CHAPTER 2

LITERATURE REVIEW

Cancer

Cancer[12, 13] is one of the leading causes of death in Thailand. The incidence of cancer is increasing and differs in sites, sex, and age. Important cancers of women in Thailand are breast, cervix, and ovarian cancers.

Etiology[12, 13]

Cancer is a group of disorder diseases in which cells have lost their normal mechanism for control of growth and proliferation. The causes of cancer are genetic and environmental factors, such as chemicals, infection, radiation, immunological, trauma, *etc.* These factors often initiate cancer. Cancer progresses by initiation, promotion, and progression. Cancer cells can also invade into distant organs.

Cancer Treatment[12, 13]

Cancer treatments include local and systemic treatments. Local treatments consist of surgery and radiotherapy, whereas immunotherapy and chemotherapy are the procedures used for systemic treatments.

Local Treatments

Surgery : This method is the most appropriate choice for solid tumors diagnosed in early stages, but useless in metastasis.

Radiotherapy : The method is suitable for cancer in which surgery is inappropiate. Radiation is mainly by X-ray, ^{131}I, *etc.*

Systemic Treatments

Immunotherapy : This method is used for stimulation of the immune system to fight against cancer. Immunotherapeutics included cytokines, tumor vaccines, and interleukins.

Chemotherapy : Chemotherapeutic methods require chemical agents with cytotoxic activity to cancer cells. The ideal chemotherapeutic agents need to be specific to cancer cells with low toxicity to normal cells. Chemical substances used in chemotherapy can be classified into two groups. If drugs are active in specific cell phases, these drugs are cell-cycle specific agents. Cell-cycle nonspecific agents are cytotoxic in all phases of the cell cycle. The treatment with cell-cycle specific agents is more appropriate in proliferating cells, while cell-cycle nonspecific agents are appropriate for the treatment of resting cells.

Classification of Cancer Chemotherapeutic Agents

- *Alkylating agents*

Drugs in this group replace hydrogen atoms in DNA with highly reactive alkyl radicals, causing cross-linking or breaking of DNA molecules. Alkylating agents *i.e.*, ifosfamide and mechlorethamine can be used against proliferation of cancer cells.

- *DNA intercalating agents*

Partial structure of these drugs is planar and polycyclic, which can intercalate into the double helix of DNA and works against DNA synthesis. Drugs in this category are such as adriamycin and daunomycin.

- *Antimetabolites*

This group of drugs is similar to essential metabolites. They can replace the normal metabolites and interfere with normal biological systems. Methotrexate and 6-mercaptopurine are widely used anticancers in this group.

- Hormones

Hormones are active by a variety of actions. Drugs in this group such as estrogens, androgens, and glucocorticoids interfere with hormone receptors on the plasma membrane of tumor cell.

- Antimitotic agents

After the entrance into cancer cells, this type of anticancers can interfere the microtubule synthesis in M phase and inhibit topoisomerase I and topoisomerase II enzymes. Effective drugs in this group are vinblastine, taxol, and etoposide.

- Miscellaneous agents

Other drugs act by a variety of toxic mechanisms. Drugs such as procarbazine and carboplatin are examples of drugs in this group.

Plant Selection

Plant selection for each research project depends on the aim of the work. The selection of particular plants for activity studies is important. The method for plants selection can be done by following of literature leads, ethnobotanical uses or randomised selection[9,12].

Methods for Plant Selection

- *Ethnobotany*

  Selection of plants is based on their therapeutical use by an ethnic group.

- *Taxonomy*

  Selection of plants is based on their taxonomy *i.e.*, family, genus, species.

- *Chemotaxonomy*

  Plants are selected according to their chemical category of substances found in a genus or family.

- *Randomised selection*

  Plants are randomly selected without consideration of the other three methods.

In this study, plants were selected by focusing on the plants of the family Rubiaceae, in which possess some members with cytotoxic activity, and then were randomly selected for species. Rubiaceous plants from Northern part of Thailand were used for this research because this family is diverse, and has no report about cytotoxic activity against MCF-7 and KB-3-1 cell lines.

Rubiaceae and Anticancer Activity

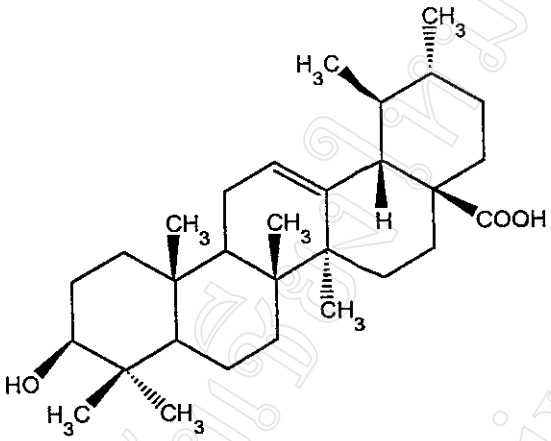
General Tracts

Rubiaceae includes vines, woody climbers, treelets, shrubs, trees and herbs. The leaves are usually opposite simple entire with interpetiolar stipules. The flowers are regular and mostly bisexual. The calyx and corolla are mostly 4-5 lobed. There are as many stamens as corolla lobes which are adnate to the corolla tube. The gynoecium consists of a single compound pistil mostly with 2 carpels with and a single inferior ovary with the number of locules equaling the number of carpels. The fruit can be drupes, capsules, and berries.[13]

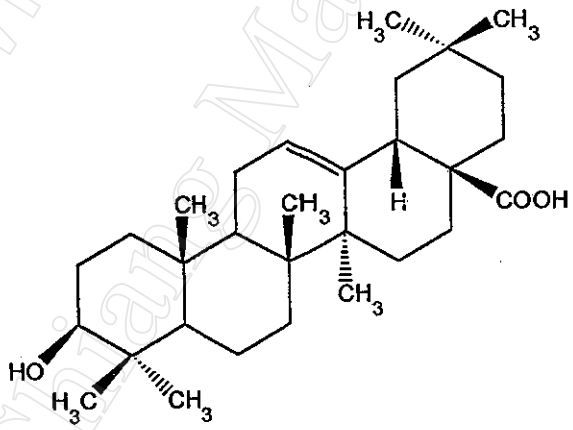
Maxwell recently reported 41 genera and 92 species of Rubiaceae in Doi Sutep-Pui National Park,[14] Chiang Mai, which is just west of Chiang Mai University and a convenient place to collect specimens.

Examples of Cancer Research on Rubiaceae

Hsu [15, 16] studied the herbs, *Hedyotis corymbosa* and *Hedyotis diffusa* and found ursolic, oleanolic, and geniposidic acids. These three compounds were tested for antitumor activity on the hepatoma cell line (Hep-2B). Ursolic and oleanolic acids displayed stronger antitumor activity than geniposidic acid (Figure 1).



ursolic acid



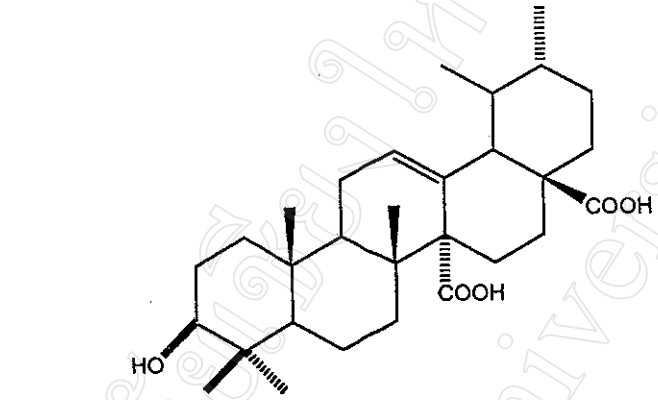
oleanolic acid

Figure 1. Structures of ursolic acid and oleanolic acid [16].

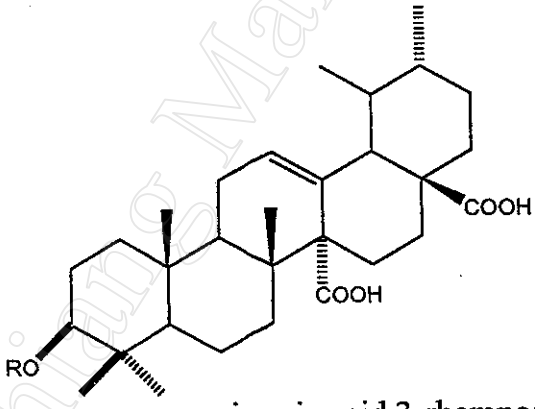
Ixora coccinea[17] extract was tested for cytotoxic activity against Dalton's lymphoma (ascitic and solid tumor, DLA), enrich ascites carcinoma (EAC) tumors, and sarcomar (S-180) cell lines at IC_{50} 18,60 and 25 µg/ml, respectively. The extract can increase the life span of DLA and EAC mice at 113 and 68 %, respectively. The extract was also toxic to transformed lymphocytes of leukemia patients with acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and K-562 suspension cell cultures. The mechanism of the extract was proven to be *via* the inhibition of cancer cell DNA synthesis.

Raffauf *et al.*[18] studied the stem bark, and wood of *Cinchona pubescens*. Chemicals were extracted with 95% ethanol following with solvents of increasing polarity. All extracts were tested for cytotoxic activity with human nasopharynx carcinoma (KB). Ether extract exhibited cytotoxic activity against KB with IC_{50} of 20 µg/ml and was purified with column chromatography. Quinovic acid and quinovic acid 3-rhamnoside was isolated, exhibited cytotoxic activity with IC_{50} of 21 and 80 µg/ml against KB cells, respectively (Figure 2).

Chapuis *et al.*[19] studied seventy-five ethnomedicinal plants collected in Africa, Panama, and Mauritius for their cytotoxic activity. All plant chemicals were extracted with many solvents, which total 260 extracts. All extracts were diluted in three concentrations such as 10, 1, 0.1 µg/ml and tested for cytotoxic activity with human colon carcinoma cells (Co115). The response was detected by the colorimetric method (*p*-nitrophenyl-*N*-acetyl-β-*D*-glucosamide), and interpreted with ED_{50}. The Rubiaceous plants tested in that research included *Crossopteryx febrifuga*, *Pavetta crassipes*, and *Spermacoce dilorachiata*. Leaves and stems of *Crossopteryx febrifuga* were extracted with dichloromethane, which were active against Co115 with ED_{50} of 7 µg/ml (from leaves) and 0.095 µg/ml (from stems).



quinovic acid



quinovic acid 3-rhamnoside

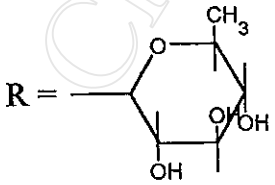
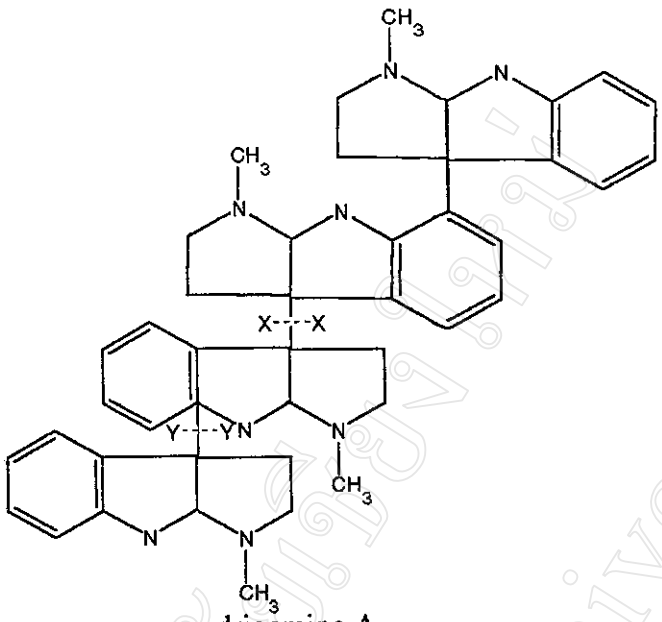


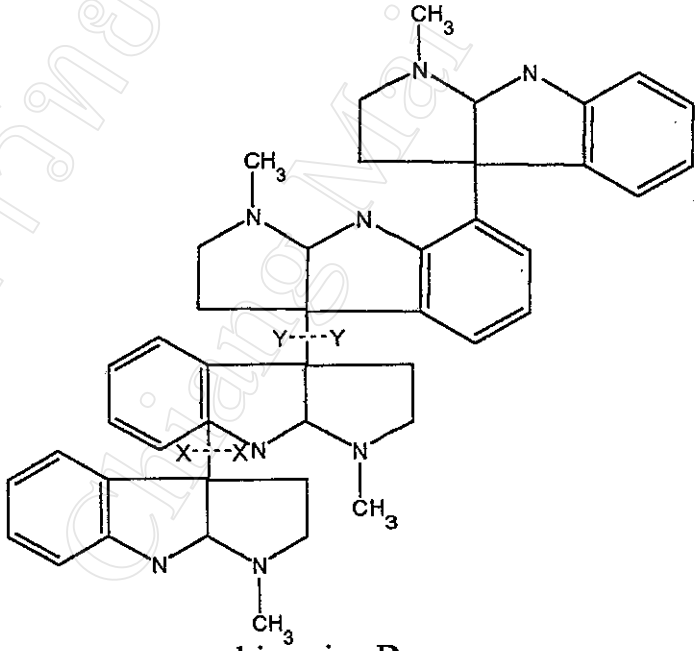
Figure 2. Structures of quinovic acid and quinovic acid 3-rhamnoside[18].

Roth *et al.* [20] studied leaf extracts of *Psychotria forsteriana* which yielded polyindoline alkaloids *i.e.*, quadrigemine A, quadrigemine B, psychotridine, and iso-psychotridine C. All compounds had cytotoxic activity against rat hepatoma cells with IC_{50} 5, 10, 2.5 and 5 μM, respectively. These activities were comparable with the standard compound leurocristine. The relative cytotoxic activity of these polyindoline compounds can be schematized as followed: psychotridine, isopsychotridine C, quadrigemine A, and quadrigemine B (Figure 3).

Erdemeier *et al.*[21] found that a 10% ammoniacal extract of *Nauclea orientalis* was active (IC_{50} of 9.5 μg/ml) against human bladder carcinoma T-24. The ammoniacal extract was purified with overpressure layer chromatography which gave 9 indole alkaloids *i.e.*, angustine, 18,19-dihydroangustine, nauclefine, angustoline, 10-hydroxyangustine, 3,14-dihydroangustine, 3,14,18,19-tetrahydroangustine, and two diastereoisomer of 3,14-dihydroangusline $[\alpha]_D$ -172.5 °, $[\alpha]_D$ -301.4°. These nine compounds inhibited human bladder carcinoma T-24 cell proliferation with IC_{50} of 3.3, 9.2, 4.7, 32.9, 3.4, 4.3, 5.0, 12.4 and 14.3 μg/ml, respectively (Figure 4).



quadrigemine A

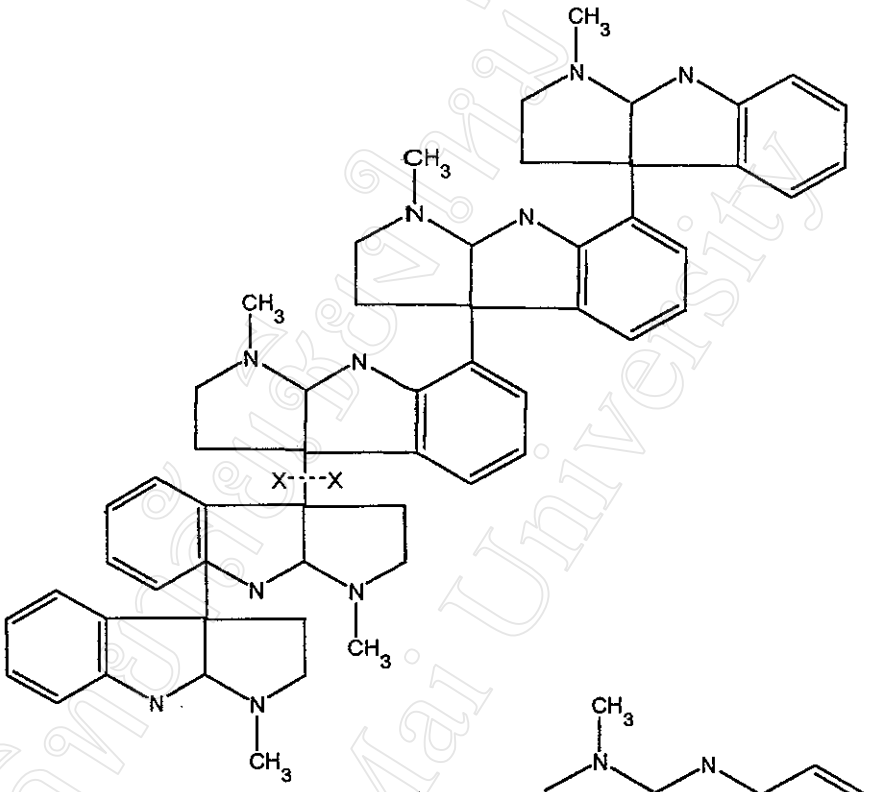


quadrigemine B

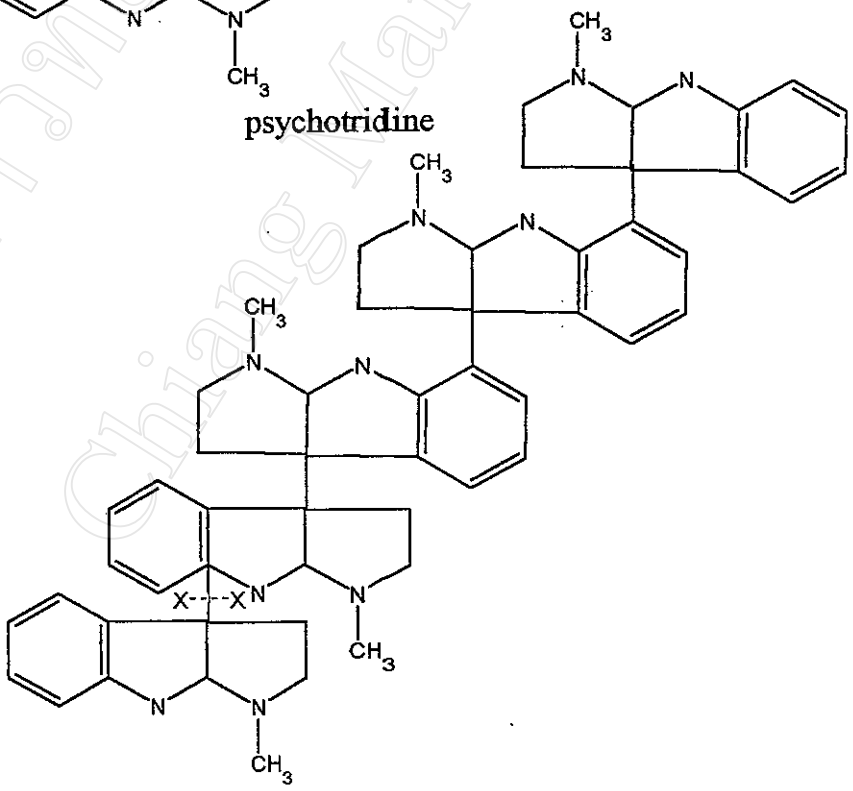
x----x example of β-β' type C-C bond

y----y example of β-phenyl type C-C bond

Figure 3. Polyindoline alkaloids isolated from leaves of *Psychotria forsteriana*[20].

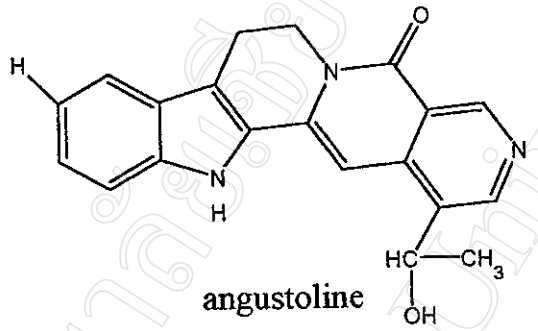


psychotridine

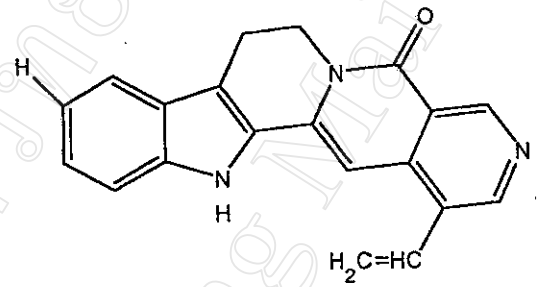


isopsychotridine C

Figure 3. Polyindoline alkaloids isolated from leaves of *Psychotria forsteriana*[20], cont.

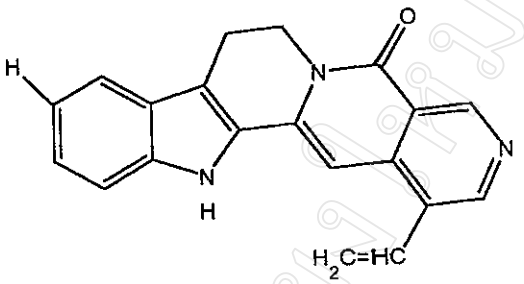


angustoline

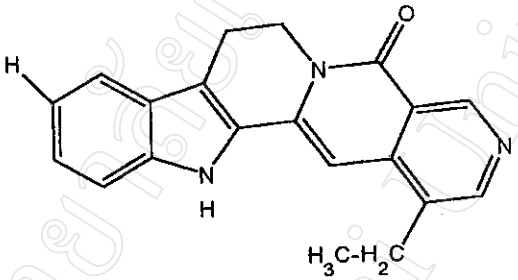


10-hydroxyangustine

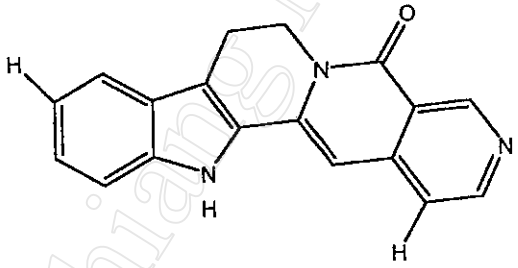
Figure 4. Alkaloids isolated from *Nauclea orientalis*[21].



angustine

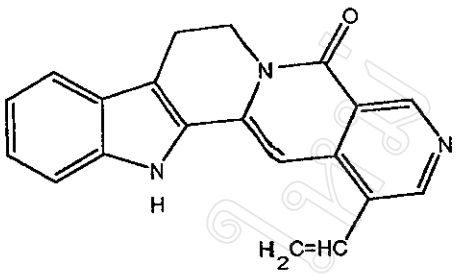


18,19-dihydroangustine

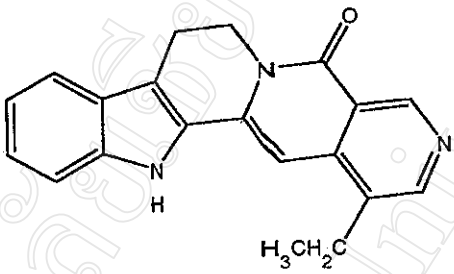


nauclefine

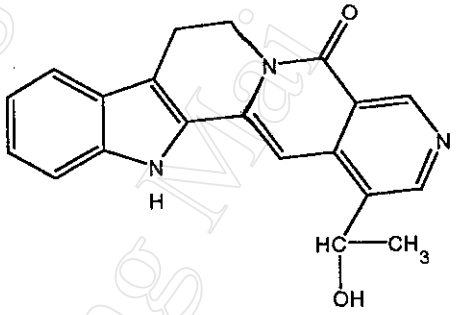
Figure 4. Alkaloids isolated from *Nauclea orientalis*[21], cont.



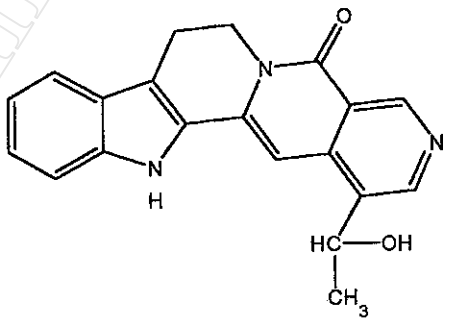
3,14-dihydroangustine



3,14,18,19-tetrahydroangustine



3,14-dihydroangustoline, $[\alpha]_D$: -172.5 ^{O}



3,14-dihydroangustoline, $[\alpha]_D$: -301.4^O

Figure 4. Alkaloids isolated from *Nauclea orientalis*[21], cont.

Silva *et al.*[22] used the leaves and stems of *Gardenia coronaria* to isolate 4 compounds *i.e.*, coronalolide methyl ester, coronalolide, coronalolic acid and methyl coronalolate acetate. The main chemical skeleton of these compounds was ring-A seco-cycloartane. Leaves of *Gardenia sootepensis* were processed and produced coronalolide methyl ester and coronalolide. Isolated compounds were tested for cytotoxic activity against hormone-dependent breast cancer cell line (ZR-75-1) and a glioma cell line (U373). Both coronalolide methyl ester and coronalolide were active against ZR-75-1 with ED_{50} of 0.6 μg/ml and against U373 with ED_{50} of 0.5 μg/ml, but coronalolic acid was inactive in these cell lines (Figure 5).

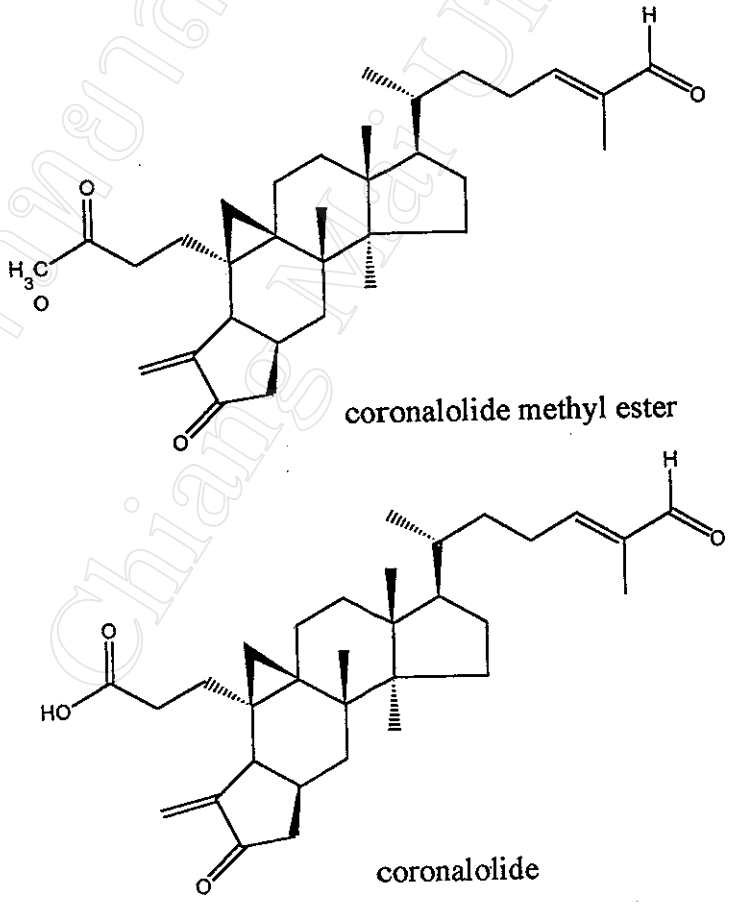
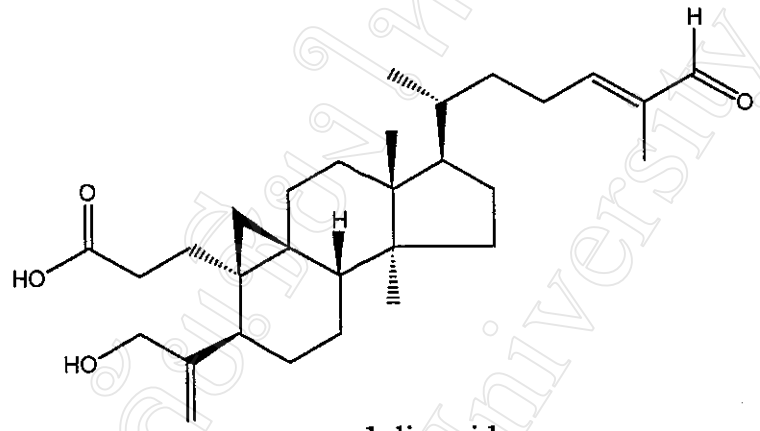
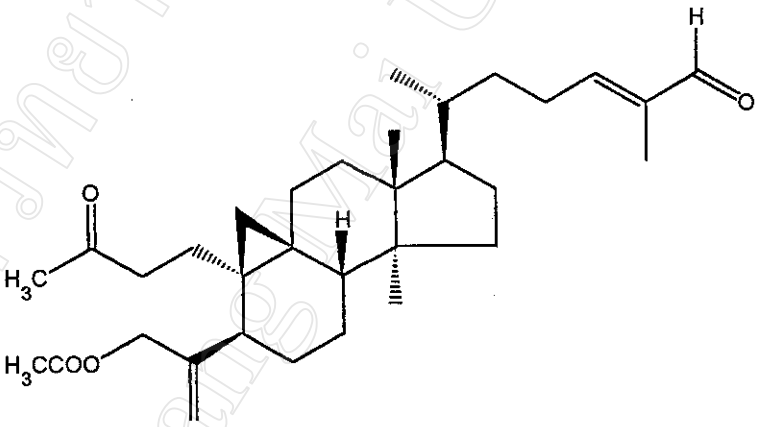


Figure 5. Triterpenes isolated from *Gardenia coronaria* and *G. sootepensis*[22]



coronalolic acid



coronalolate acetate

Figure 5. Triterpenes isolated from *Gardenia coronaria* and *G. sootepensis*[22], cont.

Cancer Research on Rubiaceae

Table 1. Biological Activity of Rubiaceae

Scientific name	Parts used	Biological activity	Reference
Anthocephalus chinensis	stem bark (India)	cytotoxic activity against CA-9KB cells by using ethanol – H_2O (1:1) extraction , ED_{50} > 20.0 μg/ml, inactive	23
Canthium glabrum	dried aerial parts (India)	cytotoxic activity against CA-nasopharyngeal cells by using ethanol – H_2O (1:1) extraction, inactive (concentration used not stated)	24
Gardenia carinata	bark, leaves, stem (Malaysia)	antitumor activity against sarcoma (YOSHIDA ASC) by intraperitoneal of methanol-H_2O extraction (1:1) with dose 1.0 gm/kg in rat, inactive	25
Gardenia gummifera	aerial parts (India)	cytotoxic activity against CA-9KB cells by using ethanol-H_2O (1:1) extraction, ED_{50} > 20.0 μg/ml, weak activity	26

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Gardenia jasminoides	commercial sample of fruits (Japan)	antitumor activity against sarcoma 180 (ASC) cells by intraperitoneal of ethanol (95%) or H_2O extraction with dose 100.0 mg/kg in mouse , inactive	27
		cytotoxic activity against HELA-S3 cells by using ethyl acetate extraction,IC_{50} 100.0 µg/ml, equivocal	28
	dried fruits (China)	cytotoxic activity against CA-JTC-26 or Cells-HE-1 by using concentration of Hot H_2O extraction 500.0 µg/ml, weak activity	29
	dried fruits (Japan)	cytotoxic activity against CA-EHRLICH-ascites by using concentration of acetone or ether or H_2O extraction 5.0% with cylinder plate method, weak activity	30
	dried fruits (South Korea)	antitumor activity against CA-EHRLICH-ascites or Leuk-SN36 or sarcoma 180 (ASC) cells by intraperitoneal of ethanol (defatted with pet ether) extraction with dose 500.0 mg/kg in mouse , inactive	27

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Gardenia jasminoides	dried fruits (South Korea)	cytotoxic activity against Leuk-L1210 cells by using benzene extraction,ED_{50} 7.4 µg/ml, active	31
	parts not specified (China)	antitumor activity against sarcoma 180 (solid)cells by intraperitoneal of polysaccharide fraction in mouse , active	32
	dried seed (China)	cytotoxic activity against CA-mammary-microalveolar cells by using concentration of H_2O extraction 250.0 µg/ml, weak activity	33
		cytotoxic activity against cells-human-embryonic HE-1 by using concentration of H_2O extraction 30.0 µg/ml, active	
Gardenia latifolia	aerial parts (India)	cytotoxic activity against CA-9KB cells by using ethanol-H_2O (1:1) extraction, ED_{50} > 20.0 µg/ml, inactive	34
Gardenia lucida	entire plant (India)	cytotoxic activity against CA-9KB cells by using ethanol-H_2O (1:1) extraction, ED_{50} > 20.0 µg/ml, inactive	34

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Gardinia turgida	fruits (India)	cytotoxic activity against CA-9KB cells by using Ethanol-H_2O (1:1) extraction, ED_{50} < 20.0 μg/ml, inactive	35
Hedyotis capitellata	leaves + stem (Malaysia)	antitumor activity against sarcoma (YOSHIDA ASC) cells by intraperitoneal of Methanol-H_2O extraction (1:1) with dose 1.0 gm/kg in rat , inactive	25
Hedyotis chrysotricha	dried parts not specified	antitumor activity against CA-EHRLICH-ascites cells by intraperitoneal of H_2O or methanol extraction with dose 150.0 gm/kg in mouse, inactive	36
Hedyotis diffusa	dried entire plant (Taiwan)	cytotoxic activity against sarcoma 180 (ASC) cells by using concentration of hexane extraction 0.59 mg/kg, weak activity	37
Ixora arborea	aerial parts (India)	cytotoxic activity against CA-9KB cells by using ethanol-H_2O (1:1) extraction, ED_{50} > 20.0 μg/ml, inactive	34
Ixora coccinea	stem (Puerto Rico)	cytotoxic activity against CA-9KB cells by using ethanol-H_2O (1:1) extraction, ED_{50} < 20.0 μg/ml, active	38

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Ixora javanica	dried flowers (India)	antitumor activity against CA-Ehrlich-ascites by subcutaneous of ethyl acetate extraction in mouse , active (dose not stated)	39
		antitumor activity against 67%ILS Lymphoma-Dalton's cells by subcutaneous of ethyl acetate extraction in mouse , active (dose not stated)	
		antitumor activity against Sarcoma 180 (ASC) cells by intragastric of petroleum benzene extraction with dose 200.0 mg/kg in mouse , active	40
		cytotoxic activity against Leuk-K562 cells by using ethyl acetate extraction, active (concentration used not stated)	39
		cytotoxic activity against Lymphoma-Dalton's cells by using ethyl acetate extraction, IC_{50} 121.0 µg/ml, active	

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Ixora javanica	dried flowers (India)	cytotoxic activity against CA-Ehrlich-ascite by using ethyl acetate extraction, IC_{50} 65.0 µg/ml, active	39
	dried flowers (India)	cytotoxic activity against Lymphocytes cells by using ethyl acetate extraction, IC_{50} 7.0 µg/ml, active	
	dried leaves (India)	cytotoxic activity against Lymphoma-Dalton's cells by using concentration ethanol- H_2O (1:1) extraction 10.0 µg/ml, active	41
		cytotoxic activity against Leuk-K562 cells by using concentration ethanol-H_2O (1:1) extraction 20.0 µg/ml, active	
		cytotoxic activity against CA-Ehrlich-Ascite by using concentration ethanol-H_2O (1:1) extraction 30.0 µg/ml, weak activity	

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Ixora javanica	dried leaves (India)	cytotoxic activity against Sarcoma 180 by using concentration ethanol-H_2O (1:1) extraction 30.0 µg/ml, weak activity	41
Ixora nigricans	aerial parts (India)	cytotoxic activity against CA-9KB by using concentration ethanol-H_2O (1:1) extraction 50.0 µg/ml, active	34
Ixora notaniana	dried aerial parts (India)	cytotoxic activity against CA-Nasopharyngeal by using ethanol-H_2O (1:1) extraction, inactive (concentration used not stated)	24
Ixora polyantha	dried aerial parts (India)	antitumor activity against Leuk-P388 cells by intraperitoneal of ethanol-H_2O (1:1) extraction with dose 250.0 mg/kg in mouse , inactive	47
		cytotoxic activity against CA-9KB by using concentration ethanol-H_2O (1:1) extraction 25.0 µg/ml, inactive	

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Morinda citrifolia	flowers (Malaysia)	antitumor activity against sarcoma (YOSHIDA ASC) cells by intraperitoneal of methanol - H_2O (1:1) extraction with dose 1.0 gm/kg in rat , inactive	25
	fresh fruits (Hawaii)	antitumor activity against 119%ILS CA-LLC cells by intraperitoneal of Juice with dose 15.0 mg/kg in mouse , strong activity	42
		antitumor activity against 40%ILS CA-LLC cells by intraperitoneal of Juice with dose 12.0 mg/kg in female mouse , active	
		antitumor activity against 119%ILS CA-LLC cells by intraperitoneal of Juice with dose 6.0 mg/kg in female mouse , active	42
	dried fruit juices (Hawaii)	antitumor activity against sarcoma 180 (ASC) cells by intraperitoneal of ethanol insoluble fraction with dose 500.0 mg/kg in mouse, active	43

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Morinda citrifolia	dried fruit juices (Hawaii)	antitumor activity against CA-Lewis lung cells by intraperitoneal of ethanol insoluble fraction in mouse (dose not state), active	43
	leaves (Malaysia)	antitumor activity against sarcoma (YOSHIDA ASC) cells by intraperitoneal of methanol - H_2O (1:1) extraction with dose 1.0 gm/kg in rat , inactive	25
	fresh leaves (Thailand)	cytotoxic activity against cells-RAJI by using concentration of methanol extraction 20.0 µg/ml, inactive	44
Morinda officinalis	dried roots (China)	cytotoxic activity against CA-mammary microalveolar cells by using concentration of H_2O extraction 500.0 µg/ml, inactive	33

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Morinda parvifolia	dried entire plant (Taiwan)	antitumor activity against Leuk-L1210 cells by intraperitoneal of ethanol (95%) extraction in mouse , active (dose not stated)	45
	dried rhizomes+ roots (Taiwan)	antitumor activity against 52% ILS Leuk-P388 cells by intraperitoneal of methanol extraction with dose 50.0 mg/kg in mouse , active	46
Morinda tinctoria	dried aerial parts (India)	cytotoxic activity against CA-9KB cells by using concentration of ethanol-H_2O (1:1) extraction 25.0 µg/ml, inactive	47
Mussaenda glabra	dried aerial parts (India)	cytotoxic activity against CA-Nasopharyngeal by using ethanol-H_2O (1:1) extraction, inactive (concentration used not stated)	24
	leaves (Malaysia)	antitumor activity against sarcoma (YOSHIDA ASC) cells by intraperitoneal of methanol - H_2O (1:1) extraction with dose 1.0 gm/kg in rat , equivocal	25

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Mussaenda glabra *var. roxburghii*	aerial parts (India)	antitumor activity against Leuk-P388 cells by intraperitoneal of ethanol-H_2O (1:1) extraction in mouse , inactive (dose not stated)	48
		cytotoxic activity against CA-9KB cells by using ethanol-H_2O (1:1) extraction, ED_{50} > 20 μg/ml, inactive	
	fruits (India)	antitumor activity against Leuk-P388 cells by intraperitoneal of ethanol-H_2O (1:1) extraction in mouse , inactive (dose not stated)	
		cytotoxic activity against CA-9KB cells by using ethanol-H_2O (1:1) extraction, ED_{50} > 20 μg/ml, inactive	

Table 1. (cont.)

Scientific name	Parts used	Biologicai activity	Reference
Nauclea latifolia	dried leaves (Guinea-Bissau)	cytotoxic activity against CA-9KB cells by using ethanol (100%) extraction, IC_{50} 19.7 µg/ml, active	49
		cytotoxic activity against CA-MDA-MB-231 cells by using ethanol (100%) extraction, IC_{50} 20.0 µg/ml, active	
		cytotoxic activity against Melanoma-B16F10 cells by using ethanol (100%) extraction, IC_{50} 29.9 µg/ml, active	
		cytotoxic activity against CA-SK-MEL-28 by using ethanol (100%) extraction, IC_{50} 32.8 µg/ml, active	
	dried roots (Guinea-Bissau)	cytotoxic activity against CA-9KB cells by using ethanol (100%) extraction, IC_{50} 20.0 µg/ml, active	

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Nauclea latifolia	dried roots (Guinea-Bissau)	cytotoxic activity against CA-A549 cells by using ethanol (100%) extraction, IC_{50} 20.0 µg/ml, active	49
		cytotoxic activity against CA-SK-MEL-28 cells by using ethanol (100%) extraction, IC_{50} 20.0 µg/ml, active	
		cytotoxic activity against CA-MDA-MB-231 cells by using ethanol (100%) extraction, IC_{50} 21.1 µg/ml, active	
	dried stem/ bark (Guinea-Bissau)	cytotoxic activity against CA-9KB cells by using ethanol (100%) extraction, IC_{50} 20.0 µg/ml, active	
		cytotoxic activity against CA-A549 cells by using ethanol (100%) extraction, IC_{50} 20.0 µg/ml, active	

Table 1. (cont.)

Scientific name	Parts used	Biological activity	Reference
Nauclea latifolia	dried stem/bark (Guinea-Bissau)	cytotoxic activity against CA-MDA-MB-231 cells by using ethanol (100%) extraction, IC_{50} 20.0 μg/ml, active	49
		cytotoxic activity against CA-SK-MEL-28 cells by using ethanol (100%) extraction, IC_{50} 20.0 μg/ml, active	
Uncaria macrophylla	dried aerial parts (India)	antitumor activity against LEUK-P388 cells by intraperitoneal of ethanol – H_2O (1:1) extraction with dose 250.0 mg/kg in mouse, inactive	50
		cytotoxic activity against CA-9KB cells by using concentration of ethanol – H_2O (1:1) extraction 25.0 μg/ml, inactive	

Rubiaceous Plants Used in This Research

1. Scientific name : *Uncaria macrophylla* Wall.[51, 52]

   Common name : เขาควายแม่หลูบ, ควายแม่หลูบ

   Botanical note : evergreen woody climber; blades dark green above, light green below

   Location : 500 m past Doi Sutep Temple, near the entrance to national park headquarter

   Properties : *stem* ; used for headache

   Compounds : Indole alkaloids : dihydrocorynantheine; corynoxine; corynoxine B; phynchophylline; rhynchophylline; iso-rhynchophylline

2. Scientific name : *Tarennoidea wallichii* Triv. & Sastre[51]

   Common name : เหล็กกี, เหล็กขี้ดิน

   Botanical note : evergreen sapling 2-3 m high, blades very dark green above, light green undernesth.

   Location : lower east side of Doi Sutep at Gu Kow Falls, Doi Sutep-Pui National Park

3. Scientific name : *Canthium glabrum* Blume[51]

   Common name : เขากวาง, ค่างเต้น, หูเสือ

   Botanical note : evergreen tree 6 m high, bark thin, very finely pustular-lenticellate, light brown, branchlets, infructescence axes, immature fruits dull green, blades dull dark green above, light green underneath

   Location : Doi Sutep National Park, south side, above Meo Doi Pui (Hmong) village

4. Scientific name : *Borreria alata* (Aubl.) DC.[51]

   Botanical note : erect, annual, weedy herb, stes, petioles, stipules, calyx light green, corollas white, blades green above, light green underneath, very common

   Location : Doi Sutep-Pui National Park, south side, Meo Doi Pui (Hmong) village

5. Scientific name : *Borreria laevis* (Lmk.) Griseb.[51]

   Common name : หญ้าเขมรเล็ก, กระดุมใบใหญ่, หญ้าเขมร

   Botanical note : erect, perennial weed, stems, branches dull green and turning dark purple, calyx green, corollas white, blades dark green above, dull light green underneath

   Location : Doi Sutep-Pui National Park , south side, Meo Doi Pui (Hmong) village

6. Scientific name : *Pavetta tomentosa* Roxb. ex Sm.[51]

   Common name : ข้าวสารป่า, เข็มแพะ

   Botanical note : deciduous treelet 50 cm high, blades dark green above, dull light green underneath

   Location : Doi Kunhtan National Park, near the canteen, below Yaw 1

7. Scientific name : *Paedaria pilifera* Hook. f.[51]

   Common name : ตดหมูตดหมา, หญ้าตดหมา

   Botanical note : deciduous, mostly trailing, vine, stems, petioles green, blades dark green above, light green underneath

   Location : Doi Sutep-Pui National Park, border of the southern side, summit of Doi Come

8. Scientific name : *Mitragyna hirsuta* Havil.[51, 53]

   Common name : กระทุ่มโคก, ตุ้มเขา

   Botanical note : deciduous tree 6 m. high, coppicing at the base, infructescences dry, blades dark green above, light green underneath

   Location : lower east side of Doi Sutep, Doi Sutep-Pui National Park, Gu Kow Falls area

   Properties : *bark* ; antihelmintic, *leaves*; emetic

   Compounds : ciliaphylline; specionoxeine

9. Scientific name : *Lasianthus kurzii* Hook. f.[51]

   Common name : ปัดมุก

   Botanical note : evergreen treelet 1-1.5 m. high, blades dull dark green above, dull light green underneath

   Location : Doi Sutep-Pui National Park, national park headquarters area, near Doi Sutep Temple

10. Scientific name : *Hymenodictyon oriexense* (Roxb.) Mabb.[51, 54]

    Common name : ส้มกบ, ส้มลุ, อุโลก

    Botanical note : deciduous sapling 50 cm. high, petioles dull green, older ones often with dull light violet, blades dull dark green above, pale light green below

    Location : Doi Khuntan National Park, near the canteen/bungalos, below Yaw 1

    Properties : *root* ; antipyretic, *heart wood* and *bark* ; antipyretic, *leaves*; diuretic, antiinflammation, analgesic, *stem*; diuretic

    Compounds : aesculin; alanine; 6-methylalizarin; anthragallol; arginine; cystine; damnacanthal; nordamnacanthal; galactose; glycine; hymexelsin; lucidin; morindone; rubiadin; rubiadin-1-methyl ether; scopoletin; soranjidiol; 2-benzyl-xanthopurpurin

Pharmacological activity : inhibited HIV-1 reverse transcriptase enzyme; decrease blood pressure; antibacterial

11. Scientific name : *Haldina cordifolia* (Roxb.) Ridsdale[51]

Common name : กระทุ่มขว้าว, กระทุ่มดง

Botanical note : deciduous tree 14 m. high, bark thickened, roughly cracked, gray, branching sympodial, peducles & petioles dull green, old styles brow, calyces light green, blades dull dark green above, dull light green below, stipules pale light green, petioles often with dark reddish

Location : Doi Sutep-Pui National Park, southern part, summit of Doi Come

12. Scientific name : *Psychotria ophioxyloides* Wall.[51]

Common name : ตาเป็ดใบยอ

Botanical note : evergreen treelet 1.5 m. high, inflorescence axes white, corollas yellow blades dark green above, light green underneath

Location : Doi Sutep-Pui National Park, national park headquarters area, near Doi Sutep Temple

13. Scientific name : *Mussaenda parva* Wall. ex G. Don[51]

Common name : แก้มนุ่ม

Botanical note : slender woody climber to scandent, enlarged calyx lobe white on both sides, corolla lobes orange inside, blades dull dark green above, dull light green underneath

Location : Doi Sutep-Pui National Park, park headquarters, near Doi Sutep Temple

14. Scientific name : *Ixora cibdela* Craib var. *puberula* Craib[51, 52]

Common name : เข็มดอย, เข็มป่า, เข็มตาไก่

Botanical note : evergreen treelet 1.5 m. high, corollas pink & white, blades dark green above, green underneath

Location : lower east side of Doi Sutep, Doi Sutep-Pui National Park, Gu Kow falls

Properties : *root;* antitussive, antituberculosis, *leaves*; antihelmint, *flower*; eyes treatment

15. Scientific name : *Gardenia obtusifolia* Roxb. ex Kurz[51]

Common name : กระมอบ, กระบอก, คำมอกน้อย

Botanical note : deciduous treelet 1.5 m. high, bark thin, smooth to shollowly cracked, grey, blades dark glossy green above, light green underneath

Location : Doi Sutep-Pui National Park, base of the east side, near TV channel 7 station

16. Scientific name : *Gardenia sootepensis* Hutch.[51, 52, 55]

Common name : คำมอกหลวง, คำมอกช้าง

Botanical note : deciduous tree 6 m. high, bark thin, very finely pustular-lenticellate, grey, branching sympodial, terminal buds with a glossy orange drop of sap, blades dark green above, light green underneath

Location : Doi Sutep-Pui National Park, lower east side, near Kow falls

Properties : seed; boil with water, use as shampoo to kill head lice

Compounds : iridoid monoterpenes : deacetylasperulosidic acid; methyl ester geniposide; geniposidic acid; scandoside methyl ester

benzenoids : benzoic acid; 4-hydroxy-3-5-dimethoxybenzoic acid; 4-hydroxy-3-methoxybenzoic acid

triterpenes : coronalolide; methyl ester coronalolide

flavones : 3'-5-7-trihydroxy-4'-5'-6-trimethoxy flavone; hispidulin; 4'-7-hydroxy flavone

sesquiterpenes : sootepdienone, steroid : β-sitosterol

Pharmacological activity : cytotoxic activity; against hormone-dependent breast cancer cell line (ZR-75-1) and against a glioma cell line (U373) by using coronalolide methyl ester and coronalolide, ED_{50} 0.6 and 0.5 μg/ml, respectively

17. Scientific name : *Ixora stricta* Roxb.[51]

Common name : เข็มแดง, เข็มญี่ปุ่น

Botanical note : evergreen woody climber; blades dark green above, light green below

Location : 500 m past Doi Sutep Temple, near the entrance to national park headquarter

18. Scientific name : *Gardenia jasminoides* Ellis[51, 56]

Common name : พุดซ้อน, เคดถา

Botanical note : shurb or small tree 2-3 m high, leaves simple; opposite, flowers white.

Location : Ban Num Lad, Chom tong, Chiang mai

Properties : *root* ; antipyretic, *leaves*; analgesic, *friut*; diuretic, *bark*; antipyretic, antispasmodic

Compounds : alkaloids : methyl anthranilate

iridoid monoterpenes: deacethyl-methyl asperulodidic acid; deacetyl asperulodidic acid; gardendiol; gardenoside; gardoside; garenoside; genipin; genipin gentiobioside *etc.*

benzenoids : benzoic acid methyl ester; benzoic acid; benzyl acetate; benzyl alcohol; 2-phenylethanol

monoterpenes : borneol; borneol-6-*O*-*β*-*D*-xylopyranosyl-*beta*-*D*-glucopyranoside *etc.*

sesquiterpenes : *α*-cadinene, farnesol

carotenoids : crocetin; all-*trans* crocetin-*β*-*D*-gentiobiosyl-*β*-*D*-glucosyl ester; all-*trans* crocetin-di (*β*-*D*-gentiobiosyl) ester; all-*trans* crocetin-mono (*β*-*D*-gentiobiosyl) ester; crocetin-mono (*β*-*D*-gentiobiosyl) ester; crocin *etc.*

triterpenes : gardenic acid; gardenoic acid B; gardenoic acid; oleanolic acid acetate; olenolid acid acetate

others : carbohydrates, lipids, flavonols, steroids *etc.*

19. Scientific name : *Gardenia erythroclada* Kurz[51, 56]

Common name : มะคังแดง, จิ้งก่าขาว

Botanical note : tree, 6-12 m. high, stem and branches reddish brown, leaves simple, opposite, elliptic or obovate, stipules interpetiolar.

Location : Mae Ping National Park, Li, Lamphun

Properties : *stem*; decoction; relief abdominal pain, improvement blood circulation; combine with *Smilax* spp., boil and drink to treat kidney disfunction with mucous, yellow or red urine.

stem bark; crush with small amount of water and topically apply to stop bleeding.

20. Scientific name : *Catunaregam spathulifolia* Triv.[51]

Common name : กะแทง, ระเวียง, หนามเค็ด

Botanical note : deciduous treelet 1.5 m high, most parts with straight thorns, blades dull dark green above, dull light green underneath

Location : Doi Sutep-Pui National Park, base of the east side, near TV channel 7 station

Assay Methods

Terminology

The exact meanings of some relevant terms are necessary for activity classification. These terms are defined by the United State National Cancer Institute[5, 57].

Anticancer refers to agents which inhibit tumor growth in human.

Antitumor or antineoplastic refers to agents which inhibit tumor growth *in vivo* or in animals.

Cytotoxic refers to agents which are toxic to cells *in vitro*. Cytotoxic agents can be classified into two types *i.e.*, cytostatic and cytocidal. Cytostatic compounds are agents which stop cell growth, while cytocidal compounds are agents which kill cells.

Role of Bioassays in Drug Discovery[5]

Bioassays play an important role in drug discovery. The NCI (USA) uses screening procedures involving prescreening, monitoring, secondary testing, and clinical trials for new chemical evaluation. Simple *in vitro* or *in vivo* prescreening were used to identify extracts with potential activity. Active compounds were screened against multiple cell lines *in vivo*. The extracts which were successful in this screening stage advanced to the monitoring stage and were isolated to obtain pure compounds. These pure compounds were tested again in the *in vivo* assays. Compounds with potential activity were advanced to secondary testing to determine whether they were suitable for clinical trial[57](Table 2).

Table 2. Comparison of *In vivo and In vitro* Assays.[57]

in vivo	*in vitro*
advantages - activity data	advantages - speed - inexpensive - sensitive - small sample size - uniform system
disadvantages - long turn around - expensive - less sensitive - large sample need	disadvantages - *in vitro* data only - activity may not correspond to *in vivo* activity

The processes of *in vitro* and *in vivo* can be divided into two types *i.e.*, cell-based and mechanism-based assays.

1. Cell-based assay[58]

   This assay measures the overall effect on the cell *i.e.*, chemical substances were tested for toxicity in tumor cells.

2. Mechanism-based assay[58]

   This assay measures only specific cell cycles *i.e.*, chemical substances can be inhibited topoisomerase I enzyme.

Cytotoxic Activity Assay

Many assay protocols have been used to quantify number of viable cells. Traditional assays are such as counting total viable cells by using a hemocytometer chamber, electronic particle counter, or colony counting. The need to process large numbers of samples have led to attempts to introduce assays which can be automated. The methods involved primary screening assays and specialized screening assays for cytotoxic activity.

Primary Screening Assays[58]

Method used in this step must be rapid, inexpensive, sensitive, reliable, and be able to identify a broad spectrum of activities. The brine shrimp lethality test, crown gall tumor bioassay, and starfish assay are examples.

Specialized Screening Assays[58]

Cell lines used in *in vitro* screening assays by the NCI involved 80 to 100 human cell lines from major tumors. The compounds showing various cytotoxic activity for a particular tumor are further studied by *in vivo* testing with the same tumor lines *i.e.*, murine P-388 leukemia cells, lewis lung carcinoma and colon-38.

Cytotoxic activity assays are used for cell proliferation or viability assay. These can be divided into four groups :[59]

- *Reproductive assay* can be used to determine the number of cells in a culture that are capable of forming colonies *in vitro*.
- *Permeability assay* involve staining damaged cells with a dye (for example, trypan blue) and counting viable cells that exclude the dye. Countings can be performed by using a hemocytometer.

- *Direct proliferation assay* uses DNA synthesis as an indicator of cell growth. These assays are performed using radioactive or nonradioactive nucleotide analogs.
- *Metabolic activity measurement* can be performed by adding dyes to cells. These dyes are converted by viable cells to different colors and the results are interpreted with detectors. Detectors can be subdivided into two groups *i.e.*, Ultraviolet-Visible (UV-Vis) spectrophotometer and spectrofluorometer.

In this research, metabolic activity was measured for viability of cells. The dye used in this research was (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, (MTT), which was analyzed with UV-Vis spectrophotometer.

Cancer Cell Lines

Cancer cell lines used in this research were breast carcinoma (MCF-7 or HTB-22) and cervix carcinoma (KB-3-1). MCF-7[63] carcinoma can be grown in a minimum essential medium Eagle of Earle's BSS and 2 mM L-glutamine, which consists of 1.5 g/L of sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 0.01 mg/ml of 90% bovine insulin, and 10% fetal bovine serum. MCF-7 was tested with cisplatin and detected by MTT assay. Cisplatin[64] was effective against MCF-7 cells with IC_{50} of 16.5 - 22.5 x 10^{-7} M. In addition, MCF-7 was tested with 5-fluorouracil[65], vinblastine[65], tamoxifen[65], and detected with sulforhodamine B, which exhibited IC_{50} values of 1.6672, 2.4660, 1.3200 µg/ml, respectively. KB-3-1[66] carcinoma can be grown in a similar medium as MCF-7 carcinoma. Vinblastine[67] is effective in this cell line with an IC_{50} of 3.31 ng/ml.

Anticancer Drugs

The chemical standards used in this study were 5-fluorouracil for MCF-7 cells and vinblastine for KB-3-1 cell lines.

5-Fluorouracil (5-FU)

The chemical name of 5-FU is 5-fluoro-2, 4(1*H*, 3*H*)-pyrimidinedione. The molecular formula is $C_4H_3FH_2O_2$, its molecular weight is 130.1[68,69] (Figure 7).

Characteristics: a white or almost white crystalline powder

Solubility [68,69]*:* sparingly soluble in water
slightly soluble in alcohol
practically insoluble in ether

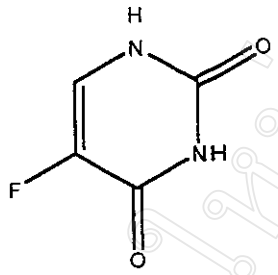


Figure 7. Structure of 5-Fluorouracil[69]

Mechanism of action: 5-Fluorouracil is a pyrimidine antimetabolite that interferes with DNA synthesis by blocking the methylation of deoxyuridylic acid. 5-Fluorouracil rapidly enters cells and is activated to the nucleotide level where 5-Fluorouracil is a powerful competitive inhibitor of thymidine synthetase, or is incorporated into RNA. The reduced folate cofactor is required for tight binding to occur between the 5-FdUMP and thymidine synthetase.[70]

Vinblastine sulphate

The chemical name of vinblastine sulphate is (3a*R*, 4*R*, 5*S*, 5a*R*, 13a*R*)-4-acetoxy-3a-ethyl-9-[(5*S*, 7*R*, 9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1, 4, 5, 6, 7, 8, 9, 10-octahydro-2*H*-3, 7-methanol-azacyclo-undecino [5,4-b]indol-9-yl]-5-hydroxy-8-methoxy-6-methyl-3a, 4, 5, 5a, 6, 11, 12, 13a-octahydro-1*H*-indolizino [8,1-cd]carbazole-5-carboxylate sulphate. The molecular formula is $C_{46}H_{58}N_4O_9.H_2SO_4$ and its molecular weight is 909.1 (Figure 8). Vinblastine is an anticancer alkaloid which is isolated from *Catheranthus roseus* (synonym; *Vinca rosea*), the periwinkle plant.[68,69]

Characteristics: a white or slightly yellowish crystalline powder, very hygroscopic

Solubility[68,69]: very slightly soluble in ethanol

insoluble in ether

one part is soluble in 10 parts of water

one part is soluble in 50 parts of chloroform

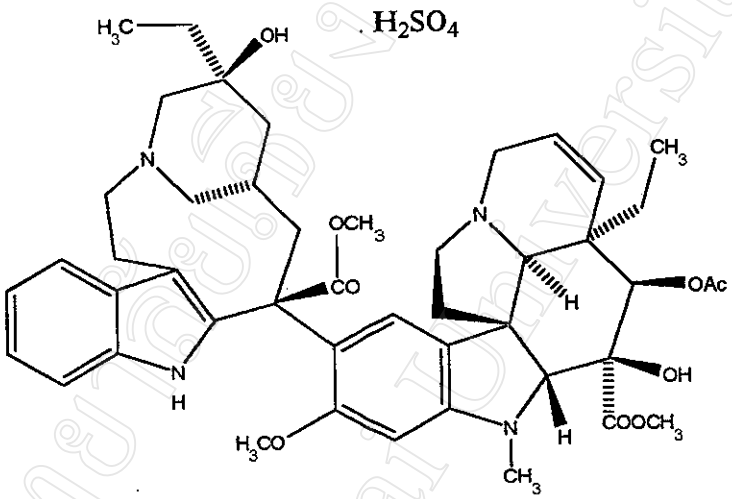


Figure 8. Structure of vinblastine sulphate[69]

Mechanism of action: Vinblastine binds to tubulin and inhibits microtubule formation, the resulting metaphase arrest causes disruption of mitotic spindles. Vinblastine may also interfere with nucleic acid and protein synthesis by blocking glutamic acid utilization. Vinblastine is specific for the M and S phases of the cell cycle[70].