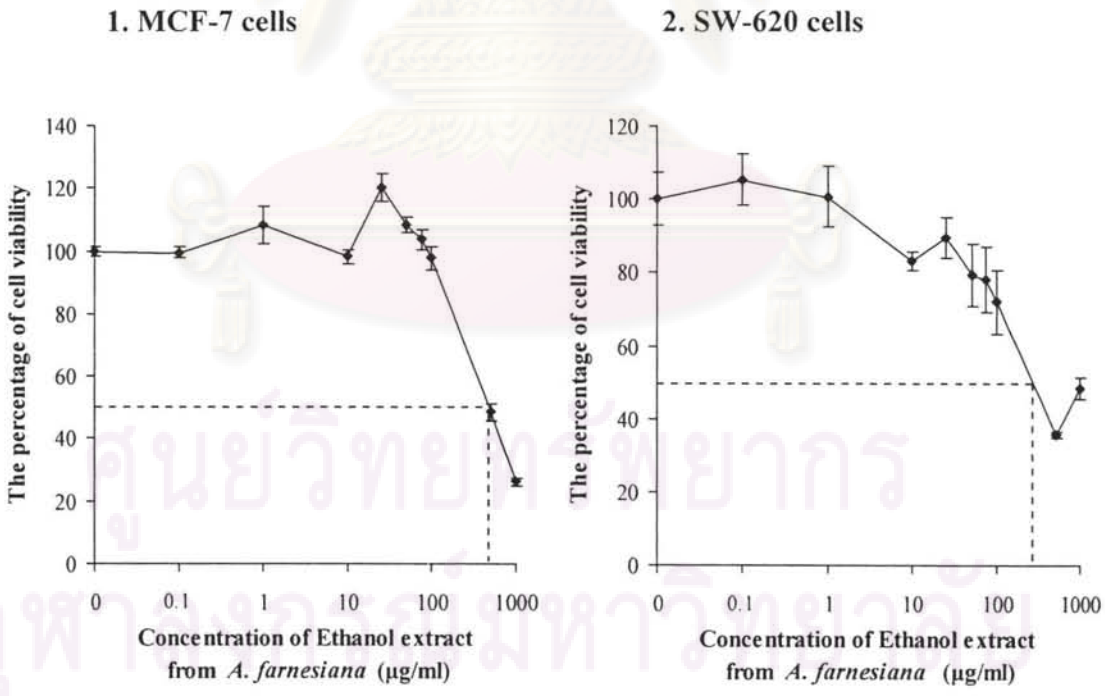

Figure 28 MTT test results of *Acacia farnesiana* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 652.39 ± 18.99 and 345.49 ± 23.63 µg/ml, respectively.

Table 22 The percentage of cell viability after treated with *Albizai procera* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 1.46	100.00 ± 3.13
0.1	110.86 ± 4.30	84.83 ± 4.54
1	110.67 ± 5.49	91.69 ± 4.85
10	119.70 ± 3.34	90.30 ± 2.12
25	107.87 ± 4.78	94.54 ± 3.35
50	94.37 ± 5.95	89.61 ± 2.06
75	90.75 ± 7.55	85.82 ± 3.74
100	81.69 ± 2.33	84.14 ± 3.47
500	59.55 ± 2.06	51.32 ± 2.16
1000	40.25 ± 1.18	12.36 ± 1.51
IC_{50} (µg/ml)	**765.23 ± 13.78**	**529.03± 15.43**

1. MCF-7 cells **2. SW-620 cells**

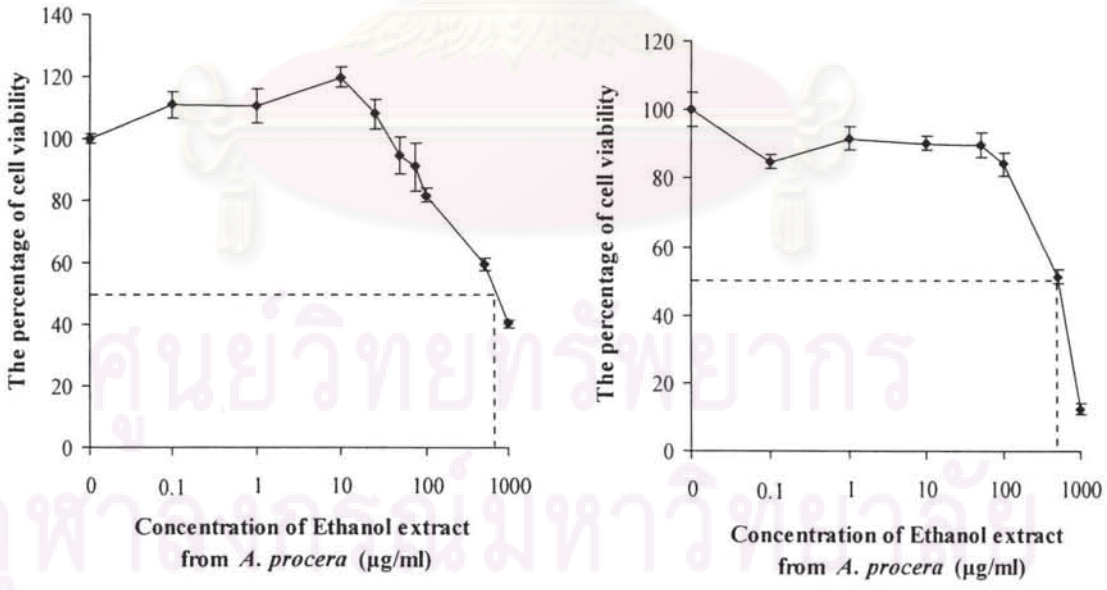

Figure 29 MTT test results of *Albizai procera* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 765.23 ± 13.78 and 529.03± 15.43 µg/ml, respectively.

Table 23 The percentage of cell viability after treated with *Anaxagorea luzonensis* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 2.88	100.00 ± 3.44
0.1	120.31 ± 4.14	105.53 ± 9.97
1	116.13 ± 5.33	113.47 ± 7.20
10	118.32 ± 6.45	107.24 ± 6.94
25	121.73 ± 5.95	57.26 ± 6.45
50	127.62 ± 7.26	30.35 ± 2.03
75	95.40 ± 5.22	29.98 ± 1.07
100	63.51 ± 2.74	26.67 ± 0.92
500	47.50 ± 2.07	37.62 ± 1.78
1000	56.98 ± 4.35	21.97 ± 1.64
IC_{50} (µg/ml)	**439.97 ± 34.99**	**36.14 ± 1.24**

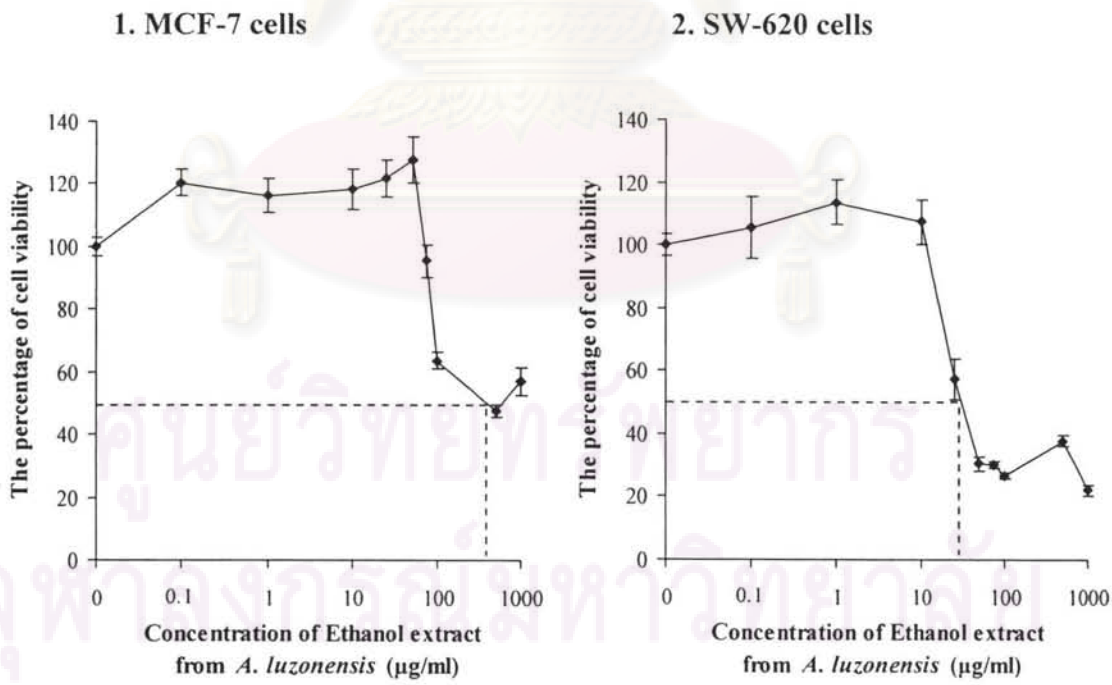

Figure 30 MTT test results of *Anaxagorea luzonensis* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 439.97 ± 34.99 and 36.14 ± 1.24 µg/ml, respectively.

Table 24 The percentage of cell viability after treated with *Betula alnoides* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 2.40	100.00 ± 7.22
0.1	97.58 ± 3.46	91.99 ± 1.52
1	120.20 ± 4.52	88.99 ± 2.38
10	123.54 ± 5.31	92.76 ± 2.85
25	140.81 ± 6.75	34.70 ± 3.76
50	144.80 ± 2.37	26.75 ± 0.99
75	115.43 ± 9.70	17.18 ± 1.80
100	88.22 ± 6.16	29.26 ± 2.93 *
500	155.27 ± 3.26*	43.77 ± 5.18*
1000	250.64 ± 9.98*	70.29 ± 3.32 *
IC_{50} (µg/ml)	**>1,000**	**23.83 ± 2.77**

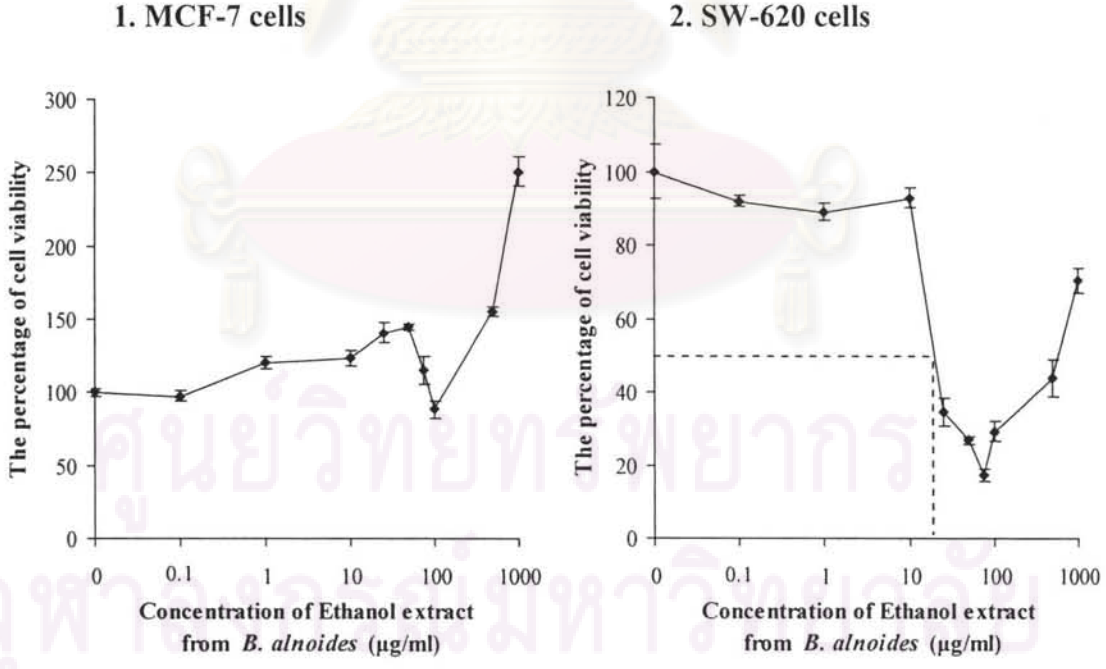

Figure 31 MTT test results of *Betula alnoides* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were more than 1,000 and 23.83 ± 2.77 µg/ml, respectively

Table 25 The percentage of cell viability after treated with *Butea superba* extract (*n*=5)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	NT	100.00 ± 4.26
0.1		104.33 ± 3.95
1		93.48 ± 3.49
10		98.70 ± 6.52
25		95.66 ± 6.07
50		91.69 ± 1.88
75		94.90 ± 9.66
100		96.22 ± 2.20
500		56.69 ± 6.25
1000		29.87 ± 2.65
IC_{50} (µg/ml)		**689.59 ± 36.01**

NT: Not test

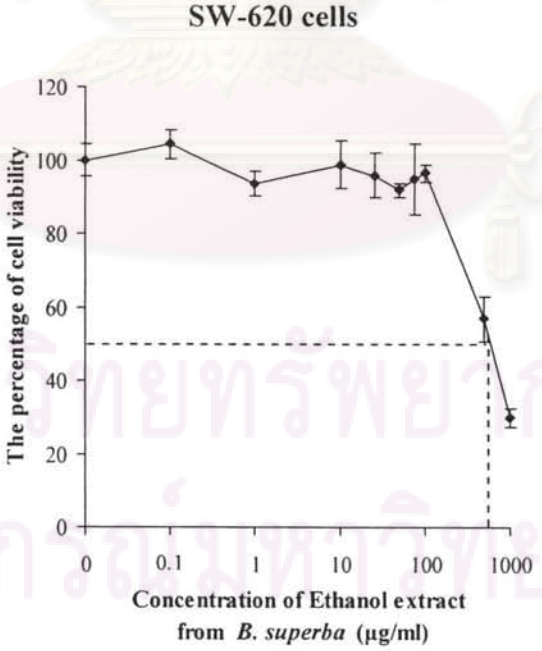

Figure 32 MTT test results of *Butea superba* extract on the growth SW-620 cell. The IC_{50} was 689.59 ± 36.01 µg/ml.

Table 26 The percentage of cell viability after treated with *Cyperus rotundus* extract ($n=5$)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 8.28	100.00 ± 4.04
0.1	103.26 ± 1.18	106.10 ± 3.64
1	101.39 ± 3.96	94.50 ± 4.30
10	89.12 ± 3.94	78.31 ± 7.14
25	73.38 ± 3.68	57.53 ± 3.75
50	70.75 ± 4.41	16.66 ± 1.49
75	22.86 ± 1.55	17.40 ± 0.61
100	16.84 ± 1.48	14.53 ± 0.74
500	49.81 ± 2.61	35.74 ± 3.27
1000	51.73 ± 2.36	50.33 ± 3.91
IC_{50} (µg/ml)	**53.46 ± 1.40**	**29.47 ± 0.20**

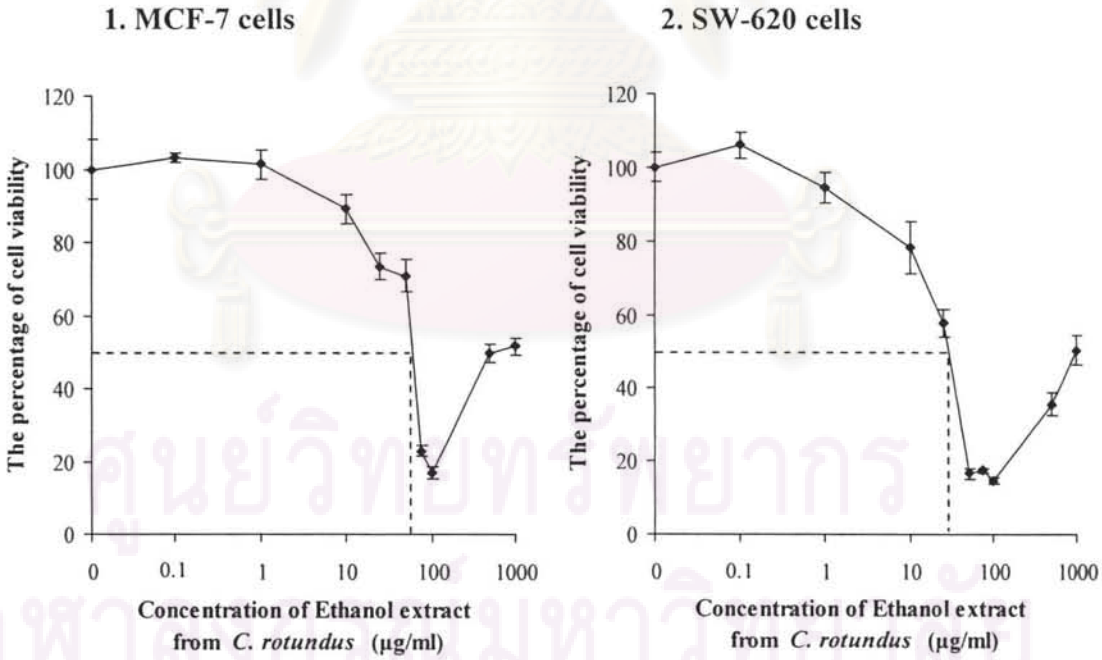

Figure 33 MTT test results of *Cyperus rotundus* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 53.46 ± 1.40 and 29.47 ± 0.20 µg/ml, respectively.

Table 27 The percentage of cell viability after treated with *Diospyros rhodcalyx* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 1.18	100.00 ± 3.51
0.1	90.44 ± 3.68	106.60 ± 8.47
1	93.68 ± 2.72	100.94 ± 6.11
10	78.46 ± 2.40	105.46 ± 10.42
25	66.47 ± 1.64	52.39 ± 9.25
50	26.98 ± 1.43	10.11 ± 0.77
75	22.31 ± 0.85	8.09 ± 1.83
100	19.52 ± 0.89	9.25 ± 0.87
500	28.43 ± 0.78*	12.99 ± 2.32*
1000	39.71 ± 1.36*	21.67 ± 4.43*
IC_{50} (µg/ml)	**33.91 ± 0.33**	**29.34 ± 1.87**

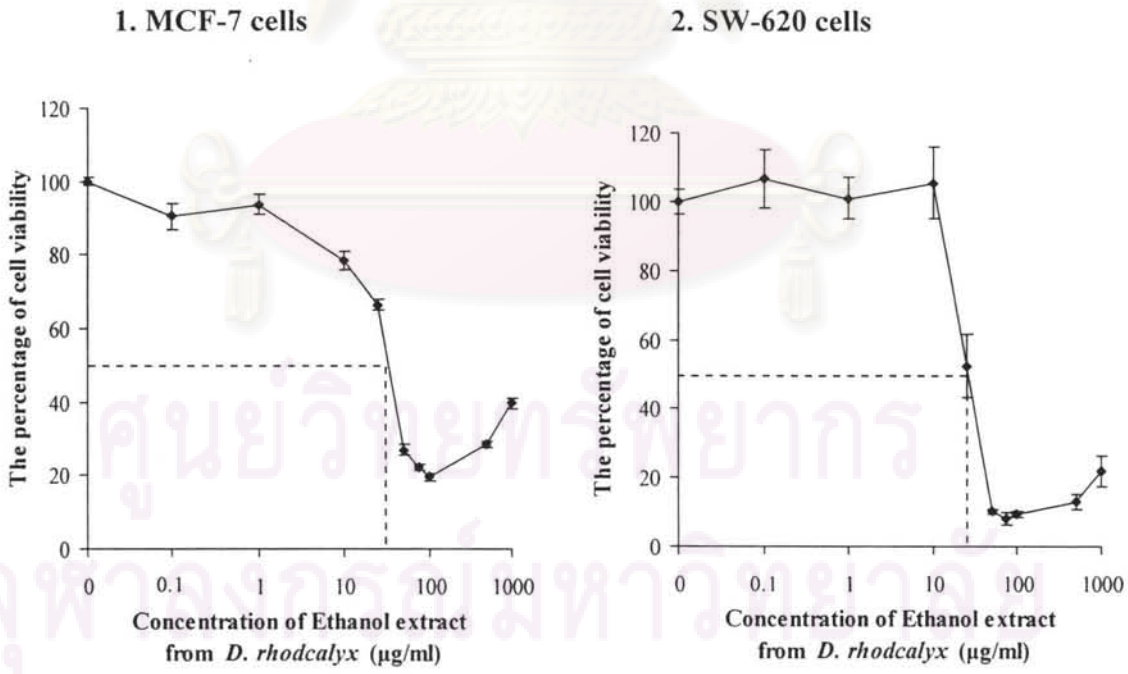

Figure 34 MTT test results of *Diospyros rhodcalyx* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 33.91 ± 0.33 and 29.34 ± 1.87 µg/ml, respectively.

Table 28 The percentage of cell viability after treated with *Dracaena conferta* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 4.09	100.00 ± 9.28
0.1	78.44 ± 5.82	94.36 ± 3.37
1	71.42 ± 2.46	111.23 ± 8.13
10	73.90 ± 5.02	133.94 ± 8.86
25	88.89 ± 6.11	138.98 ± 11.72
50	88.21 ± 2.62	101.86 ± 13.48
75	98.02 ± 2.05	99.99 ± 14.71
100	86.91 ± 6.17	72.26 ± 10.43
500	77.75 ± 6.11	31.71 ± 3.00
1000	77.97 ± 3.52	28.83 ± 2.95
IC_{50} (µg/ml)	**>1,000**	**407.37 ± 48.11**

1. MCF-7 cells **2. SW-620 cells**

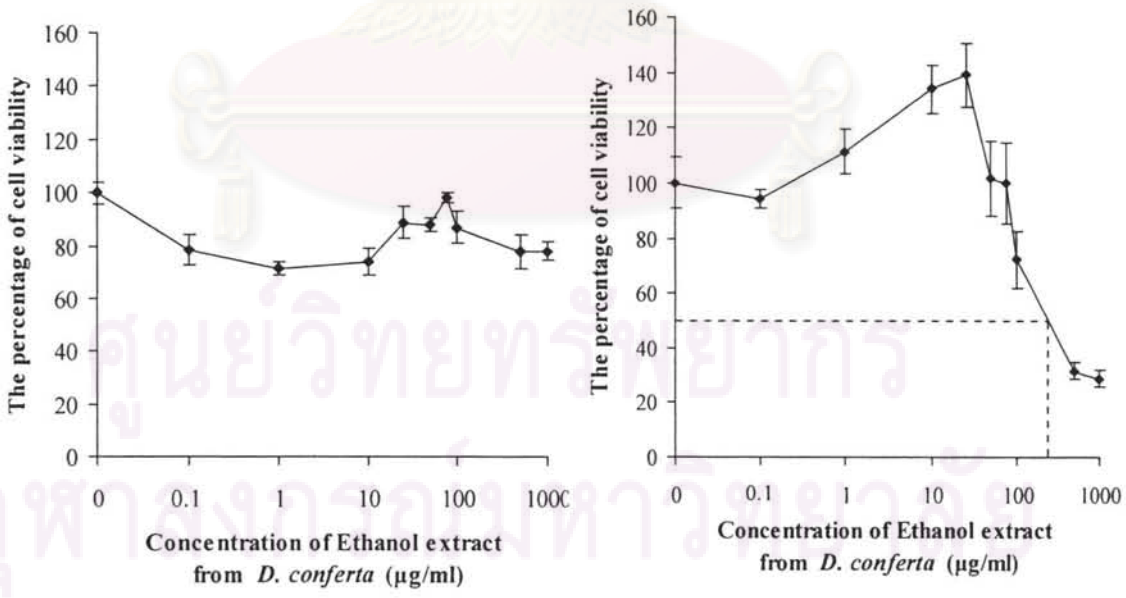

Figure 35 MTT test results of *Dracaena conferta* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were more than 1,000 and 407.37 ± 48.11 µg/ml, respectively.

Table 29 The percentage of cell viability after treated with *Fagraea fragrans* extract (*n*=5)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 1.74	100.00 ± 4.52
0.1	92.04 ± 4.48	100.64 ± 6.33
1	99.21 ± 4.49	91.21 ± 10.57
10	96.60 ± 2.91	92.52 ± 9.73
25	94.89 ± 6.01	94.42 ± 5.03
50	91.36 ± 6.73	89.23 ± 4.64
75	65.04 ± 3.83	93.57 ± 4.57
100	68.25 ± 1.77	89.81 ± 6.52
500	79.73 ± 5.69	46.37 ± 3.30
1000	73.07 ± 0.46	71.29 ± 8.55*
IC_{50} (µg/ml)	**>1,000**	**468.18 ± 19.12**

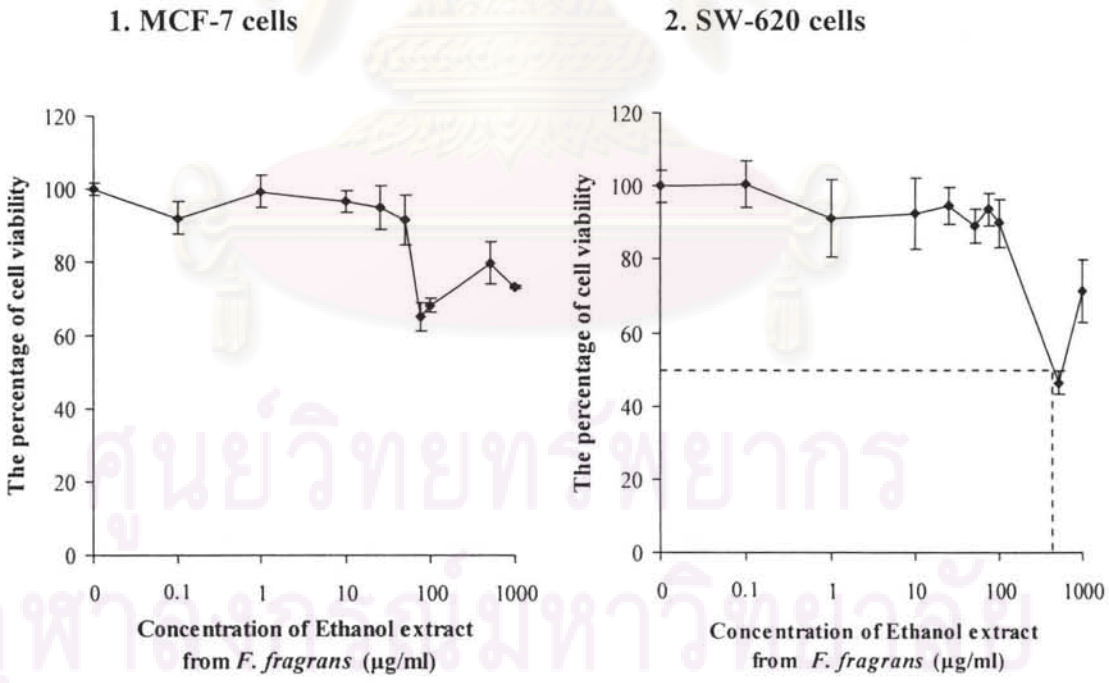

Figure 36 MTT test results of *Fagraea fragrans* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were more than 1,000 and 468.18 ± 19.12 µg/ml, respectively.

Table 30 The percentage of cell viability after treated with *Kaempferia parviflora* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 1.46	100.00 ± 1.31
0.1	103.85 ± 6.16	96.48 ± 9.29
1	94.76 ± 6.64	108.56 ± 9.11
10	88.80 ± 4.01	100.75 ± 7.36
25	93.72 ± 3.24	105.82 ± 6.90
50	90.19 ± 1.67	93.93 ± 6.07
75	88.25 ± 6.52	91.09 ± 11.87
100	87.16 ± 3.50	84.01 ± 9.25
500	57.14 ± 2.44	10.35 ± 0.56
1000	33.23 ± 1.67	11.91 ± 0.62
IC_{50} (µg/ml)	**694.92 ± 18.22**	**282.29 ± 22.71**

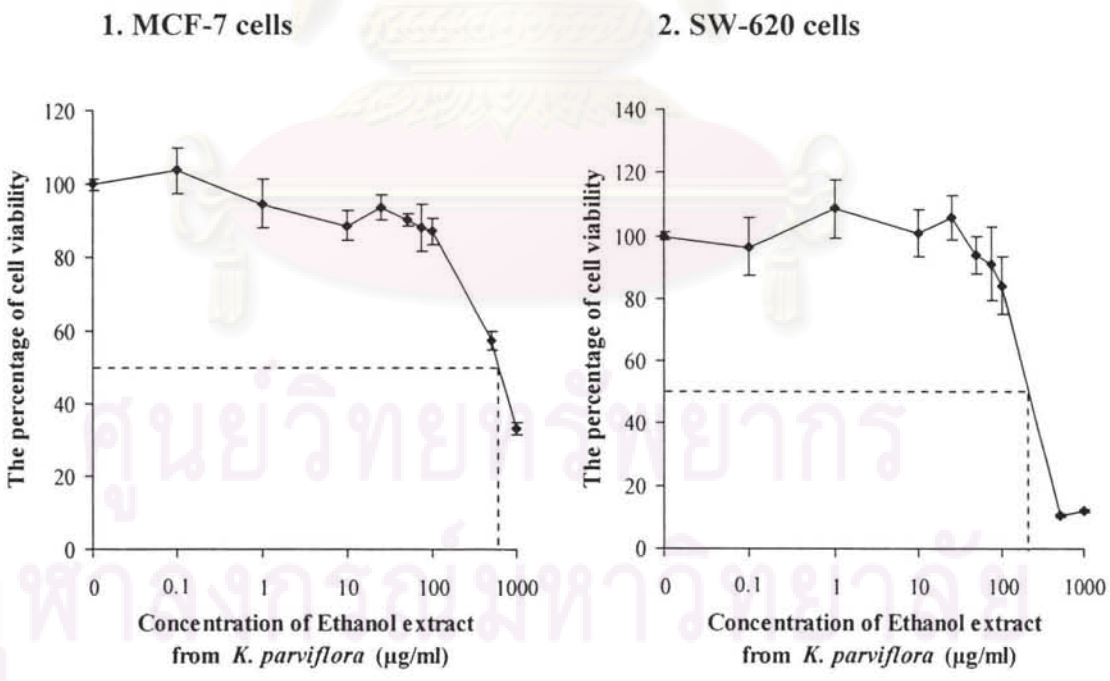

Figure 37 MTT test results of *Kaempferia parviflora* extract on the growth of MCF-7 and SW-620 cells.The IC_{50} were 694.92 ± 18.22 and 282.29 ± 22.71 µg/ml, respectively.

Table 31 The percentage of cell viability after treated with *Leucaena leucocephala* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 2.28	100 ± 4.37
0.1	96.56 ± 4.09	101.52 ± 6.97
1	107.34 ± 5.44	113.87 ± 3.68
10	102.34 ± 3.73	132.06 ± 4.04
25	84.74 ± 3.45	126.31 ± 6.59
50	94.39 ± 2.34	120.57 ± 3.32
75	95.89 ± 4.76	118.31 ± 2.85
100	88.06 ± 4.38	116.87 ± 7.50
500	89.56 ± 3.49	48.47 ± 4.25
1000	140.05 ± 6.07*	52.75 ± 4.24
IC_{50} (µg/ml)	**> 1,000**	**482.51 ± 7.25**

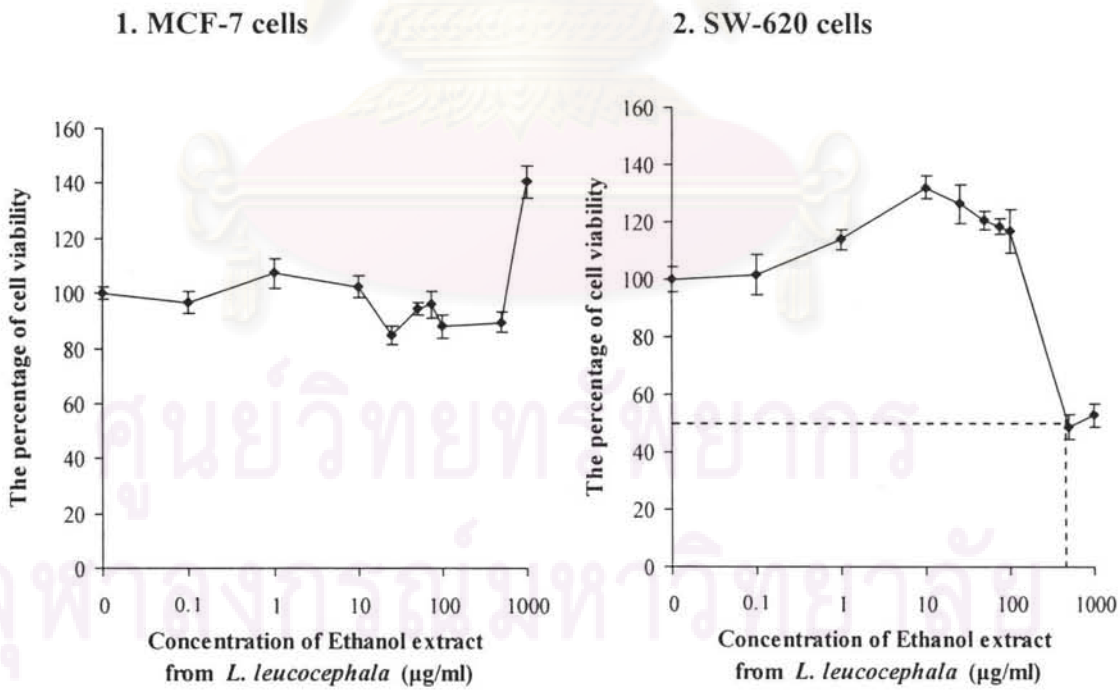

Figure 38 MTT test results of *Leucaena leucocephala* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were more than 1,000 and 482.51 ± 7.25 µg/ml, respectively.

Table 32 The percentage of cell viability after treated with *Melia azedarach* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 2.10	100.00 ± 7.19
0.1	102.21 ± 4.54	118.81 ± 9.60
1	103.67 ± 3.23	132.77 ± 7.82
10	93.88 ± 3.09	122.86 ± 5.94
25	90.66 ± 5.86	101.54 ± 7.04
50	86.84 ± 3.19	88.41 ± 4.52
75	67.71 ± 4.38	87.98 ± 7.37
100	70.62 ± 2.84	90.63 ± 8.97
500	97.31 ± 2.62	125.14 ± 8.36
1000	154.47 ± 6.62	146.52 ± 7.16
IC_{50} (µg/ml)	**> 1,000**	**> 1,000**

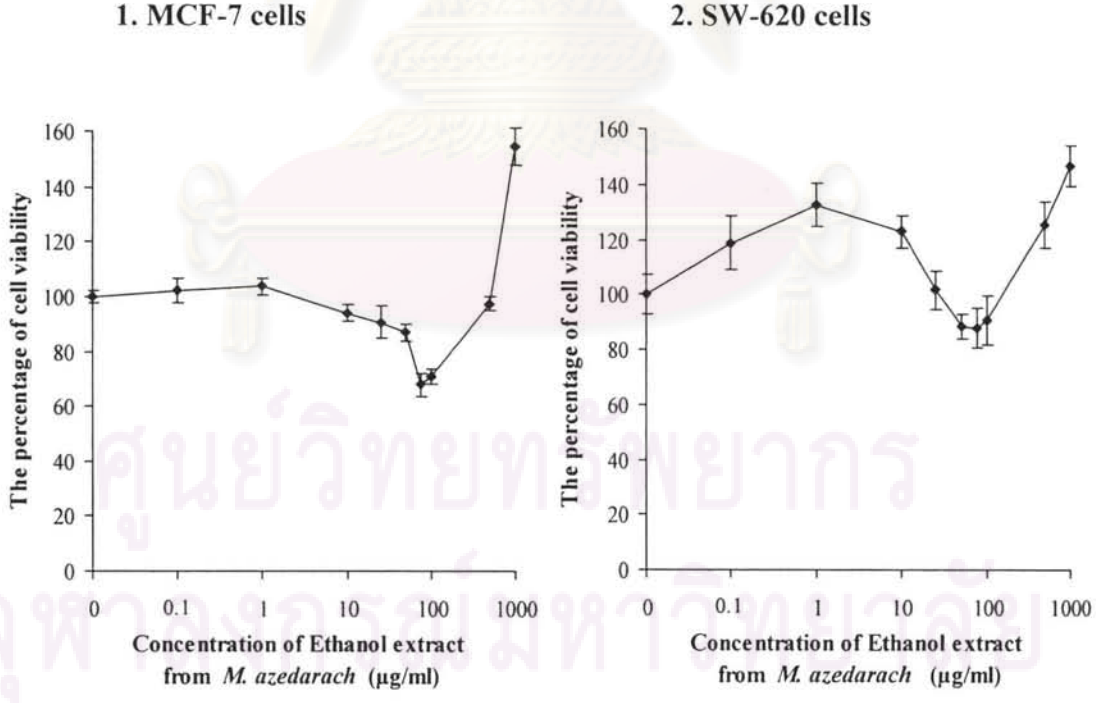

Figure 39 MTT test results of *Melia azedarach* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were more than 1,000 µg/ml same.

Table 33 The percentage of cell viability after treated with *Mucuna colletti* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	NT	100.00 ± 5.43
0.1		89.52 ± 6.44
1		89.76 ± 5.67
10		87.78 ± 4.76
25		83.62 ± 3.89
50		87.75 ± 7.21
75		80.45 ± 6.73
100		83.40 ± 6.40
500		28.27 ± 2.23
1000		35.81 ± 3.26
IC_{50} (µg/ml)		**332.86 ± 19.42**

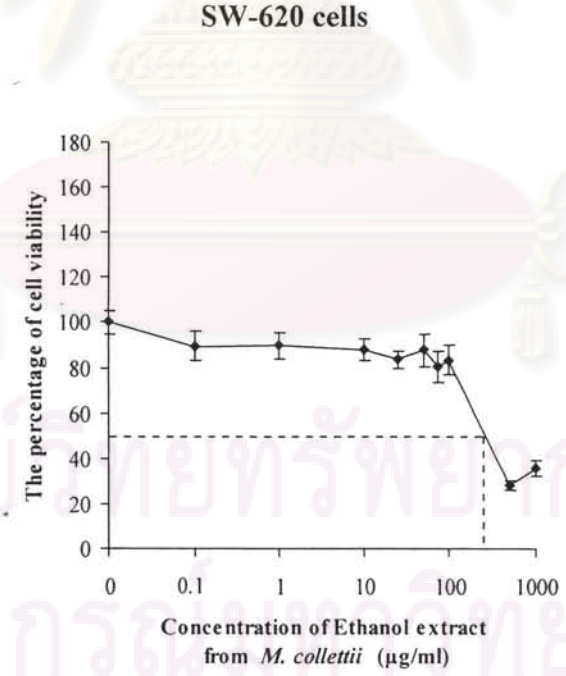

Figure 40 MTT test results of *Mucuna colletti* extract on the growth of SW-620 cell The IC_{50} was 332.86 ± 19.42 µg/ml.

Table 34 The percentage of cell viability after treated with *Phyllanthus emblica* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 2.37	100.00 ± 1.86
0.1	95.91 ± 2.61	107.91 ± 3.83
1	97.56 ± 1.85	103.99 ± 2.45
10	84.04 ± 5.64	80.06 ± 6.14
25	91.65 ± 3.26	59.52 ± 6.31
50	73.73 ± 1.22	25.55 ± 1.16
75	67.87 ± 2.03	28.25 ± 1.05
100	77.68 ± 4.61	34.88 ± 3.49
500	80.59 ± 4.99	39.16 ± 6.29
1000	83.32 ± 3.35	38.38 ± 2.89
IC_{50} (µg/ml)	**> 1,000**	**33.06 ± 0.84**

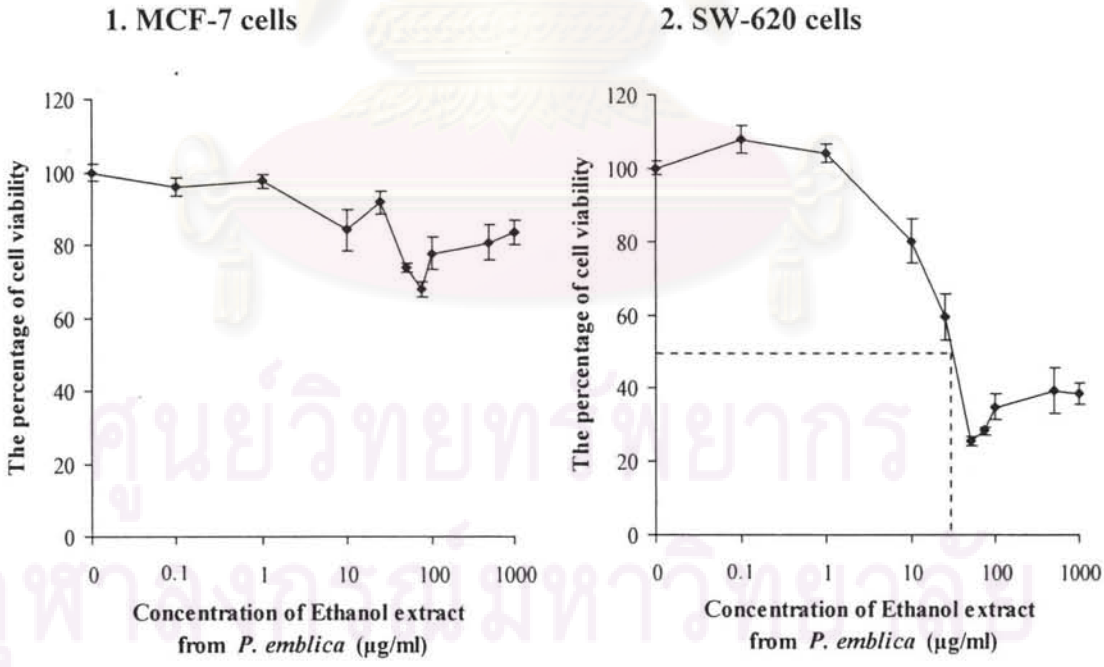

Figure 41 MTT test results of *Phyllanthus emblica* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were more than 1,000 and 33.06 ± 0.84 µg/ml, respectively.

Table 35 The percentage of cell viability after treated with fruits of *Piper nigrum* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 2.16	100.00 ± 3.08
0.1	93.67 ± 3.93	88.12 ± 2.31
1	94.38 ± 2.14	90.87 ± 4.85
10	92.96 ± 3.77	75.74 ± 3.62
25	79.96 ± 5.89	13.62 ± 0.88
50	28.53 ± 3.31	11.11 ± 1.53
75	11.74 ± 0.96	8.24 ± 0.39
100	8.78 ± 0.38	8.19 ± 0.31
500	26.58 ± 2.02	21.18 ± 0.88
1000	47.12 ± 3.10	38.44 ± 1.73
IC_{50} (µg/ml)	**39.61 ± 2.86**	**14.78 ± 0.39**

1. MCF-7 cells **2. SW-620 cells**

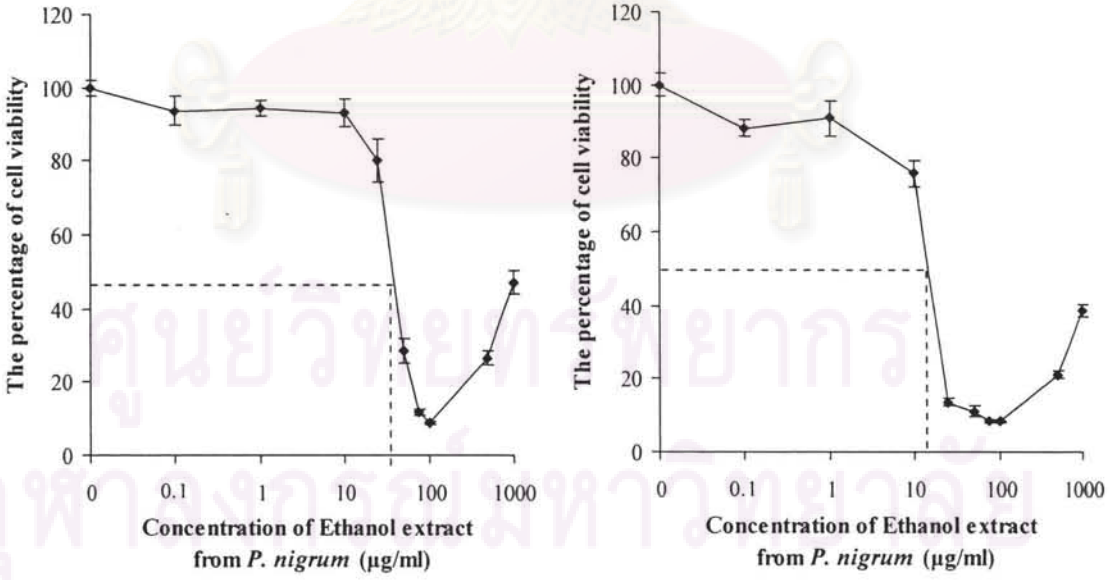

Figure 42 MTT test results of *Piper nigrum* fruits extract on the growth of MCF-7 and SW-620. cells. The IC_{50} were 39.61 ± 2.86 and 14.78 ± 0.39 µg/ml, respectively.

Table 36 The percentage of cell viability after treated with seeds of *Piper nigrum* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 2.23	100.00 ± 2.88
0.1	104.11 ± 3.23	101.89 ± 3.68
1	100.68 ± 5.80	98.43 ± 2.07
10	92.88 ± 3.85	52.79 ± 2.41
25	77.61 ± 5.56	19.84 ± 3.02
50	24.08 ± 4.36	10.96 ± 2.66
75	16.24 ± 0.76	7.57 ± 1.28
100	13.51 ± 1.29	7.31 ± 0.10
500	33.96 ± 1.51	23.07 ± 2.56
1000	57.16 ± 0.60	30.39 ± 2.92
IC_{50} (µg/ml)	**37.02 ± 2.44**	**14.60 ± 0.73**

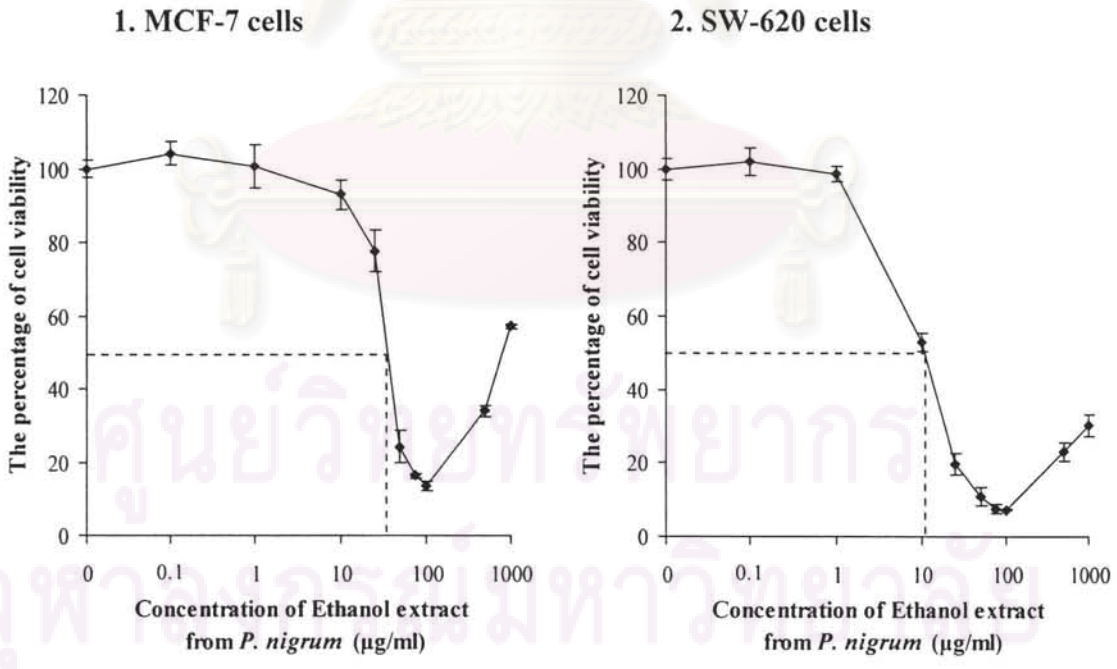

Figure 43 MTT test results of *Piper nigrum* seeds extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 37.02 ± 2.44 and 14.60 ± 0.73 µg/ml, respectively.

Table 37 The percentage of cell viability after treated with *Pueraria mirifica* extract (*n*=5)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	NT	100.00 ± 6.26
0.1		103.47 ± 2.98
1		102.05 ± 4.26
10		96.03 ± 5.68
25		84.91 ± 6.27
50		86.94 ± 7.98
75		80.59 ± 3.92
100		87.45 ± 2.91
500		79.52 ± 7.57
1000		59.20 ± 3.93
IC_{50} (µg/ml)		**> 1,000**

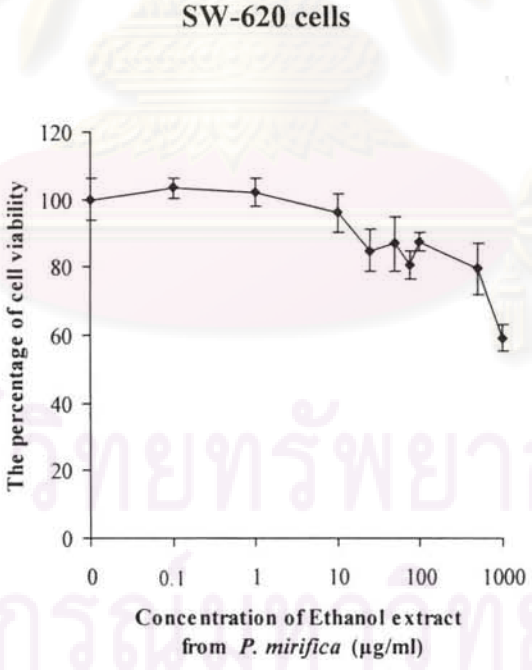

Figure 44 MTT test results of *Pueraria mirifica* extract on the growth of MCF-7 and SW-620 cells.The IC_{50} were more than 1,000 µg/ml same.

Table 38 The percentage of cell viability after treated with *Stephania erecta* extract (*n*=5)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 2.36	100.00 ± 2.55
0.1	90.34 ± 4.03	87.11 ± 2.10
1	91.88 ± 2.82	77.14 ± 0.46
10	70.32 ± 1.30	10.59 ± 0.68
25	66.10 ± 2.85	7.91 ± 0.32
50	32.11 ± 3.62	6.88 ± 0.85
75	18.30 ± 1.39	5.55 ± 0.34
100	11.73 ± 2.15	6.26 ± 0.37
500	22.40 ± 1.77	15.47 ± 0.74
1000	25.76 ± 1.11	12.78 ± 1.00
IC_{50} (µg/ml)	**35.34 ± 1.22**	**5.08 ± 0.07**

1. MCF-7 cells **2. SW-620 cells**

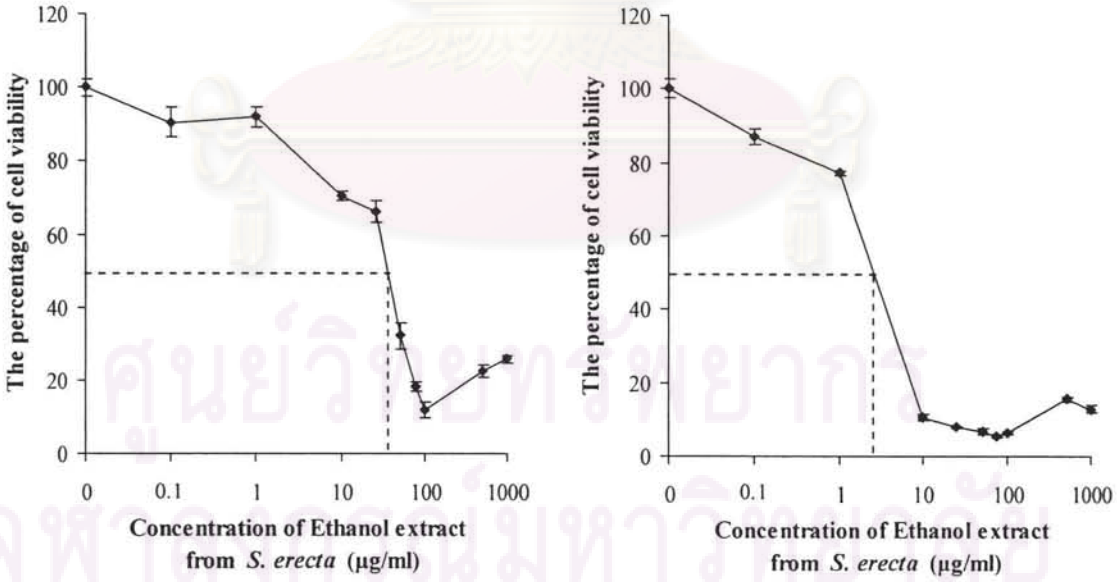

Figure 45 MTT test results of *Stephania erecta* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 35.34 ± 1.22 and 5.08 ± 0.07 µg/ml, respectively.

Table 39 The percentage of cell viability after treated with *Stephania venosa* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 5.06	100.00 ± 2.87
0.1	111.44 ± 5.62	99.24 ± 2.54
1	104.59 ± 6.95	89.53 ± 4.48
10	99.76 ± 7.54	67.65 ± 4.62
25	85.65 ± 2.50	21.69 ± 1.11
50	83.60 ± 3.93	17.48 ± 3.85
75	54.65 ± 2.54	7.51 ± 1.02
100	22.77 ± 1.67	6.46 ± 0.93
500	12.80 ± 0.71	12.12 ± 1.76
1000	20.65 ± 1.70	14.01 ± 1.02
IC_{50} (µg/ml)	**76.15 ± 1.14**	**15.73 ± 0.41**

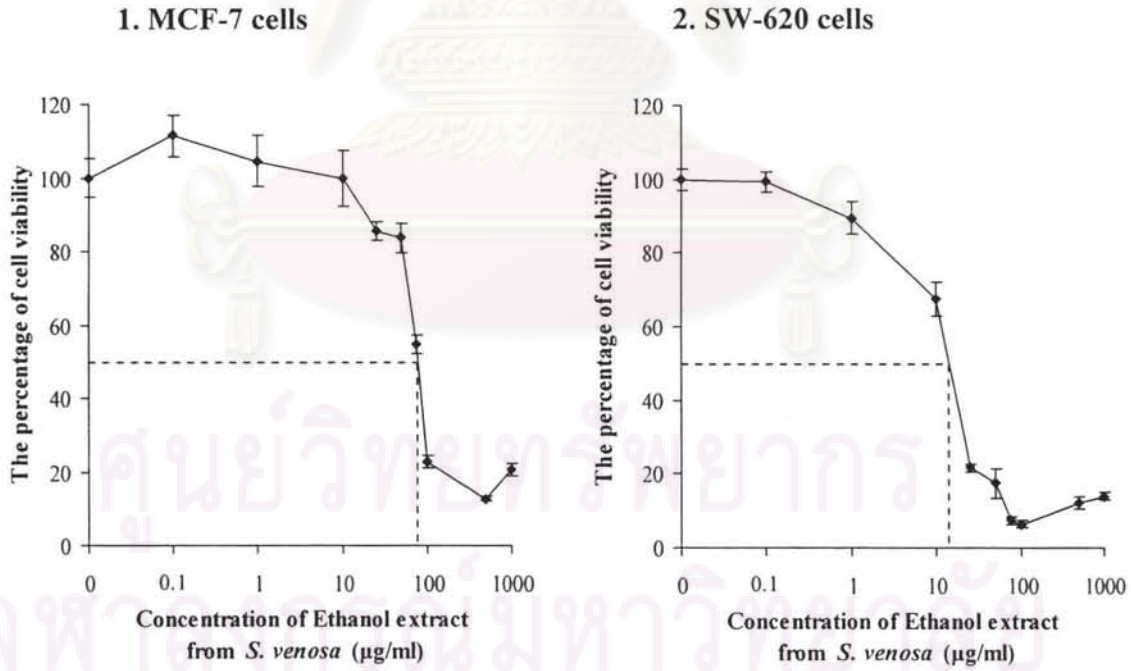

Figure 46 MTT test results of *Stephania venosa* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 76.15 ± 1.14 and 15.73 ± 0.41 µg/ml, respectively.

Table 40 The percentage of cell viability after treated with *Streblus asper* extract. (*n*=5)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 2.42	100.00 ± 5.23
0.1	100.69 ± 4.32	87.70 ± 4.45
1	92. 20 ± 3.79	84.20 ± 3.36
10	56.25 ± 1.38	14.56 ± 0.60
25	51.98 ± 1.73	5.54 ± 0.24
50	38.16 ± 3.17	4.73 ± 0.11
75	40.76 ± 1.02	4.48 ± 0.31
100	36.03 ± 1.04	4.91 ± 0.46
500	28.32 ± 2.80	8.94 ± 0.59
1000	35.88 ± 1.88	11.75 ± 0.31
IC_{50} (µg/ml)	**46.28 ± 1.71**	**5.55 ± 0.09**

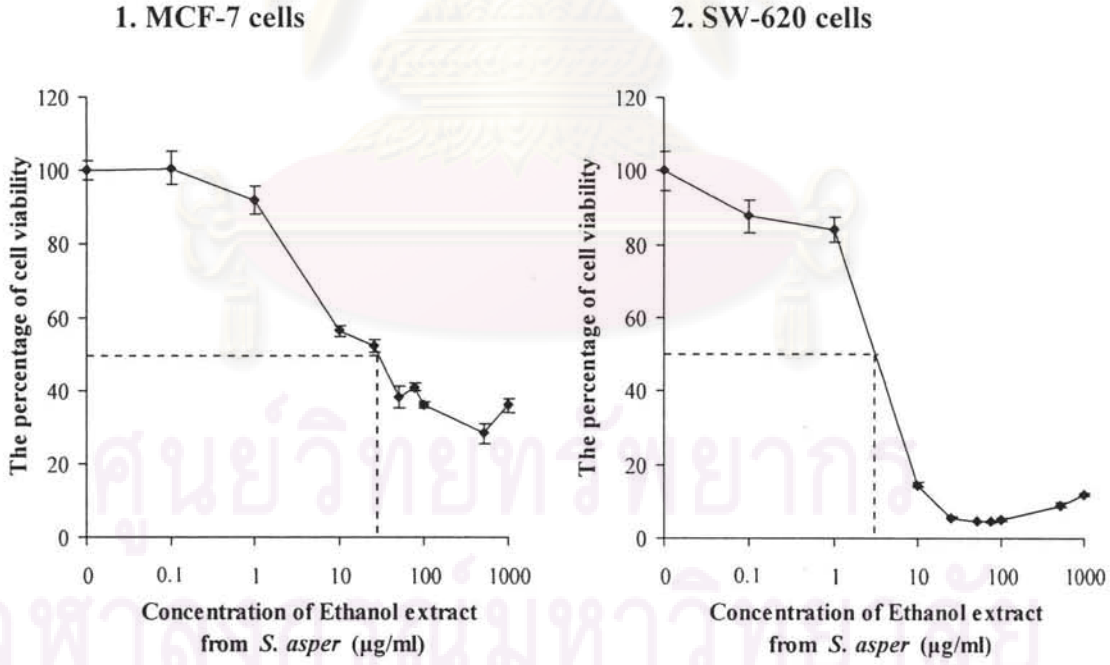

Figure 47 MTT test results of *Streblus asper* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 46.28 ± 1.71 and 5.55 ± 0.09 µg/ml, respectively.

Table 41 The percentage of cell viability after treated with leaves of *Suregada multiflorum* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 3.45	100.00 ± 4.85
0.1	101.46 ± 4.33	101.43 ± 5.75
1	101.57 ± 5.13	97.33 ± 3.68
10	90.09 ± 6.16	13.19 ± 0.54
25	43.83 ± 0.52	13.16 ± 1.79
50	21.24 ± 2.36	16.66 ± 1.23
75	18.02 ± 1.79	13.67 ± 1.06
100	17.61 ± 2.62	11.22 ± 1.54
500	17.88 ± 3.01	9.66 ± 1.03
1000	21.40 ± 0.74	11.76 ± 1.70
IC_{50} (µg/ml)	**23.79 ± 0.90**	**5.90 ± 0.15**

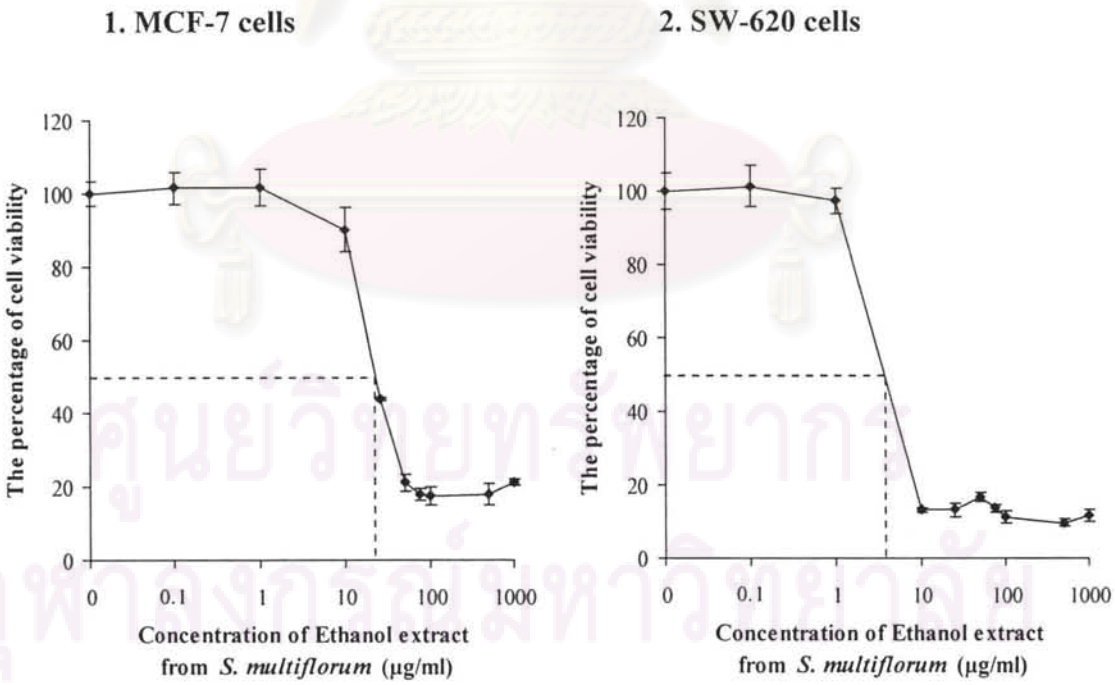

Figure 48 MTT test results of *Suregada multiflorum* leaves extract on the growth of MCF-7 and SW- 620 cells. The IC_{50} were 23.79 ± 0.90 and 5.90 ± 0.15 µg/ml, respectively.

Table 42 The percentage of cell viability after treated with stems of *Suregada multiflorum* extract (*n=5*)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 3.42	100.00 ± 7.82
0.1	99.29 ± 8.69	94.24 ± 7.38
1	124.75 ± 6.78	103.36 ± 7.10
10	129.89 ± 4.14	104.71 ± 6.88
25	98.56 ± 4.45	117.39 ± 7.67
50	93.88 ± 4.25	114.81 ± 6.90
75	71.01 ± 3.41	125.44 ± 4.73
100	68.46 ± 6.21	119.45 ± 9.48
500	19.84 ± 1.71	20.05 ± 1.12
1000	26.57 ± 3.51	24.03 ± 1.07
IC_{50} (µg/ml)	**299.64 ± 12.55**	**377.05 ± 8.04**

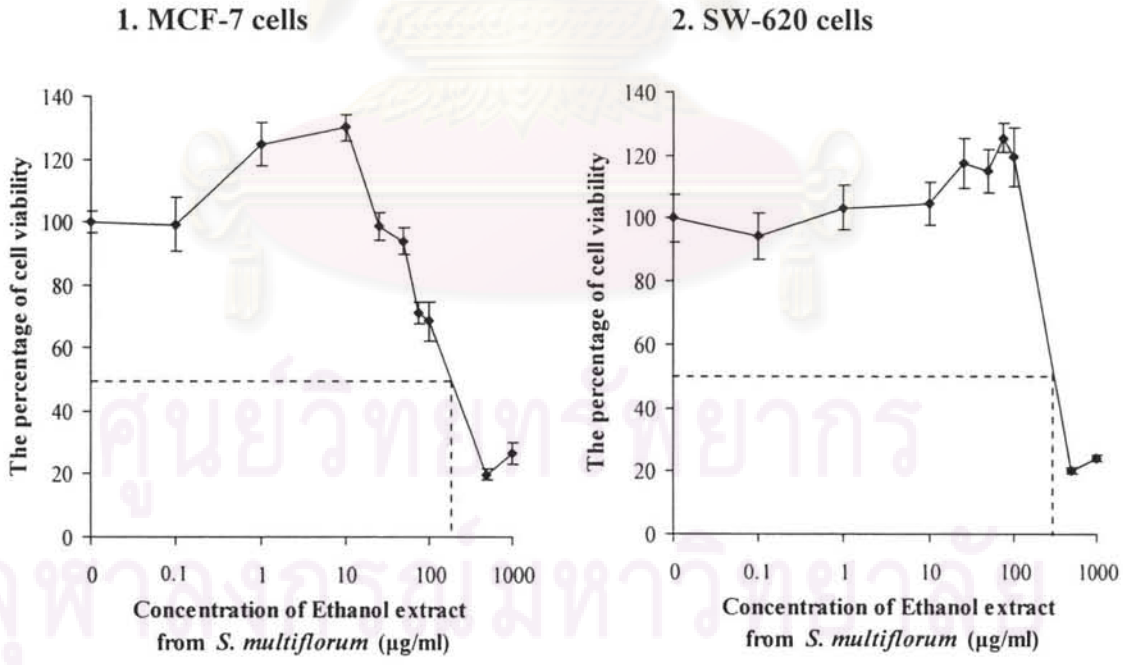

Figure 49 MTT test results of *Suregada multiflorum* stems extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 299.64 ± 12.55 and 377.05 ± 8.04 µg/ml, respectively

Table 43 The percentage of cell viability after treated with *Tinospora crispa* extract (*n*=5)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 6.13	100.00 ± 2.05
0.1	102.60 ± 5.34	103.67 ± 7.51
1	91.06 ± 3.65	104.35 ± 7.40
10	83.32 ± 2.12	99.08 ± 8.48
25	72.73 ± 3.49	107.18 ± 9.02
50	66.14 ± 2.83	92.47 ± 2.97
75	66.46 ± 4.16	81.41 ± 4.57
100	67.46 ± 2.61	84.35 ± 2.95
500	25.52 ± 1.06	13.22 ± 0.74
1000	31.75 ± 2.12	21.07 ± 0.80
IC_{50} (µg/ml)	**279.03 ± 8.63**	**290.86 ± 7.31**

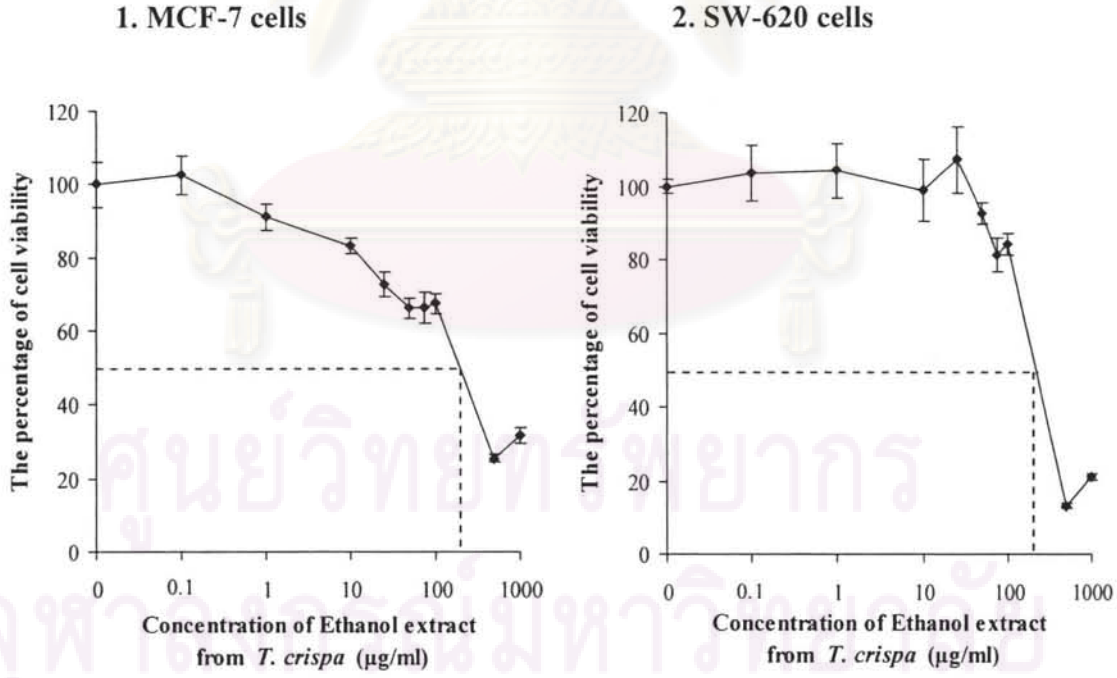

Figure 50 MTT test results of *Tinospora crispa* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were 279.03 ± 8.63 and 290.86 ± 7.31 µg/ml, respectively.

Table 44 The percentage of cell viability after treated with *Vitex negundo* extract (*n*=5)

Concentrations (µg/ml)	The percentage of cell viability	
	MCF-7	SW-620
0	100.00 ± 1.82	100.00 ± 2.31
0.1	101.18 ± 5.84	95.91 ± 6.05
1	103.43 ± 2.01	98.13 ± 2.80
10	111.36 ± 2.35	97.27 ± 2.67
25	112.60 ± 4.20	97.36 ± 2.46
50	99.26 ± 3.27	93.56 ± 5.98
75	96.08 ± 2.10	108.24 ± 5.67
100	98.37 ± 1.92	111.85 ± 2.42
500	94.78 ± 3.07	23.04 ± 2.92
1000	86.62 ± 5.15	23.84 ± 3.25
IC_{50} (µg/ml)	**> 1,000**	**362.25 ± 10.21**

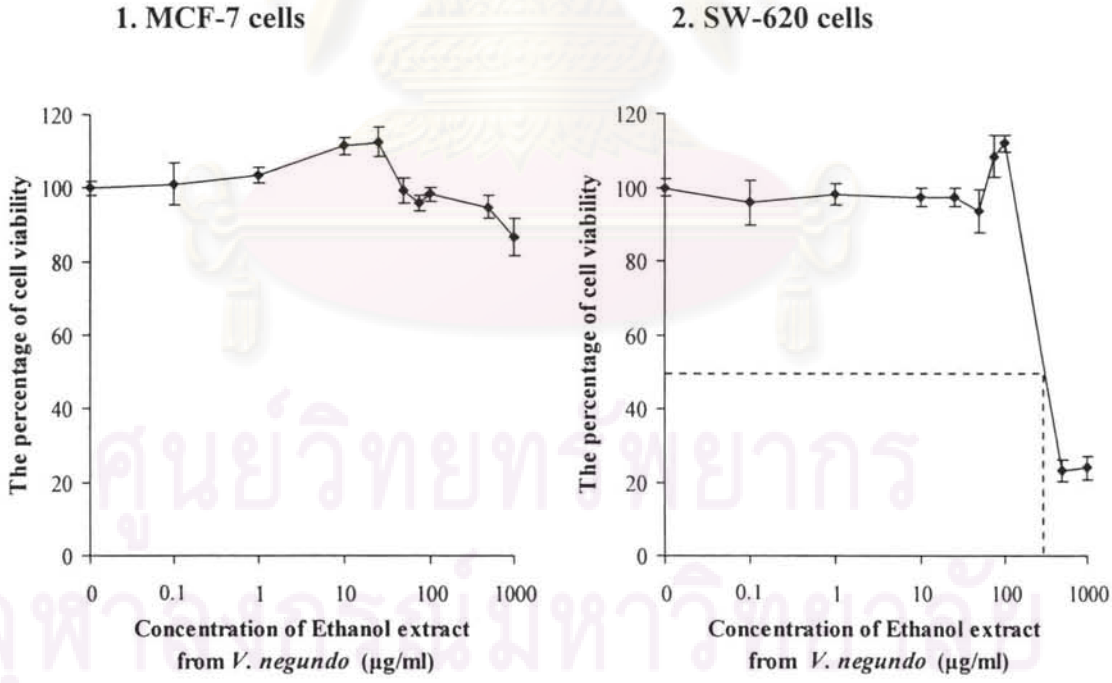

Figure 51 MTT test results of *Vitex negundo* extract on the growth of MCF-7 and SW-620 cells. The IC_{50} were more than 1,000 and 362.25 ± 10.21 µg/ml, respectively.

Table 45 Cytotoxicity of plant extracts from the Thai rejuvenating herbs against MCF-7 (Mean ± S.E.M.) (n = 5)

Ranking order	Plant	IC_{50} (µg/ml)
1	*Suregada multiflorum* (leaf)	$23.79 \pm 0.90^{a*}$
2	*Diospyros rhodocalyx*	$33.91 \pm 0.33^{a,b}$
3	*Stephania erecta*	$35.34 \pm 1.22^{a,b}$
4	*Piper nigrum* (seed)	$37.02 \pm 2.44^{a,b}$
5	*Piper nigrum* (fruit)	$39.61 \pm 2.86^{a,b}$
6	*Streblus asper*	$46.28 \pm 1.71^{a,b}$
7	*Cyperus rotundus*	53.46 ± 1.40^{b}
8	*Stephania venosa*	76.15 ± 1.14^{c}
9	*Tinospora crispa*	279.03 ± 8.63^{d}
10	*Suregada multiflorum* (stem)	299.64 ± 12.55^{d}
11	*Anaxagorea luzonensis*	439.97 ± 34.99^{e}
12	*Acacia farnesiana*	652.39 ± 18.99^{f}
13	*Kaempferia parviflora*	694.92 ± 18.22^{g}
14	*Albizia procera*	765.23 ± 13.78^{h}
15	*Betula alnoides*	$>1,000^{i}$
16	*Dracaena conferta*	$>1,000^{i}$
17	*Fagraea fragrans*	$>1,000^{i}$
18	*Leucaena leucocephala*	$>1,000^{i}$
19	*Melia azedarach*	$>1,000^{i}$
20	*Phyllanthus emblica*	$>1,000^{i}$
21	*Vitex trifolia*	$>1,000^{i}$

*The difference at the level of ^{a,b,c,d,e,f,g,h,i} is verified by Duncan's test.

Table 46 Cytotoxicity of plant extracts from the Thai rejuvenating herbs against SW-620 (Mean ± S.E.M.) (n = 5)

Ranking order	Plants	IC_{50} (μg/ml)
1	*Stephania erecta*	5.08 ± 0.07[a]
2	*Streblus asper*	5.55 ± 0.09[a]
3	*Suregada multiflorum* (leaf)	5.90 ± 0.15[a]
4	*Piper nigrum* (seed)	14.60 ± 0.73[a]
5	*Piper nigrum* (fruit)	14.78 ± 0.39[a]
6	*Stephania venosa*	15.73 ± 0.41[a]
7	*Betula alnoides*	23.83 ± 2.77[a]
8	*Diospyros rhodocalyx*	29.34 ± 1.87[a]
9	*Cyperus rotundus*	29.47 ± 0.20[a]
10	*Phyllanthus emblica*	33.06 ± 0.84[a]
11	*Anaxagorea luzonensis*	36.14 ± 1.24[a]
12	*Kaempferia parviflora*	282.29 ± 22.71[b]
13	*Tinospora crispa*	290.86 ± 7.31[b,c]
14	*Mucuna collettii*	332.86 ± 19.42[c,d]
15	*Acacia farnesiana*	345.49±23.63[d]
16	*Vitex trifolia*	362.25 ± 10.21[d,e]
17	*Suregada multiflorum* (stem)	377.05 ± 8.04[d,e]
18	*Dracaena conferta*	407.37 ± 48.11[e]
19	*Fagraea fragrans*	468.18±19.12[f]
20	*Leucaena leucocephala*	482.51 ± 7.25[f]
21	*Albizia procera*	529.03± 15.43[g]
22	*Butea superba*	689.59 ± 36.01[h]
23	*Melia azedarach*	>1,000[i]
24	*Pueraria mirifica*	>1000[i]

*The difference at the level of [a,b,c,d,e,f,g] is verified by Duncan's test.

2.1.3 The selectivity index (SI)

The selectivity index (SI) was calculated from the IC_{50} ratio in MCF-7 versus SW-620 cells (Bézivin et al., 2003). SI value indicates selectivity of the sample to the cell lines tested. Any sample which has SI value higher than 3 will be considered to have high selectivity, results summarized in Table 47.

- SI value were higher than 3 ($SI \geq 3$)

The 8 extracts including *B. alnoides*, *P. emblica*, *A. luzonensis*, *S. asper*, *S. erecta*, *S. venosa*, *S. multiflorum* (leaves) and *M. collettii* shown high selectivity between MCF-7 and SW-620.

- SI values were higher than between 2.76-1.45($SI > 2.76$ to $SI > 1.45$)

The 5 extracts including *V. trifolia*, *D. conferta*, *F. fragrans*, *L. leucocephala* and *B. superba* shown SI value were higher than between 2.76-1.45.

- SI values were less than 3 ($SI < 3$)

The 11 extracts including *P. nigrum* (fruits), *P. nigrum* (seeds), *K. parviflora*, *A. farnesiana*, *C. rotundus*, *A. procera*, *D. rhodocalyx*, *M. azedarach*, *P. mirifica*, *T. Crispa* and *S. multiflorum* (stems) showed no selectivity between MCF-7 and SW-620 .

In consideration of the cytotoxicity and selectivity result, the samples could be classified into 5 categories.

- Firstly, potentially cytotoxic (IC_{50} in SW-620 <100 μg/ml) and high selectivity ($SI \geq 3$); *A. luzonensis*, *B. alnoides*, *P. emblica*, *S. erecta*, *S. venosa*, *S. Asper* and *S. multiflorum* (leaves)
- Secondly, potentially cytotoxic (IC_{50} in SW-620 <100 μg/ml) but less selectivity ($SI < 3$); are *C. rotundus*, *D. rhodocalyx* and *P. nigrum* (fruits and seeds)
- Thirdly, moderate cytotoxic (100 μg/ml < IC_{50} in SW-620 <1000 μg/ml) and high selectivity ($SI \geq 3$); are *D. conferta*, *F. fragrans*, *L. leucocephala* and *V. trifolia*
- Fourthly, moderate cytotoxic (100 μg/ml < IC_{50} in SW-620 ≤ 1000 μg/ml) but less selectivity ($SI < 3$); are *A. farnesiana*, *A. procera*, *K. parviflora*, *S. multiflorum* (stems), *T. crispa*, *V. trifolia*, *D. conferta*, *F. fragrans* and *L. leucocephala*
- Finally, non toxic in both cell lines (IC_{50}>1000 μg/ml) is *M. azedarach*.

Table 47 Cytotoxicity and selectivity results in human mammary cancer (MCF7) and human colon cancer (SW620) cell line of 24 plants ethanol extracts

Plants in this study	IC_{50} (µg/ml)		Selectivity index
	MCF-7	SW-620	
Acacia farnesiana	652.39	345.49	1.89
Albizia procera	765.23	529.03	1.45
Anaxagorea luzonensis	439.97	36.14	12.17
Betula alnoides	>1,000	23.83	>41.96
Butea superba	N/A	689.59	N/A
Cyperus rotundus	53.46	29.47	1.81
Diospyros rhodocalyx	33.91	29.34	1.16
Dracaena conferta	>1,000	407.37	>2.45
Fagraea fragrans	>1,000	468.18	>2.14
Kaempferia parviflora	694.92	282.29	2.46
Leucaena leucocephala	> 1,000	482.51	>2.07
Melia azedarach	> 1,000	> 1,000	1.00
Mucuna collettii	N/A	332.86	N/A
Phyllanthus emblica	> 1,000	33.06	>30.25
Piper nigrum (fruits)	39.61	14.78	2.68
Piper nigrum (seeds)	37.02	14.60	2.54
Pueraria mirifica	N/A	> 1,000	N/A
Stephania erecta	35.34	5.08	6.96
Stephania venosa	76.15	15.73	4.84
Streblus asper	46.28	5.55	8.37
Suregada multiflorum (leaves)	23.79	5.90	4.03
Suregada multiflorum (stems)	299.64	377.05	0.79
Tinospora crispa	279.03	290.86	0.96
Vitex trifolia	> 1,000	362.25	>2.76

CHAPTER V

DISCUSSION

Pharmaceutical agents have been usually discovered by screening of natural products from plant materials, for examples vincristine, camptothecin and taxol which are anticancer agents (Shoeb, 2006). Vincristine, the plant alkaloid inhibits microtubule assembly, induces cell cycle arrest in G2/M-phase and induces apoptosis in several tumor cell lines such as gastric, breast and lung cancers (Papamichael, 2000, Cragg and Newman, 2005). Camptothecin, isolated from *Camptotheca acuminate* Decne (Nyssaceae) and semi-synthetic derivatives of camptothecin are used for treatment of ovarian and small cell lung cancers as well as colorectal cancers (Creemers et al., 1996; Bertino, 1997). Different cell lines might exhibit different sensitivities towards a cytotoxic compound, the use of more than one cell line is therefore considered necessary in the detection of cytotoxic compounds (Kamuhabwa *et al.*, 2000)

In the present study, we evaluate the cytotoxicity effect of 24 ethanolic extracts from 22 plants belonging to 15 families, on human breast cancer (MCF-7) and human colon cancer (SW-620). Twenty-two plant materials have been presented as ingredients in the Thai traditional rejuvenating remedies, with some plants, *Piper nigrum*, *Suregada multiflorum*, *Diospyros rhodocalyx*, *Cyperus rotundus* and *Tinospora crispa* that are already used as ingredients in the treatment of cancer formulary (แก้ว กาญจนา 2548). MTT assay, the principle based on the ability of living cells to cleave the yellow tetrazolium salt into a blue color product (formazan) by the mitochondrial enzyme, succinate dehydrogenase, was applied in this study. With the aid of spectrophotometer, the number of living cells in each sample could be analyzed.

The results from the study indicated that the test plant extracts had differenct degrees of anti-proliferative activity against the two cell lines.

Suregada multiflorum (leaves) extract showed the highest cytotoxic activity against breast cancer cells (IC_{50} = 23.79 µg/ml), but ranked third against colon cancer cells (IC_{50} = 5.90 µg/ml). In the previous reports, triterpenoids isolated from the bark of the plant was effective against human breast cancer cells (Bourinbaiar and Lee-Huang,

1996). On the contrary, *S. multiflorum* stem extract showed weak activity against breast and colon cancer cells (IC_{50} = 299.64 and 377.05, respectively). The water extracts of *S. multiflorum* stem showed weak activity against COR-L23 (large cell lung carcinoma), LS-174T (human colon adenocarcinoma) and MCF-7 cells (IC_{50} = 103.5, 101.6 and 108.7, respectively) (Itharatet al., 2004).

The ethanolic extracts of *Stephania erecta* (tuberous root), *Streblus asper* (seed), *Piper nigrum* (seed), *Piper nigrum* (fruit), *Stephania venosa* (tuber), *Diospyros rhodocalyx* (stem bark) and *Cyperus rotundus* (rhizome) showed high cytotoxic activity against colon cancer cells with IC_{50} values 5.08, 5.55, 14.60, 14.78, 15.73, 29.34 and 29.47, respectively and showed moderate cytotoxic activity against breast cancer cells with IC_{50} values 35.34, 46.28, 37.02, 39.61, 76.15, 33.91 and 53.46, respectively

S. asper has been reported to possess anticancer activity (Rastogi and Dhawan, 1990). Two cytotoxic cardiac glycosides, strebloside and mansonin, were isolated from *S. asper* stem bark with significant activity against KB (human epidermoid carcinoma) cells with IC_{50} values of 32 and 42 µg/ml, respectively. The volatile oil isolated from fresh leaves of *S. asper* showed significant anticancer activity (IC_{50} << 30 µg/ml) against P388 (mouse leukemia) cells (Phutdhawong *et al.*, 2004).

Tuber from *S. venosa* was used as an antitumor and anti-proliferation of breast cell culture. Palmatine and crebanine isolated from *S. venosa*, possess high cytotoxic activity against MCF-7 with IC_{50} values in the range of 5-6 µg/ml (Keawpradub *et al.*, 2001,). The 80% ethanol crude extract from *S. venosa* showed moderate cytotoxic activity all tumor cell lines tested, with an IC_{50} of 35.11 µg/ml for SKOV3 (human ovarian cancer cell line) and 39.67 µg/ml for SKBR3 (human breast cancer cell line).

Extracts of seed and fruit of *P. nigrum* showed cytotoxic activity against colon cancer cells with IC_{50} values of 14.60 and 14.78 µg/ml, respectively and against breast cancer cells with IC_{50} values of 37.02 and 39.61 µg/ml, respectively. *P. nigrum* (fruit), black pepper is obtained from the dried unripe fruit and *P. nigrum* (seed), white pepper is obtained when the pericarp is removed (Craib, 1992). This demonstrated that substances containing in pericarp of *P. nigrum* are not different in sensitivity on colon and breast cancer cells.

The ethanolic extracts of *Betula alnoides* (whole stem), *Phyllanthus emblica* (fruit) and *Anaxagorea luzonensis* (whole stem) extract showed cytotoxic activity against colon cancer cells (IC_{50} = 23.83, 33.06 and 36.14 μg/ml, respectively) but no activity against breast cancer cells (IC_{50} > 1,000 μg/ml and *A. luzonensis* = 439.97 μg/ml). Thus, the plant ethanolic extract was selectively toxic against cancer cells. The extract of *P. emblica* fruits showed moderate cytotoxic activity in all tested tumor cell lines, with IC_{50} of 63.3 μg/ml for B-16 (murine melanoma), 66.5 μg/ml for HCT-8 (human colon carcinoma), 70.4 and 33.2 μg/ml for CEM and HL-60 (leukemia) tumor cell lines, respectively. *P. emblica* roots possess antiproliferative activities against MK-1 (human gastric adenocarcinoma), HeLa (human uterine carcinoma), and B16F10 (murine melanoma) cells (Zhang, *et al.*, 2004).

The ethanolic extracts of *Mucuna collettii* (whole stem), *Butea superba* (tuberous root) and *Pueraria mirifica* (tuberous root) showed weak cytotoxic activity against SW-620 cells with an IC_{50} value of 332.86, 689.59 and no activity against SW-620 with an IC_{50} value more than 1,000 μg/ml, respectively. The ethanolic extracts of *M. colletti* had anti-proliferation effect on the growth of MCF-7 cells with an ED_{50} value of 85.36 μg/ml. While *B. superba* and *P. mirifica* exhibited no activity against MCF-7 cell lines with an IC_{50} value of 370.91 and 642.83 μg/ml, respectively (Cherdshewasart *et,al* 2004). Hopeaphenol (which is present in *M. collettii*) exhibited cytotoxicity against KB (human epidermoid carcinoma) cell line (Ohyama, *et al.* 1999). Formononetin and prunetin isolated from *B. superba*, showed moderate cytotoxic activity against KB cells with IC_{50} values 37.3 and 71.1 μM and on BC (breast cancer) cell lines with IC_{50} values 32.7 and 47.3 μM, respectively (Ngamrojanavanich *et al.*, 2007)

The ethanolic extracts of *Acacia farnesiana* (root), *Albizia procera* (stem bark), *Kaempferia parviflora* (rhizome) and *Tinospora crispa* (whole stem) showed weak cytotoxic against MCF-7 and SW-620 cells. Saponins isolated from the bark of *A. procera* exhibited cytotoxicity against HepG2 cells with IC_{50} value 9.13 μg/ml (Melek, *et al.*, 2007).

The ethanolic extracts of *Melia azedarach* (whole stem) showed no activity against and activate proliferation of both cell lines (IC_{50} > 1,000 μg/ml).

However, according to the criteria of cytotoxicity activity for the crude extracts, as established by the American National Cancer Institute (NCI); 50% inhibition values (IC_{50}) of proliferation at the less than 30 μg/ml were considered "active" in the preliminary assay (Suffness and Pezzuto, 1990). In consideration of the cytotoxicity (IC_{50} < 30 μg/ml) and selectivity result of SW-620 versus MCF-7 cell lines (SI ≥ 3), the samples of *B. alnoides* (> 41.96), *S. erecta* (6.96), *S. venosa* (4.84), *S. asper* (8.37) and *S. multiflorum* (leaves) (4.03), this result suggested that the plant ethanolic were selectively toxic against colon cancer cell line.

In summary, the ethanolic extracts of plants used in Thai traditional rejuvenating remedies were screened for cytotoxic activity against MCF-7 and SW-620 cancer cell lines. Out of 22 plants tested, 8 plants including *Stephania erecta*, *Streblus asper*, *Suregada multiflorum, Piper nigrum, Stephania venosa*, *Betula alnoides*, *Diospyros rhodocalyx* and *Cyperus rotundus* exhibited potential cytotoxicity against human colon cancer cells (SW-620) *in vitro* and only *Suregada multiflorum* exhibited a potential cytotoxicity against human breast cancer cells (MCF-7) *in vitro*.

The extracts from natural products such as fruits, vegetables and medicinal herbs initiates positive effects against cancer in comparison with chemotherapy or the recent hormonal treatments (Wu *et al,* 2002). The chemicals isolated from plants as well as the crude extract *per se* harbor pharmacologically active constituents. However the presence of the active chemicals in the crude extract would vary according to plant materials or solvent used or extraction conditions. Water and alcohol is a common solvent used in plant extraction. In the Thai traditional medicines preparation from plants, boiling of the plant materials with water or soaking in alcohol is the commonly used methods. Water is a prime choice solvent due to low cost and results in the separation of polar compounds. However water extraction leaves undesired substances including sugars and starch which is a good nutrient for microbes. Alcohol is a better choice for separation of both polar and non-polar compounds, and also effective in suppressing the growth of microorganisms. Alcoholic extraction is used in this experiment, thus the anti-proliferation activity of the plant extracts should derived mainly from their polar and non-polar constituents.

The oriental traditional medicines are increasing popularity in the Western countries. China and India are the 2 big players in this type of market. Even many government organizations have tried hard to promote the exportation of the Thai herbal plant products, the demand is still low due to lack of credit in hygiene production as well as poor quality control. Researches on bioactive chemicals would initiate more interests. Organic farming of the selected cultivar of the well-studied herbal plants would help guarantee of the standard raw materials. So far, *Pueraria mirifica* cultivar with high isoflavones has been selected and submitted to organic farming in a huge area to guarantee the unique supply of standardized raw material for manufacturing of traditional medicines, dietary supplements, beverages, foods and cosmetic products.

The potent Thai rejuvenating herbal plants screened from this study including *Stephania erecta*, *Streblus asper*, *Suregada multiflorum, Piper nigrum, Stephania venosa*, *Betula alnoides*, *Diospyros rhodocalyx* and *Cyperus rotundus* are well grown in many places in Thailand. Thus, our data could help support the promotion of both consumption and organic plantation of these plants.

PERSPECTIVE

Anti-proliferation assay in cell cultures of the plant extracts is a conventional and rapid screening method. Thus, more plant samples in other groups of traditional remedies should be brought to this type of investigation. More different cell type is also needed to verify other bioactivities of the plant materials in this group. Even the selected plant crude extracts could be manufactured into traditional medicines, beverages, foods or cosmetics, however, the crude extract *per se* is not convincing for development of medicines for a specific treatment. Thus, identification as well as purification of bioactive chemicals is urgent needed in the further study. The new intellectual property should derive from the further study. Finally, new commercial products should be obtained from this massive study.

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APPENDICES

MEDIA AND REAGENTS

1. RPMI 1640 medium

RPMI 1640 medium (Biochrom)	10.43 g
(with L-glutamine and 25 mM HEPES buffer, without NaHCO3)	
$NaHCO_3$	2 g
10,000 units/ml penicillin and 10,000 μg/ml streptomycin	1 ml
Sterile water	1000 ml

Adjust pH to 7.0 - 7.2 with 1 N HCl or 1 N NaOH before adjust volume with water and sterilize by nitrocellulose membrane (pore size 0.22 μm). Dispense the filtrate into bottles. All bottled mediums are stored in 4°C until use.

2. DMEM medium (Dulbecco's Modified Eagle Medium)

DMEM powder medium (Sigma)	9.58 g
HEPES	1.5 g
$NaHCO_3$	1 g
10,000 units/ml penicillin and 10,000 μg/ml streptomycin	1 ml
Sterile water	1000 ml

Adjust pH to 7.2 - 7.4 with 1 N HCl or 1 N NaOH before adjust volume with water and sterilize by nitrocellulose membrane (pore size 0.22 μm). Dispense the filtrate into bottles. All bottled mediums are stored in 4°C until use.

3. EMEM medium (Eagle's Minimum Essential Medium)

EMEM powder medium (BioWhittaker)	9.58 g
HEPES	1.5 g
$NaHCO_3$	1 g
10,000 units/ml penicillin and 10,000 μg/ml streptomycin	1 ml
Sterile water	1000 ml

Adjust pH to 7.2 - 7.4 with 1 N HCl or 1 N NaOH before adjust volume with water and sterilize by nitrocellulose membrane (pore size 0.22 μm). Dispense the filtrate into bottles. All bottled mediums are stored in 4°C until use.

4. Phosphate buffer saline (PBS)

NaCl	8 g
KCl	0.2 g

Na_2HPO_4	1.15 g
KH_2PO_4	0.2 g
Distilled water	1 L

Adjust pH to 7.4 with 1 N HCl or 1 N NaOH before adjust volume with water. Dispense into bottles and autoclave for 20 minute.

5. 0.4% Trypan blue dye

Trypan bue	1.6 g
NaCl	3.24 g
KH_2PO_4	0.24 g
Distilled water	400 ml

All ingredients were mixed altogether, heat and stirred with magnetic stirre until completely dissolved. Adjust pH to 7.2-7.3 (by add 7.5% $NaHCO_3$ and/or 1% HCl).Then dispensed into light protecting bottles.

6. MTT solution

MTT:	5 mg
3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide	
0.89% Normal saline	1 ml

Add MTT 5 mg into 1 ml of 0.89% normal saline. All ingredients were mixed and sterilized by filter. Dispense into light protecting bottles and freshly prepared for every experiment.

7. Sorensen's glycine buffer

0.1 M glycine	7.507 g
0.1 M NaCl	5.844 g
Distilled water to make	1000 ml

Adjust pH to 10.5 with 0.1 NaOH before adjust volume with water and sterilize by nitrocellulose membrane (pore size 0.22 μm).

8. Hypertonic buffer

10 mM Tris pH 7.4 (1.2 g/100 ml)	10 ml
400 mM NaCl (5.84 g/100 ml)	40 ml
5 mM $CaCl_2$ (0.55 g/100 ml)	10 ml
10 mM $MgCl_2$ (2.03 g/100 ml)	10 ml

Distilled water to make 100 ml

All ingredients were mixed altogether and sterilize by nitrocellulose membrane (pore size 0.22 μm). Dispense into bottles and autoclave for 20 minute.

BIOGRAPHY

Miss Jiraphat Charoenkupt was born on December 17th, 1983 in Bangkok, Thailand. She graduated with a Bachelor degree of Science in Biology, Faculty of Science, Chulalongkorn University in 2005. She has enrolled in the Graduate School, Chulalongkorn University for Master Degree of Science in Biotechnology during 2006-2009.

Research presentation

Charoenkupt, J., Cherdshewasart, W. and Panriansaen, R. 2008. Comparison of the total phenolic contents ethanol and water extract from the Thai traditional plants used in rejuvenating remedies. Abstract *the 13th Biological Sciences Graduate Congress*, 15th-17th December, National University of Singapore, Singapore.

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