CHAPTER 1

INTRODUCTION

1.1 Introduction

1.1.1 Rationale and background for investigation

Nowadays, AIDS is one of the top causes of death of people in the world. From data between 1984 to 2006 of Ministry of Public Health found that the total of AIDS patients in the Thailand were 297,507 and the total of the death from HIV of Thailand were 83,906 (Ministry of Public Health, 2006).

Acquired immunodeficiency syndrome (AIDS) has evolved rapidly in to an epidemic and worldwide public health crisis. Following the identification of this human retrovirus called human immunodeficiency virus type 1 (HIV-1), many researches have been carried out intensively to discover some active compounds as anti HIV-1 agents and it enzyme inhibitors. Three HIV-1 enzymes are essential for the life cycle of the virus (Figure 1-1). HIV-1 reverse transcriptase (RT) is crucial for viral replication. HIV-1 protease (PR) processes viral proteins in to functional enzymes and structural proteins, thereby, facilitating maturation and infectivity of virion particles, whereas HIV-1 integrase (IN) mediates the integration of the transcribed double strand DNA into the host genome (Ng *et al.,* 1997). For the first two enzymes, many synthetic inhibitors have been intensively used for AIDS treatments as a combination regimen or using alone. There are many available antiviral agents as HIV-1 PR inhibitors such as saquinavir (SQV), nelfinavir (NFV) and amprenavir (APV), while those of HIV-1 RT inhibitors are zidovudine (AZT), didanosine (DDI) and abacavir (1592U89) (Hirsch *et al.,* 1998). However HIV-1 integrase is interesting target for new anti-AIDS agent because it can integrate DNA of virus into host cell. Therefore, searching for HIV-1 IN inhibitors from natural sources and from synthetic compounds have been intensively carried out. The compounds such as flavones, caffeic acid phenyl esters (CAPE), quercetagetin (Fesen *et al.,* 1994), curcumin (Mazumder *et al.,* 1995), tyrphostins (Mazumder *et al.,* 1996),

CAPE amides, lignanolides (Cushman *et al.,* 1995), arylamides, hydrazides, napthoquinones (Fesen *et al.,* 1993), diaryl sulfones (Neamati *et al.,* 1997), chicoric acid (Lin *et al.,* 1999) and lamellarin α 20-sulfate (Reddy *et al.,* 1999) have been reported to show HIV-1 IN inhibitory effect. Surprisingly, there is no HIV-1 IN inhibitory substances from medicinal plants is promising in this regard, althought the multiplate integration assay is used as an appropriate method for screening a large number of samples on HIV-1 IN inhibitory activity.

Hau-Khao-Yen is a Thai medicinal plant which was found in many preparations of Thai traditional medicine textbooks (The Association of Traditional Medicine, 1952 and 1978; Pongboonrod, 1976; The palm leaf text studies program, 1982) and is an ingredient in as many as 2449 formulae (Division of Medical Research, 1986). These formulae are used to treat lymphopathy, dermopathy, venereal diseases, leprosy, cancer and AIDS as well as inflammatory conditions associated with diseases such as rheumatism, infectious diseases and other pain-causing conditions. Selective interviews of traditional doctors of Southern Thailand (Itharat *et al*, 1998) found that they used Hua-Khao-Yen as an ingredient in their drug formulae for HIV patients. The selective interview was found that it was used to treat HIV patients (26.1 %), blood disease and abscess (43.5 %). It was found that 5 species were called in the same name, such as *Dioscorea burmanica* Pierre ex. Prain & Burkill, *Dioscorea membranacea* Pierre ex. Prain & Burkill, *Smilax corbularia* Kunth, *Smilax glabra* Roxb. and *Pygmaeopremna herbacea* Roxb. They were tested on biological activity against HIV-1 integrase and HIV-1 protease and showed that *Smilax corbularia* was a best type for anti HIV-1 integrase. The ethanolic and water extract showed IC_{50}= 1.9 and 5.4 μg/ml respectively (Tewtrakul *et al.,* 2006). However, the active compounds in the extract have not been reported. Therefore the aim of this study were to investigate the active compounds for anti HIV-1 integrase and antioxidant activities from isolation of this plant extract.

1.1.2 HIV-1 integrase assay

HIV-1 integrase (IN) is becoming an interesting target for development of new anti-AIDS agents. Viral IN is the enzyme that integrates the viral reverse transcribed DNA into host-cell DNA. During viral infection, IN catalyzes the excision of the last two nucleotides from each

3′-end, leaving the terminal dinucleotide CA-3′-OH at the resessed 3′-ends (3′-processing). After transport to the nucleus as a nucleoprotein complex, IN catalyzes a DNA strand transfer reaction involving the nucleophilic attack at these ends on the host DNA, which is called stand transfer or joining (Fujiwara and Mizuuchi, 1988; Katz and Skalka, 1994; Vink and plasterk, 1993)

Recently, there are many reports on HIV-1 IN inhibitory assay using isotope-labelled substrate and denaturing gel separation of reaction products (Fesen *et al.,* 1994; Mazumder *et al.,* 1997, 1995; Neamati *et al.,* 1997; Reddy *et al.,* 1999; Burke *et al.,* 1995). These *in vitro* methods are referred as standard integration assays and give clear results. However, they are inconvenient and time consuming, especially when screening inhibitors from many samples. Lately, an assay for HIV-1 IN activity using DNA-coated plates has been reported in a few papers (Chang *et al.,* 1996; Hazuda *et al.,* 1994; Vink *et al.,* 1994). It is a non-radioisotopic technique and can be used for screening the inhibitory activity of plant extracts or any compounds against HIV-1 IN.

In this method, 96 well plates were used for the screening test called a multiplate integration assay (MIA). It is simple, convenient and accurate and doses not require the centrifugation, electrophoresis or other DNA denaturation steps. This assay screens for both 3′-processing and 3′-strand transfer and can be used without any exposure to radioisotopes. In this study, we therefore used this assay method for screening the HIV-1 IN inhibitory substances. MIA is the method to measure the incorporation of digoxigenin-labelled target DNA in to long terminal repeat (LTR) donor DNA. For this assay, a biotin-labelled donor DNA is added into each well, which strongly bind with a streptavidin coated-well plate, followed by addition of digoxiginin-labelled target DNA, integrase enzyme and sample solution. After integration process, the ligated two double-stranded DNA is immobilized on streptavidin-coated wells and subsequently bound with an alkaline phosphatase (AP)-labelled anti-digoxigenin antibody. Finally, it is colorized by adding p-nitrophenylphosphate as a substrate. In basic solution (pH 9.5), AP hydrolyzes *p*-nitrophenylphosphate to *p*-nitrophenol which exhibits a yellow color.

The screening of medicinal plants for HIV-1 IN inhibitory activity has been a promising approach to find compounds that interfere with retroviral replication, until now.

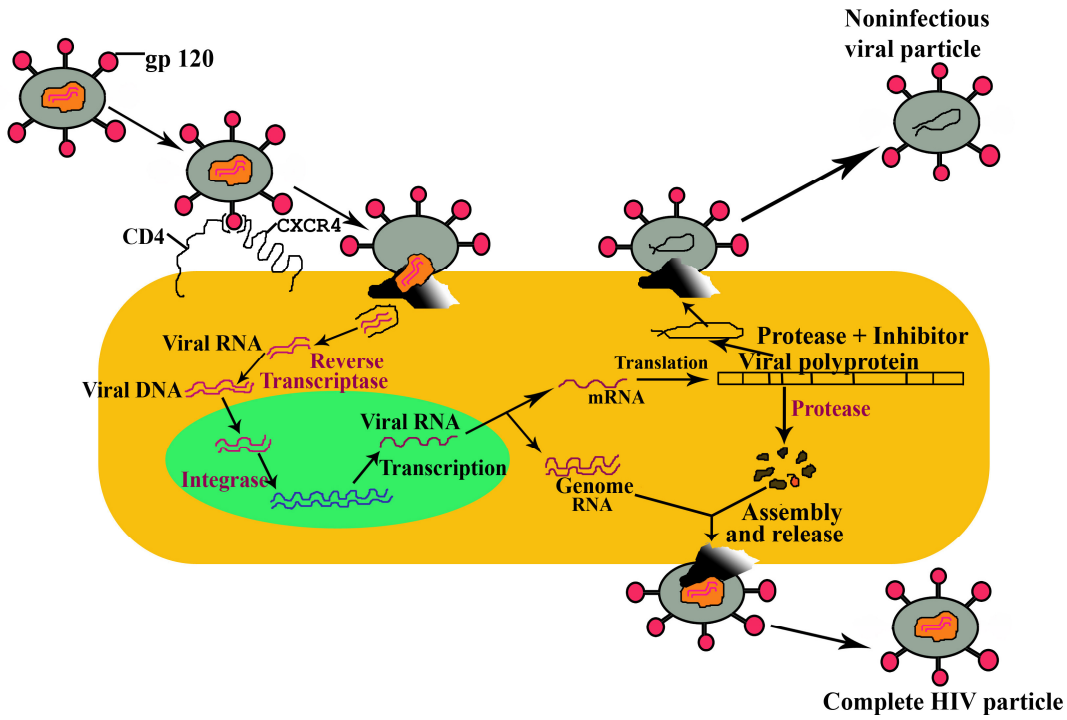


Figure 1-1 HIV-1 life cycle

1.1.3 Relative of Antioxidants with AIDS patients

In recent years the role of free radicals and reactive oxygen species (ROS) in human degenerative diseases of aging such as cancer, cardiovascular diseases, cataracts, brain dysfunction and immune system decline has become apparent. Free radicals or oxidants are energetically unstable and highly dangerous molecules which are constantly generated during body functions such as respiration, oxidative energy metabolism and immune activity. Free radicals are also produced from other sources (UV radiation, smoke, pollution, heavy metals, rancid fatty acid, etc.). Molecule oxygen is an oxidizing agent, that is it takes electron from another species (Halliwell and Gutteridge, 1999). The destructive effects of free radicals are far reaching, including cell membrane destruction via the interaction of fatty acid with oxygen to

from dangerous peroxides (lipid peroxidation); genetic damage via DNA mutation; decline in immune function; increased inflammatory conditions; growth and spread of cancers; oxidation of LDL cholesteraol leading to atherosclerosis hormone disruption, contributing to diabetes and other systemic disorders (Halliwell and Gutteridge, 1990; Benzie, 2000).

Oxygen is a double-edged sword: although we require oxygen to survive, certain oxygen species (such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen) are toxic to the body. In healthy aerobic organisms, production of reactive oxygen species is approximately balanced by the antioxidant defence system in the body. These endogenous antioxidants can protect from damage caused by these harmful molecules, as well as from free radicals mention above. The body has evolved its own natural free radical scavengers, which include the antioxidant vitamins (Vitamin A and beta-carotene, several of the B-complex vitamins, Vitamin C and Vitamin E), the mineral selenium and the antioxidant enzyme systems such as SOD (superoxide dismutase), glutathione peroxidase and catalase, which are the backbone of the cellular antioxidant defence system. Damage from free radicals can be prevented and even reversed if there are sufficient concentrations of antioxidants, which work individually and together in the body. However, the endogenous antioxidant system in body is not able to respond to a rapid increase in oxidative stress so the small exogenous antioxidant molecule such as α-tocopherol, β-carotene, ascorbic acid or antioxidants from plant foods can prevent effects damage from oxygen free radicals (Dreher and Junod, 1996, Thurnham, 1993).

Acquired immune deficiency syndrome (AIDS) is a clinical disorder caused by a retrovirus infection and represents the end point in a progressive sequence of immunosuppressive changes. Vitamins can enhance disease resistance in animals and humans. As such they are important co-factors in optimal functioning of the immune systems. The effects of murine and human retrovirus infection on vitamin status are analyzed as co-factors in the development of severe immune dysfunction, AIDS. The properties of immunoenhancing antioxidative vitamins, vitamin A, B_6, B_{12}, C, E, and □-carotene, which are frequently low in AIDS patients, are evaluated relative to the development of immunodeficiency during retrovirus infection. Vitamin A, E, and B_{12} deficiency accelerated the development of AIDS with low T cells, whereas their normalization retarded the development of immune dysfunction. The interactions between these vitamins and the immune system in human AIDS patients and animal models of AIDS are

reviewed. Our purpose is to provide data on how retrovirus infection can cause nutritional deficiencies that accentuate immune damage and to evaluate the potential therapeutic role of vitamins in the treatment of immune dysfunctions in AIDS patients. (Liang *et al,* 1999)

Antioxidants are in various forms. They are classified broadly in to two groups: antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase etc. and molecular antioxidants such as vitamin (C and E), mineral (selenium, zinc and manganese), carotenoids and flavonoids which are found in plants.

Therefore, the discovery of antioxidant compounds from plants is necessary especially the plants which was used as dietary food.

Table1-1 Thai medicinal plants for inhibition of HIV-1 IN (Tewtrakul *et al.,* 2003)

Botanical name	**Family**	**Part used**	**Extract**	**HIV-1 IN IC_{50} (μg/ml)**
Acacia consinna DC.	Mimosaceae	Leaf	Water	3.8± 0.4
Adhatoda vasica Nees	Acanthaceae	Leaf	Ethanol	12.0 ± 2.1
Andrographus paniculata	Acanthaceae	Leaf	Ethanol	12.0 ± 2.9
Wall ex. Ness			Water	1.5 ± 0.3
Baleria lupulina Lindl.	Acanthaceae	Leaf	Ethanol	10.0 ± 2.0
			Water	10.0 ± 1.8
Bixa orellana L.	Bixaceae	Leaf	Ethanol	2.2 ± 0.4
			Water	0.7 ± 0.1
Bixa orellana L.	Bixaceae	Seed	Ethanol	3.0 ± 0.6
			Water	0.3 ± 0.1
Calophyllum inophyllum L.	Guttiferea	Leaf	Ethanol	4.5 ± 0.8
			Water	4.0 ± 0.5
Cassia angustifolia Vahl	Caesalpiniaceae	Leaf	Ethanol	4.9 ± 1.4
Cassia fistula L.	Caesalpiniaceae	Fruit	Ethanol	10.0 ± 2.0
			Water	2.8 ± 0.5
Clinacanthus nutans Lindau	Acanthaceae	Leaf	Ethanol	2.8 ± 0.2
			Water	2.5 ± 0.3
Coleus parvifolius Benth.	Labiatae	Arial parts	Ethanol	9.2 ± 2.9
			Water	2.0 ± 0.6
Combretum quadrangulare	Combretaceae	Leaf	Ethanol	2.5 ± 0.2
Kurz			Water	2.9 ± 0.6
Croton sublyratus Kurz	Euphorbiaceae	Leaf	Ethanol	3.0 ± 0.4
Derris scandens Benth.	Papilionaceae	Leaf	Ethanol	3.9 ± 1.2

Hibiscus sabdariffa L.	Malvaceae	Flower	Water	1.4 ± 0.2
Lawsonia inermis L.	Lythraceae	Leaf	Ethanol	2.1 ± 0.4
			Water	3.3 ± 0.4

Table 1-1 (Continuted)

Botanical name	**Family**	**Part used**	**Extract**	**HIV-1 IN IC_{50} (μg/ml)**
Morinda citrifolia L.	Rubiaceae	Leaf	Ethanol	1.2 ± 0.3
			Water	6.0 ± 1.2
Myristica fragrans L.	Myristicaceae	Leaf	Ethanol	3.0 ± 0.4
			Water	2.3 ± 0.3
Ocimum basilicum L.	Labiatae	Leaf	Water	6.0 ± 2.0
Ocimum canum Sims	Labiatae	Leaf	Water	1.6 ± 0.3
Piper betle L.	Piperaceae	Leaf	Ethanol	4.0 ± 0.4
Piper nigrum L.	Piperaceae	Fruit	Water	8.0 ± 1.2
Piper ribesoides Wall. (A*)	Piperaceae	Stem	Water	0.9 ± 0.2
Piper ribesoides Wall. (A*)	Piperaceae	Leaf	Ethanol	0.6 ± 0.3
			Water	0.5 ± 0.1
Piper ribesoides Wall. (B**)	Piperaceae	Stem	Water	0.4 ± 0.2
Piper ribesoides Wall. (B**)	Piperaceae	Leaf	Ethanol	0.1 ± 0.2
			Water	4.1 ± 0.5
Piper sarmentosum Roxb.	Piperaceae	Leaf	Ethanol	1.2 ± 0.4
Plumbago indica L.	Plumbaginaceae	Leaf	Ethanol	6.0 ± 1.2
			Water	2.9 ± 0.4
Psidium guajava L.	Myrtaceae	Leaf	Ethanol	2.5 ± 0.5
			Water	1.7 ± 0.3

Quisqualis indica L.	Combretaceae	Leaf	Ethanol	2.0 ± 0.2
			Water	1.2 ± 0.2
Rhinacanthus nasutus Kurz	Acanthaceae	Leaf	Ethanol	0.8 ± 0.1
			Water	0.7 ± 0.1
Terminalia citrina Roxb.	Combretaceae	Fruit	Ethanol	2.7 ± 0.5
ex. Flemming			Water	0.3 ± 0.1
Theobroma cacao L.	Sterculiaceae	Leaf	Ethanol	8.0 ± 1.0
			Water	2.5 ± 0.6

Table 1-1 (Continuted)

Botanical name	**Family**	**Part used**	**Extract**	**HIV-1 IN IC_{50} (μg/ml)**
Thevetia peruviana Schum.	Apocynaceae	Leaf	Water	8.8 ± 1.0
Thunbergia laurifolia L.	Thunbergiaceae	Leaf	Ethanol	3.0 ± 0.4
			Water	2.8 ± 0.3
Tribulus terristris L.	Zygophyllaceae	Arial parts	Ethanol	8.0 ± 1.4
Zingiber officinale Roscoe	Zingiberaceae	Rhizome	Ethanol	4.0 ± 0.8
			Water	1.8 ± 0.3
Zingiber zerumbet Smith	Zingiberaceae	Rhizome	Water	2.8 ± 0.4

The results are the mean ± S.D (n=4)

*A= lanceolate shaped leaf and **B= cordate shaped leaf, IC_{50}= 50 % inhibitory concentration on HIV-1 Integrase

Table 1-2 Anti-HIV-1 integrase activity of compounds from the plants

Botanical name	**Compounds**	**HIV-1 IN [IC_{50} (μM)]**	**References**
Acer okamotoanum	Quercetin 3-*O*-(2″-gall-oyl)-α-L-arabinopyranoside	18.1 ± 1.3 μg/ml	Kim *et al.,* 1998
	Quercetin 3-*O*-(2″, 6″ -*O*-digalloyl)-β-D-galactopyranoside	24.2 ± 6.6 μg/ml	
Agastache rugosa	Rosmarinic acid	10 μg/ml	Kim *et al.,* 1999
Chrysanthemum - morifolium	Apigenin 7-*O*-β-D-(4″-caffeoyl) glucuronide	7.2 ± 3.4 μg/ml	Lee *et al.,* 2003
Coleus parvifolius	Luteolin 5-*O*-β-D-glucopyranoside	58.0 ± 8.2	Tewtrakul *et al.,* 2003
	Luteolin	11.0 ± 0.8	
	Luteolin 7-methyl ether	11.0 ± 1.5	
	Luteolin 5-*O*-β-D-glucuronide	20.0 ± 0.7	
	5-*O*-β-D-Glucopyranosyl-luteolin-7- methyl ether	70.0 ± 6.4	
	Rosmarinic acid	5.0 ± 0.9	
	Rosmarinic acid-methyl ether	3.1 ± 0.8	

Eclipta prostrata	Orobol	8.1 ± 0.5	Tewtrakul *et al.,*
	Wedelolactone	4.0 ± 0.2	2007
Lindera chunii	Hernandonine	16.3	Zhang *et al.,* 2002
	Laurolistine	7.7	
	7-Oxohernangerine	18.2	
	Lindechunine A	21.1	

Table 1-2 (Continuted)

Botanical name	**Compounds**	**HIV-1 IN [IC_{50} (μM)]**	**References**
Paeonia suffruticosa	(methanol extract)	15 μg/ml	Au *et al.,* 2001
Prunella vulgaris	(aqueous extract)	45 μg/ml	Au *et al.,* 2001
Salvia miltiorrhiza	Lithospermic acid	0.83	Abd-Elazem
	Lithospermic acid B	0.48	*et al.,* 2002
Thevetia peruviana	Quercetin	15	Tewtrakul *et al.,*
	Kaempferol	40	2002
	Kaempferol 3-*O*-[β-D- glucopyranosyl-(1→2) - [β-D- glucopyranoside]	59	
	Quercetin 3-*O*-[(6-*O*- sinapoyl)-β-D-glucopyranosyl -(1→2)-β-D- galactopyranoside	7	
	Kaempferol 3-*O*-[(6-*O*- sinapoyl)-β-D-glucopyranosyl -(1→2)-β-D- galactopyranoside	30	

	Quercetin 3-*O*-[(6-*O*-feruloyl)-β-D-glucopyranosyl-(1⟶2)-β-D-galactopyranoside	5
	Kaempferol 3-*O*-[(6-*O*-feruloyl)-β-D-glucopyranosyl-(1⟶2)-β-D-galactopyranoside	31

Table 1-2 (Continuted)

Botanical name	**Compounds**	**HIV-1 IN [IC_{50} (μM)]**	**References**
Thevetia peruviana	Quercetin 3-*O*-[β-D-glucopyranosyl-(1⟶2)-[β-D- glucopyranoside]	45	Tewtrakul *et al.,* 2002
	Kaempferol 3-*O*-[β-D-glucopyranosyl-(1⟶2)-[α-L-rha-monopyranosyl-(1⟶6)]-β-D-galactopyranoside	43	

1.2 Review of Literatures

1.2.1 Description and literature search of *Smilax corbularia* Kunth

Smilax corbularia (Smilacaceae), its synonym are *S. hypoglauca* Benth, *S. corbularia* Kunth var. *hypogauca* (Benth) T. Koyama, *S. Peguana* A.DC., *S. balansaeana* H.Bon ex Gagnep., *S. pseudochina* Lour. It is Thai vernacular names are Hua Khao Yen Wok, Hua Khao Yen Nuea. It is rather widely distributed in south-eastern Asia from Southern China and upper Burma through Thailand and Indonesia southwards to Malay Peninsula and south eastwards to Borneo (Koyama, 1975). It is found in tropical evergreen and lower mountain forests from sea level to ca. 2000 m. In Thailand, the rhizome is used to treat venereal disease (Perry, 1980) and cancer (Vimonkunakorn, 1979 and Pornprasert *et al.,* 1986).

The description of *S. corbularia* Kunth which is shown in Figure 1-2 is climber 2 to 4 m long; with woody stem, rather densely branched; branches straightish, internodes 3-10 cm long. Leaves are highly variable in shape and thickness; blades elliptic, 3-10 cm long, 1.5-5 cm wide, cuneate, rounded or shallowly cordate at base, the apex acuminate tip, coriaceous, fresh-green and shiny on upper surface, strongly glaucous and more or less white-powdery beneath, petioles short, 7-15 mm long, tendrils developing only on sterile branches and stems. Flowering branches 5-20 cm long, upper leaves reduced to bracts. Umbels with peduncles 5-12 mm long, staminate umblels 10 to 40 flowered, pistillate 8 to 20 flowered. Staminate perianth reddish, tepals free. Stamen 6, nearly sessile; anther elliptic, 1.3 mm long. Pistillate perianth greenish to yellowish, 1.5-2 mm long; tepals oblique ovary ellipsoid, contracted at apex, 2 mm long, 1.5 mm wide, capped with 3-lobed stigma. Staminodes 3, needle-like, 1.25 mm long. Berries globose, 6-8 mm across, purplish-black, 1-to-3 seeded. (Koyama, 1975)

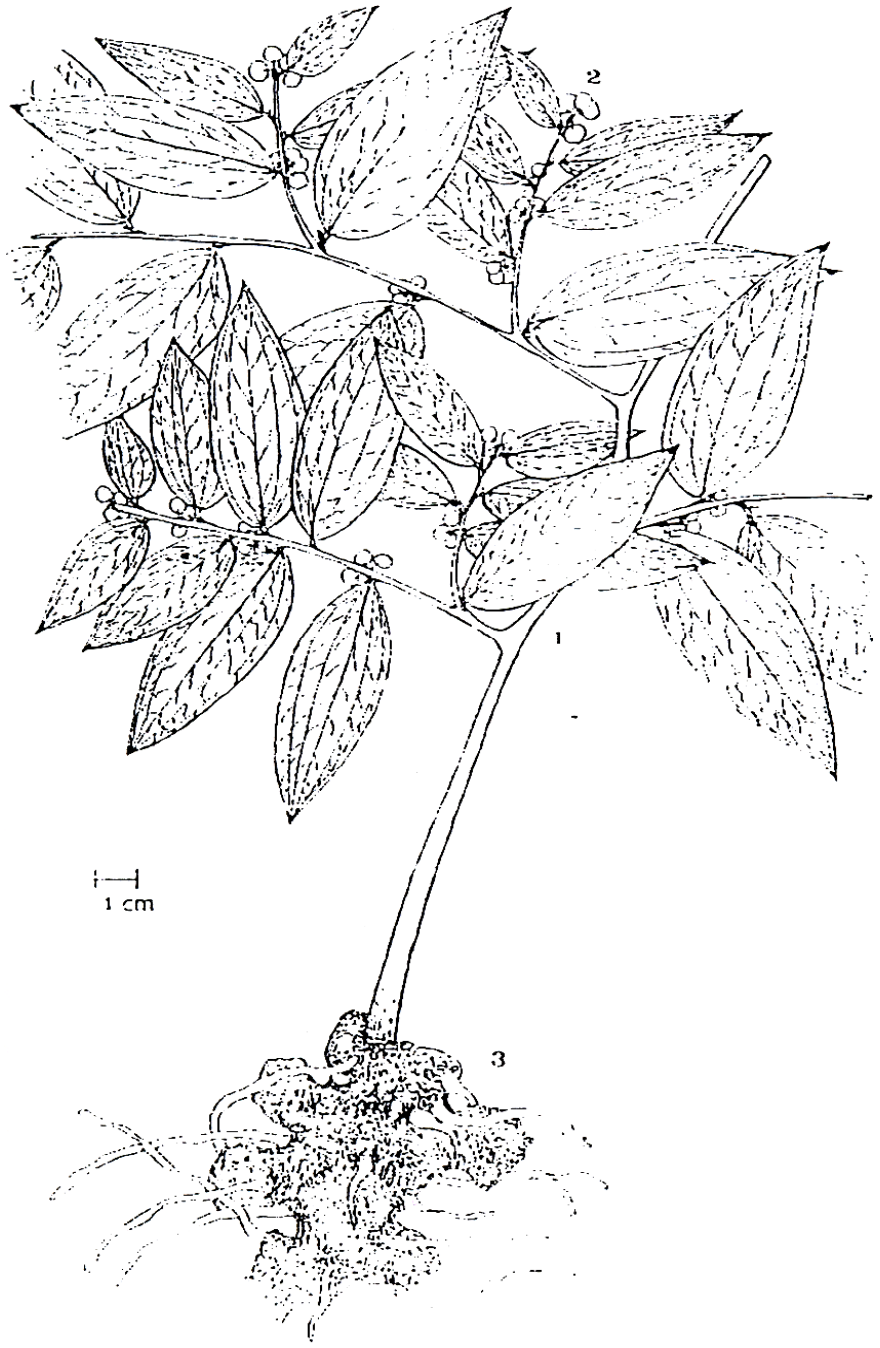


Figure 1-2 *Smilax corbularia* Kunth; 1. branch; 2. fruit; 3. rhizome

(From Booyarattanakornkit and Chantaptavan, 1993)

1.2.2 General data of the Genus *Smilax*

The genus *Smilax* is composed of 350 species, which are widely distributed in the tropical and temperate zones throughout the world and especially in tropical regions of East Asia, South and North America, such as *Smilax corbularia, Smilax china, Smilax glyciphylla, Smilax glabra, Smilax macrophylla, Smilax medica and Smilax bracteata* (Bernardo *et al.,* 1996).

Smilax, a large genus of climbers of the family Smilacacea and the plants in this genus were mostly woody climbers with reticilate-veined broad leaves and differ from Liliaceae in having true vessels in the conducting tissue of leaves. The possession of such vessels in leaves has so far not been seen in the order Monocotyledons and became this character in association with usually dioecious flowers it is suggested that a new family named Smilacaceae should be informed (Koyama, 1981)

The rhizomes or tuberous root of several species of the genus *Smilax* are called Radix Sarsaparilla (Chadha *et al*., 1972). Sarsaparilla (USP 1920 to 1955, NF 1955 to 1965) is the dried root of the following *Smilax* species (Clause and Tyler, 1965 and Osol *et al.,* 1950)

Smilax aristolochiaefolia Miller, known in commerce as Mexican, Vera Cruz, Tampico or Gray Sarsaparilla.

S. regelii Killip et Morton known in commerce as Hondurus or Brown Sarsaparilla.

S. febrifuga Kunth known in commerce as Ecuadorian Sarsaparilla.

S. ornate Hook.f. known as camaica, Costa Rica known as central American or Red Sarsaparilla.

The rhizome of *Smilax china* Linn., a native of China and Japan, has been employed under the name of China root for similar purposes with official sarsaparilla. *Smilax glabra* Roxb. has been official in the chinese Pharmacopoiea (1988).

Sarsaparilla was used in the treatment of syphilis and skin diseases (Chadha *et al.,* 1972). The chief constituents are sarsasaponin, smilagenin and other phytosterols (β-

sitosterol, stigmasterol), parillin and smilacin, previously reported as glycosides (Clause and Tyler, 1965).

1.2.3 *Smilax* species in Thailand

The Smilacaceae in Thailand were surrveryed by Tetsue Koyama (Koyama, 1981) and proposed to include two genera *Smilax* (24 species and 8 subspecies) and *Heterosmila* (3 species). The recorded vernacular names of *Smilax* (Smithinand, 2001 and Koyama, 1981) and uses are as follows (Table 1-3).

Figure 1-3 *Smilax corbularia* Kunth leaves and flowers

Figure 1-4 *Smilax corbularia* Kunth

Table1-3: *Smilax* species in Thailand, their vernacular names and uses

***Smilax* species**	**Vernacular name**	**Uses**	**References**
S. biumbellata T. Koyama	Khueang bai lai เขืองใบลาย	-	-
S. blummei A.DC.	Kram Chang กรามช้าง	Root used for anti-diarrhea and sore throat in Northern Thailand	Chuwsakul, 1997
S. bracteata Presl ssp.	Khueang Lueng เขืองเหลือง	Root was used to treat post partum haemorrhage as a depurative, as emmenagogue, antisyphilitic and uterine tonic in Phillipines	Perry, 1980
S. calophylla A.DC.	Hua Ya Kum Lung หัวยากำลัง	Root was used as aphrodisiac, treat gonorrhea and	Burkill, 1996

		tonic in Sumatrana and Malay Peninsular	

Table1-3 (continued)

Smilax species	Vernacular name	Uses	References
S. china Linn.	Khao-Yen Nuea ข้าวเย็นเหนือ	Root was used for syphilis, ganorrhea and as a tonic in Malay Penisular, as aphrodisiac stimulant in India	Kirtikar *et al.,* 1981
		Rhizome used as aphrodisiac and antidote against mercurial poisoning in Eastern Asia	Perry, 1980
S. china Linn.	Khao-Yen Nuea ข้าวเย็นเหนือ	Root was used for treatment of cancer in Thailand	Wasuwat, 1967
S. corbularia Kunth ssp. corbularia	Hua-khao-yen-nuea	Rhizome used to be remedy for treating	Pornsiriprasert 1986

	หัวข้าวเย็นเหนือ	cancer	
S. corbularia Kunth ssp Synandra (Gagnep.)	Khao-Yen-Loei ข้าวเย็นเลย	-	-
S. davidiana A.DC.	Khueang Thon เขืองโทน	-	-

Table 1-3 (continued)

***Smilax* species**	**Vernacular name**	**Uses**	**References**
S. extensa Wall.ex A.DC.	Khueang Phangnga เขืองพังงา	-	-
S. glabra Roxb.	Yahua, Khaoyentai ยาหัว หัวข้าวเย็นใต้	Rhizome used as a tonic	Chien and Adam, 1979
		antirheumatic in Vietnam	
		Root used for veneral disease and sores in India	Kapur, 1983
		Rhizome was used for chronic skin disease and syphilis in China	Fukunaga *et al.,* 1997

S. griffithi A.DC.	Khaoyennuan หัวข้าวเย็นนวล	-	-
S. lancifolia Roxb.	Thaoyangdong (South-easthern) เถายั้งดง Dao, Namdao, (Northern) เดา หนามเดา	Root was used for rheumatism and rheumatic pain as a poultice over the affect part	Chadha, 1972

Table 1-3 (continued)

***Smilax* species**	**Vernacular name**	**Uses**	**References**
S. luzonensis Presl	Khuang (Eastern) -Yanthat, Falaep (Peninsular) เขือง ย่านทาด ฟ้าแลบ	-	-
S. megacarpa A.DC.	Khueang Luk Daeng เขืองลูกแดง	Root was used as galactogogue and for childbirth in Indo-China	Perry, 1980
S. microchina T. Koyama	Hua Khaoyen หัวข้าวเย็น	-	-

S. microphylla C.H. Wright ssp. microphylla	-	Root was used for galactogogue and for childbirth in Indo-China	Perry, 1980
S. microphylla C.H. Wright ssp *elongata* (Warb.) T. Koyama	Khueang Phu เขืองภู	-	-
S. inversa T. Koyama	Yan Khot ย่านคด	-	-

Table1-3 (continued)

***Smilax* species**	**Vernacular name**	**Uses**	**References**
S. myosotifolia A.DC.	Khao-Yen Bai Bang ข้าวเย็นใบบาง	Rhizome, leaves and fruit was used for aphrodisiac and as remedy for syphilis on Malay	Burkill, 1966
S. ovalifolia Roxb.	Thaowan yang เถาวัลย์ยัง	Rhizome was use as a substitute for sarsaparilla in India and for treating venereal diseases on Malay Penisular	Perry, 1980

S. perfoliata Lour	Kamlungkhwaithuk (Peninsular) กำลังควายถึก	Fresh leaves used for menorhagia in India	Nagaraju and Rao, 1990
		Root was used for treatment venereal disease in India	Chadha, 1972
S. pottingeri Prian	Khueang Haeng Tomdum เขืองแห้งต้นดำ	-	-

Table1-3 (continued)

***Smilax* species**	**Vernacular name**	**Uses**	**References**
S. rigida Kunth ssp. *rigida*	-	-	-
S. rigida Kunth ssp *myrtillus* (A.DC.) T. Koyama	Khueng Bai Phum เขืองใบพุ่ม	-	-
S. siamensis T. Koyama	Khueng Sayam เขืองสยาม	Root used as decoction for body pain in	Anderson, 1986

		Thailand	
S. verticalis Gagnep	Khruadao (Northern) เครือเดา	-	-
S. zeylanica Linn.	Khueang Phuang Klom เขืองพวงกลม	Root was used for treatment of venereal disease and skin diseases In India, for gonorrhoea and other discharges from mucous membrane in Nepal	Kirtikar *et al.,* 1981

Table1-3 (continued)

***Smilax* species**	**Vernacular name**	**Uses**	**References**
S. zeylanica Linn. ssp.	Khueang Phuang Klom เขืองพวงกลม	Root used to enhance fertility in India	Girach *et al.,* 1994
S. zeylanica Linn. ssp. *hemsleyana* T. Koyama	Khueang Phuang Klom Doi เขืองพวงกลมดอย	-	-

1.3 Biological activity of *Smilax* species

The investigation of biological activities of *Smilax* species summarized in Table 1-5 were found that almost results of studying are cytotoxic activity, and anti-inflammatory activity.

The dried rhizome of *Smilax corbularia* Kunth which was identified to be one of Hua-Khao-Yen is used in Thai traditional formulae against cancer by a decoction made with four other plants species (*Polygala chinensis, Ludwigia hyssopifolia, Cinnacanthus siamensis,* and *Canna indica*). This preparation was tested for cytotoxicity againt CA-9KB cells and in the rat. Results showed that this preparation was inactive against KB cell (*in vitro*) but active with *in vivo* test by IP injection (Pornsiriprasart *et al*, 1986). This preparation showed immunostimulant activity and cytotoxic activity against K562 erythroleukemic cells. It also increased the release of tumor necrosis factor-alpha from monocyte/macrophages (Vongsakul and Ketsaard, 1995).

Studied on anti-HIV-1 integrase of plant in *Smilax* species were shown in Table 1-4. It was found that the ethanolic extract of *Smilax corbularia* exhibited the highest activity against HIV-1 IN (IC_{50}= 1.9 μg/ml) (Tewtrakul *et al.,* 2006).

Studies on anti-HIV and related activities of *Smilax* species such as anti-inflammatory, antihepatotoxic, antiallergic, antianalphylactic, antibacterial activities are shown in Table 1-5.

Table 1-4 Anti-HIV-1 integrase activity of different species of Hua-Khao-Yen (Tewtrakul *et al.,* 2006).

Botanical name	Family	Extract	IC_{50} μg/ml)±S.D. HIV-1 IN
Dioscorea burmanica Pierre ex. Prain & Burkill	Dioscoreaceae	Water	4.5 ± 0.8
Dioscorea burmanica		Ethanol	4.7 ± 0.4
Dioscorea membranacea Pierre ex. Prain & Burkill	Dioscoreaceae	Water	>100
Dioscorea membranacea		Ethanol	>100
Smilax corbularia Kunth	Smilacaceae	Water	5.4 ± 0.5
Smilax corbularia		Ethanol	1.9 ± 0.2
Smilax glabra Roxb.	Smilacaceae	Water	8.5 ± 0.8
Smilax glabra		Ethanol	6.7 ± 0.4
Pygmaeopremna herbacea Roxb.	Verbenaceae	Water	>100
Pygmaeopremna herbacea		Ethanol	>100
Suramin (positive control for HIV-1 IN)			3.4 ± 0.1

The results are IC_{50} ± S.D., n=4 for HIV-1 IN inhibitory activity

The results indicated that the ethanolic (EtOH) extract of *Smilax corbularia* possessed the most potent inhibitory activity against HIV-1 IN with an IC_{50} value of 1.9 μg/ml, the water extract of *Smilax corbularia* (IC_{50} = 5.4 μg/ml), the ethaanolic extract of *Smilax glabra* (IC_{50} = 6.7 μg/ml) and the water extract of *Smilax glabra* (IC_{50} = 8.5 μg/ml). The ethanolic extract of *Smilax corbularia* exhibited the highest anti-HIV-1 IN activity with IC_{50} value (1.9 μg/ml) approximately two-fold higher activity than suramin (IC_{50} = 3.4 μg/ml), a positive control.

Table 1-5 *Smilax* species and biological activity

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
S. anceps	Dried aerial part	Antibacterial	Methanolic extract weakly active against *Staphyllococcus aureas, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* but inactive against *Candida albicans*	Jalagur *et al.,* 1998
S. aspera	Aerial part	Cytotoxic	EtOH:Water (1:1) extract inactive against CA-9 KB cell line (IC_{50} >20 μg/ml)	Bhakuni *et al.,* 1971
	Dried leaves	Antioxidant	Hexane extract showed antioxidant activity but methanolic extract showed weak activity	Chevolleau *et al.,* 1992
*S. china**	Dried rhizome	Antitumor	Ethanolic extract (defatted with petroleum ether) inactive CA-Ehrlich-Ascites, Sarcoma 180 and Leuk-SN3 at dose 250 mg/ml (IP mouse)	Woo *et al.,* 1977

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
*S. china**	Dried rhizome	Cytotoxic	Ethanolic extract (defatted with petroleum ether) against Hela cell (IC_{50}= 33 μg/ml)	Woo *et al.,* 1977
	Dried entire	Antitumor	Water extract inactive with CA-Enrich Ascites at dose 150 mg/kg (IP mouse)	Kosuge *et al.,* 1985
	Dried root	Cytotoxic	Chloroform extract inactive against Hela and human SNU-1cell (IC_{50}> 30 μg/ml)	Park *et al.,* 1993
	Dried stem	Cytotoxic	Water extract inactive against CA-mammary micro alveolar and weakly active against cell human embryonic HE-1	Sato, 1989

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
S. china*	Dried leaves	Cytotoxic	Methanolic extract inactive against CA-9KB cell line at concentration 50 μg/ml	Arisawa, 1994
	Dried rhizome	Cytotoxic	Water extract showed weak activity against HE-1 at concentration 50 μg/ml and active against CA-JCT-26 cell line at concentration 120 μg/ml	Sato, 1990
	Dried stem	Cytotoxic	EtOH extract of *S. china* exhibited cytotoxicity against the KB, Hela and DLD-1 cell lines	Kuo, *et al.,* 2005

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
*S. corbularia**	Dried root	Antitumor	Water extract active *in vivo* against tumor in rat (IP)	Pornsiriprasert *et al.,* 1986
	Dried root	Immuno-stimulant activity	A water extract of Thai remedy which had this plant component on the effect or cells involved in cancer immunity, natural killer (NK) cell and monocyte/macrophages, was studied in 13 breast cancer patients. Treatment of the patients with the extract for 2 week significantly increased NK cell. It also increased the release of tumor necrosis factor	Vongsakul and Ketsaard, 1995

			from monocyte/macrophages (dose 0.5 l/day in female). The extract showed cytotoxic activity against K 562 erythroleukemic cells	

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
*S. corbularia**	Dried root	Cytotoxic	Water extract weak activity against CA-KB	Pornsiriprasert *et al.,* 1986
	Dried rhizome	HIV-1 integrase and protease	Ethanolic and water extract showed high inhibit HIV-1 integrase (IC_{50}= 1.9 and 5.4 μg/ml respectively)	Tewtrakul *et al.,* 2006
*S. glabra**	Dried rhizome	Psoriasis treatment	Water extract of plant mixture (*smilax graba* and 7 plants) was used in 108 human subjects	Zhu *et al.,* 1981

			with psoriasis. The result showed effectiveness after 3-4 weeks of administration	

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
*S. glabra**	Dried root	Prostaglandin synthetase inhibition	Hot water extract showed active at 750 μg/ml	Kiuchi *et al.*, 1983
	Dried rhizome	Antiallergic	Decoction of multi-component preparation which contained this plant showed activity by given oral route in rat	Kakimoto *et al.*, 1984

	Dried root	Antitumor	Water extract and methanolic extract inactive against CA-Ehrich-Ascites at dose 150 mg/kg (IP mouse)	Kosuge *et al.*, 1985

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
*S. glabra**	Dried rhizome	Anti-inflammatory	Decoction of multi-component preparation of this plant showed activity when given by oral route in rat by adjuvant induced arthritis but inactive with carrageenan-induce pedal edema and mustard-induced swelling	Kakimoto *et al.*, 1984

	Dried root	Cytotoxic	Water extract and methanolic extract inactive against Hela cell at concentration 0.1 mg/ml	Kosuge *et al.,* 1985
	Tuber	Antioxidant	Methanolic extract inactive at concentration 50 μg/ml	Kim *et al,* 1994

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
*S. glabra**	Dried rhizome	Antibacterial	Hot water extract active against *Klebsiella pneumoniae* (MIC 1.6 mg/ml) but inactive against *Escherichia coli, Proteus vulgaris, Pseudomonas aeroginosa, Salmonella typhimurium, Staphyllococcus aureus, Streptococcus faecalis* and *Candida albicans* (MIC > 1.6 mg/ml)	Franzblau and Cross 1986

	Dried root	Antiviral	Hot water extract active against Herpes virus type1 at 100 μg/ml (*in vitro*)	Zheng, 1988
	Dried rhizome	Apoptosis induction at concentration	Water extract active against NPC 10 μg/ml	Xu *et al.,* 2000

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
*S. glabra**	Dried rhizome	HIV-1 integrase and protease	Ethanolic and water extract showed high activity against HIV-1 integrase (IC_{50}= 6.7 and 8.5 μg/ml respectively)	Tewtrakul *et al.,* 2006

Dried root	Antihepatotoxic activity	Water soluble fraction exhibited activity (dose 100 mg/kg)	Xu *et al.,* 2000
Dried rhizome	Anti-inflammatory	Water extract active at dose 400 mg/ml intragastric in rat (adjuvant-induced arthritis, caragennan-induced pedal edema and cotton pellete granuloma)	Jiang *et al.,* 1997

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
*S. glabra****	Rhizome	Cytotoxic	The effects of 24 h incubation with different concentrations (0-50 mg/ml) of the extracts on HepG2 cells were	Thabrew *et al.,* 2005

			determined	
	Dried root	Antihyperglycemia	Methanolic extract showed activity at dose 20 mg/kg injected IP to male mice in NIDDM model	Fukunaga *et al.,* 1997
	Dried root	Anti-inflammatory	Water extract inactive orally in rat (formalin pedal edema)	Jiang *et al.,* 1997

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**

S. glaucophylla	Entire plant	Antispasmodic	Ethanolic-water (1:1) extract active in guinea pig ileum (acetylcholine and histamine-induced spasms	Dhar *et al.,* 1968
S. glyciphylla	Leaves	Antioxidant	A hot water extract of the Australian native sarsaparilla *Smilax glyciphylla* inhibited peroxidation of phosphatidylcholine liposomes initiated by Fe^{2+}/ascorbate (IC_{50}= 10 μg/ml)	Cox *et al.,* 2005
S. lanceolata	Dried root	Antibacterial	Ethanolic extract active against *Bacillus subtilis* (concentration used 1.0 μg/spot), *Escherichia coli* (concentration used 20.0 μg/spot)	Heinrich *et al.,* 1992

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
S. laurifolia	Dried aerial Part	Antibacterial	Cyclohexane extract active against *Bacillus subtilis,* ethyl acetate extract active against *Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus,* water extract was active against *Staphylococcus aureus*	Mc Chesney and Adam, 1985
S. lundellii	Dried rhizome	Antibacterial, antifungal and antiyeast	Ethanolic extract active against *Staphylococcus aureus* (MIC= 1 μg/ml), *Pseudomonas aeruginosa* (MIC= 5 μg/ml) and water extract active against *Staphylococcus aureus* (MIC= 10 μg/ml). Ethanolic extract active against *Aspergillus flavus, Mycosporum gypseum, Cryptococcus neoformans* (MIC= 0.5 μg/ml) and *Candida albicans* (MIC= 5 μg/ml)	Caceres *et al.,* 1998

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
S. medica	Dried rhizome	Antifungal	Compounds of this extract demonstrated weak antifungal activity against the human pathogenic yeasts *Candida albicans, C. glabrata, and C. tropicalis,* with MIC values between 12.5 and 50 μg/ml	Sautour *et al.,* 2005
*S. ovalifolia**	Dried entire plant	Anti-inflammatory	Water extract showed activity with carragenan induced pedal edema (gastric intubation of rat at dose 500 mg/kg)	Tariq *et al.,* 1985
S. regelli	Dried root	Antihepatotoxic	Ethanolic extract exhibited activity (carbon tetrachloride induced hepatotoxic and given intragastric route of rat (500 mg/kg)	Rafatullah *et al.,* 1991

* *Smilax* species found in Thailand

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
S. regelli	Dried root	Antifungal	Hot water extract active against *Epidermophyton floccosum, Microsporum canis a*nd *Trichophyton mentagrophytes* (MIC= 1 μg/ml)	Caseres *et al.,* 1991
S. riparia	Seed	Cell differeniation induction	Ethanolic extract active in Leuk-HL60 at concentration 25 μg/ml	Hata *et al.,* 1998
S. riparia var ussuriensis	Dried aerial part	Cytotoxic	Methanolic extract inactive with CA-9KB cell at concentration 50 μg/ml	Arisawa *et al.,* 1994

Table 1-5 (continued)

***Smilax* species**	**Plant part**	**Activity**	**Result of biological activity**	**References**
S. sarsaparilla	Dried rhizome	Anti-inflammatory	Ethanolic extract active with carrageenan-induced pedal edema and cotton pellet granuloma (intragastric rat at dose 500 mg/ml)	Ageel *et al.,* 1989
S. sieboldii	Dried aerial	Cytotoxic	Water extract inactive against CA-human colorectal SNU-C4 and CA-human-colorectal-SNU-1 at concentration 300 μg/ml	Hyun *et al.,* 1994

S. spinosa	Dried root	Antibacterial	Ethanolic extract active against *Escherichia coli, Staphylococcus aureus* (30 μg/disc)	Casares *et al.,* 1987

1.4 Chemical constituents of *Smilax* species

The chemical constituents previously isolated from *Smilax* species of *Smilax* compounds isolated from their roots are sapogenins and their glycosides, whilst flavonoids and flavonoid glycoside compounds have been found in mainly the leaves.

There are no report for isolated compounds of anti-HIV-1 integrase and antioxidant activities.

The chemical compounds and structures which have been isolated from all *Smilax* are summarized in Table 1-6.

Table 1-6: Chemical investigation of *Smilax* species

Botanical name	Plant part	Chemical constituents	References
S. aristolochiaefolia	Roots	Steroid sapogenin [sarsasapogenin, parillin, smilagenin], Sterol [sitosterol, stigmasterol], Steroid saponin [sarsaparilloside]	Tschesche *et al.,* 1960
		Saponin [asparagoside A, desglucodesrhamnoparillin, desglucoparillin]	Mahato *et al.,* 1982
S. aspera	Roots	Sapogenin [tigogenin] Sarsasapogenin	Laorga and Pinar, 1960
		29-Norcycloartanol	Chada, 1972
		Asperagenin	Tschesche *et al.,* 1974
		Yamogenin	Mahato *et al.,* 1982
		Flavonoid phenolic [cyanidin-3-*O*-rutinoside,	Longo and Vasapllo, 2006

		pelargonidin-3-*O*-rutinoside]	

Table 1-6 (continued)

Botanical name	Plant part	Chemical constituents	References
S. bockii	Tubers	Maltol glucosides [bockioside A, bockioside B]	Guo *et al.,* 2004
S. bracteata	Rhizomes	(2s, 3s)-5-*O*-β-D-glucopyranosyloxy-6-methyl-3′-methoxy-3,7,3′-trihydroxyflavan, (2s, 3s)-5-*O*-β-D-glucopyranosyloxy-6-methyl-4′-methoxy-3,7,4′-trihydroxyflavan, 3β-{3′,5′-dihydroxyphenyl}-2α-{4′′-hydroxyphenyl}-dihydrobenzofuran-5-carbaldehyde, {1-p-*O*-coumaroyl-6-*O*-feruroyl}-β-D-fructofuranosyl-α-D-glucopyranoside, {1-p-*O*-coumaroyl-3,6-di-*O*-feruroyl}-β-D-fructofuranosyl	Li *et al.,* 2002

		-α-D-glucopyranoside, {6-*O*-feruroyl}-β-D-fructofuranosyl-{6-*O*-acetyl} -α-D-glucopyranoside	

Table 1-6 (continued)

Botanical name	**Plant part**	**Chemical constituents**	**References**
*S. china**	Tubers	Smilacin Sarsasaponin Diosgenin	Kawasaki *et al.,* 1966
*S. china**	Tubers	N^2-(2-hydroxysuccinoyl) arginine	Kaisai *et al.,* 1983
*S. china**	Rhizomes	Dioscin, Gracillin, Methyl protogracillin, Methyl protodioscin	Kim *et al.,* 1989
	Rhizomes	Smilasides A-F	Kuo *et al.,* 2005
*S. corbularia**	Rhizome	Diosgenin	Sukdayan *et al.,* 1985
S. excelsa	Roots	Tigogenin	Iskenderov *et al.,* 1970

		Diosgenin	Mahato *et al.,* 1982
*S. glabra**	Rhizomes	Sterol [campesterol, stigmasterol, diosgenin, β-sitosterol]	Tsukamoto *et al.,* 1963

**Smilax* species found in Thailand

Table 1-6 (continued)

Botanical name	**Plant part**	**Chemical constituents**	**References**
*S. glabra**	Leaves	Flavonoid [quercetin, kaemferol]	Chien *et al.,* 1979
	Rhizomes	Flavonoid [astilbin, engeletin]	
	Rhizomes	Flavonoid [astilbin, daucosterol, isoengeletin]	Cao *et al.,* 1993
	Rhizomes	Flavonoid [isoastilbin, isoengeletin]	Chen *et al.,* 1996
		Chromone [smiglabin]	Li *et al.,* 1996
		6,7-dihydroxy-3-methoxyl-isoflavone	Yi *et al.,* 1998

		Chromone [eurryphin] Flavonoid [smitilbin, dihydroquercetin, resveratrol, 5-*O*-caffeoylshikimic acid]	Chen *et al.,* 1999
		Phenylpropanoid [helonioside A, smilaglaside A, smilaglaside B]	Chen *et al.,* 2000

**Smilax* species found in Thailand

Table 1-6 (continued)

Botanical name	**Plant part**	**Chemical constituents**	**References**
*S. glabra**	Rhizomes	Milaglaside C Smilaglaside D Smilaglaside E	Chen *et al.,* 2000
		Flavonoid [smitilbin] Astibin Eurryphin Engeletin Dihydroquercetin Resveratrol 5-*O*-caffeoylshikimic acid	Ng and Yu, 2001
	Rhizomes	Astilbin Isoastilbin	Du *et al.,* 2005

S. glycyphylla	Leaves	Glycyphyllin or Phloretin 2-rhamnoside	Williams, 1967
S. lebrunii	Roots	Spirost-5-ene-3,17,27-triol	Ju *et al.,* 1993
S. medica	Roots	Sarsasapogenin	Shoppee, 1964
		Pollinasterol	Devys *et al.,* 1969

**Smilax* species found in Thailand

Table 1-6 (continued)

Botanical name	**Plant part**	**Chemical constituents**	**References**
S. medica	Roots	(25R)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl -(1→2)-[β-D-glucopyranosyl -(1→4)]-β-D - glucopyranoside, (25R)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-	Sautour *et al.,* 2005

		glucopyranosyl -(1→4)]-β-D - glucopyranoside, (25R)-3β,5β,22α-methoxyfurostann-3β, 26-diol-3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl -(1→2)-[β-D-glucopyranosyl -(1→4)]-β-D -glucopyranosyl 26-*O*-β-D-glucopyranoside,	

Table 1-6 (continued)

Botanical name	Plant part	Chemical constituents	References
S. medica	Roots	(25R)-5β-spirostan -3β-ol 3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl -(1→2)]-β-D-glucopyranoside	Sautour *et al.,* 2005

S. menisperoidea	Roots	Spirost-5-ene-3, 17, 27-triol	Ju *et al.,* 1992
S. officinalis	Roots	Sasasapogenin	Shoppee, 1964
	Rhizomes	Sarsasapogenin 3-*O*-β-D -glucopyranosyl-(1→4) -[α-L-arabinopyranosyl- (1→6)-β-D- glucopyranoside]	Bernardo *et al.,* 1996

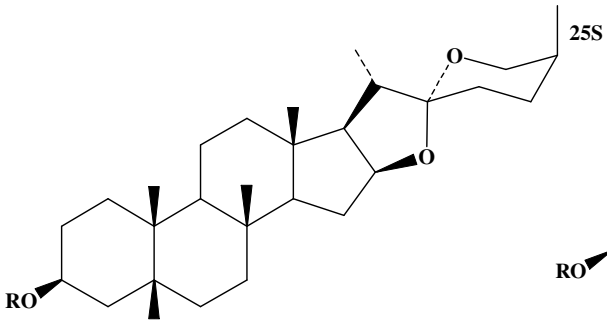
Table 1-6 (continued)

Botanical name	Plant part	Chemical constituents	References

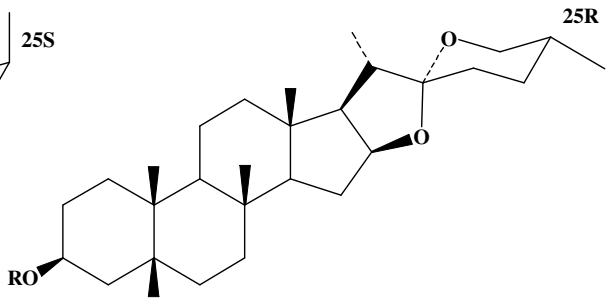
S. officinalis	Rhizomes	Neotigogenin 3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside], 25S-spirostan-6-β-ol 3-*O*-β-D-glucopyranosyl -(1→4)-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside	Bernardo *et al.,* 1996
S. ornata	Roots	Smilagenin Sarsasapogenin β-sitosterol Smilacin	Shoppee, 1964
S. parvifolia	Roots	Diosgenin Diosgenin-3-*O*-β-D-glucopyranoside	Sharma *et al.,* 1980
S. pseudochina	Roots	Essential oil Hexose Tannin Alkaloids Phytosterol β-linolic Oleic acid	Chu *et al.,* 1945

Table 1-6 (continued)

Botanical name	Plant part	Chemical constituents	References
S. sieboldii	Roots	Sieboldin A Sieboldin B	Woo *et al.,* 1992
S. sieboldii	Roots	Smilaxin A Smilaxin B Smilaxin C Tigogenin Neotigoginin Laxogenin	Akahori *et al.,* 1963



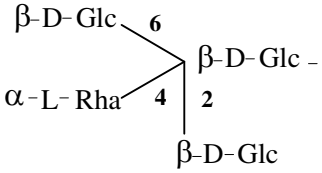
Sarsasapogenin or Pargenin (R=H)



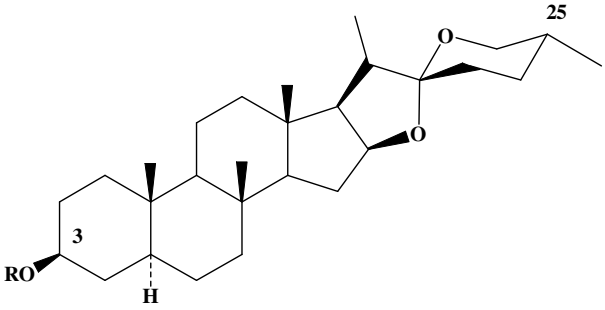
Smilagenin

Parillin

R=



Asparagoside A (R= β-D-Glc-)



Desglucodesrhamnoparillin

R β-D-Glc —(6)— β-D-Glc -

Tigogenin

Desglucoparillin

R=

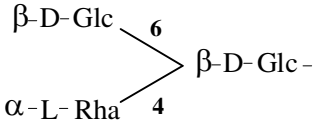
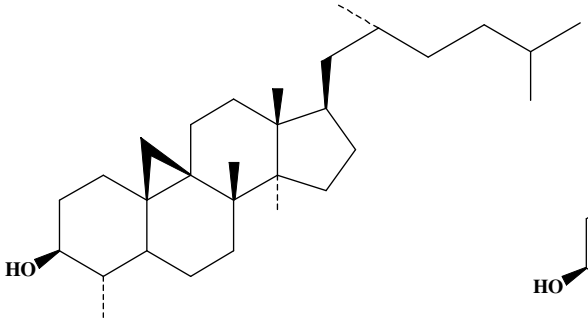
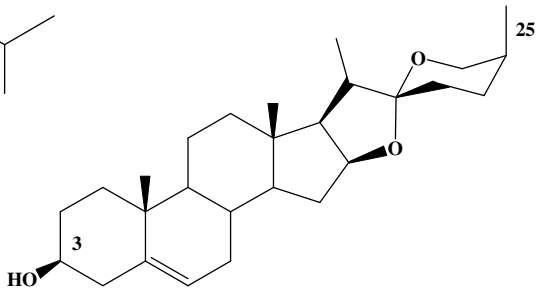


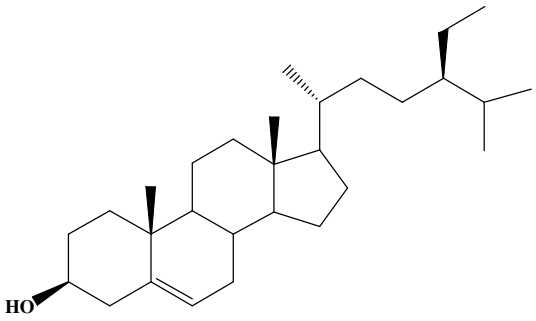
Figure 1-5 Structures of some chemical constituents of *Smilax* species



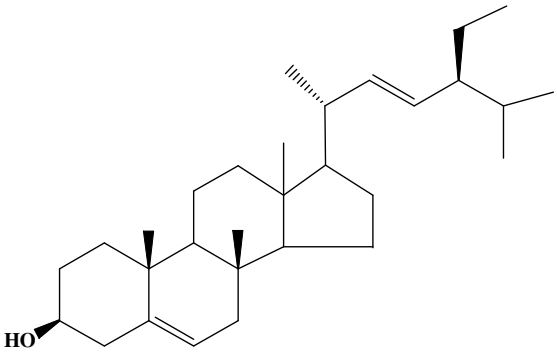
29-Norcycloartanol



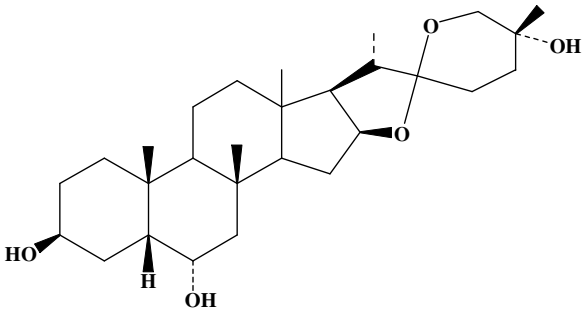
Yamogenin



β-Sitosterol

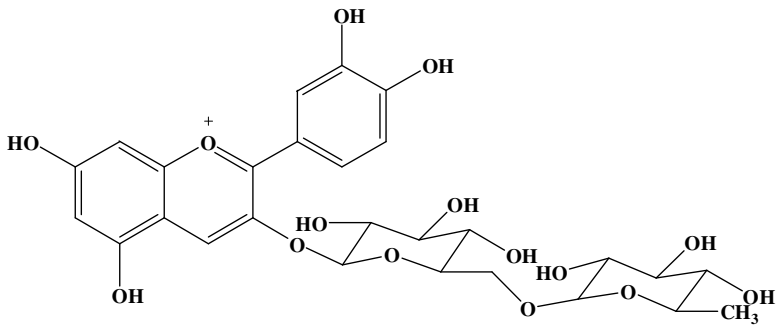


Stigmasterol

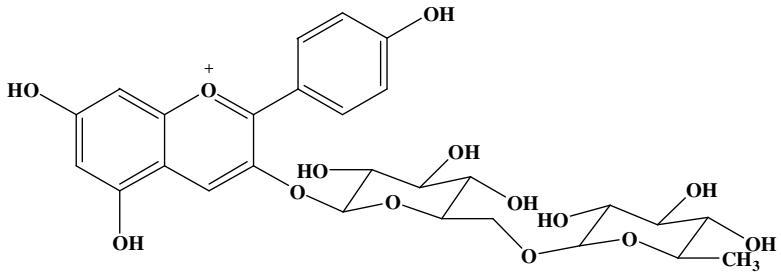


Asperagenin

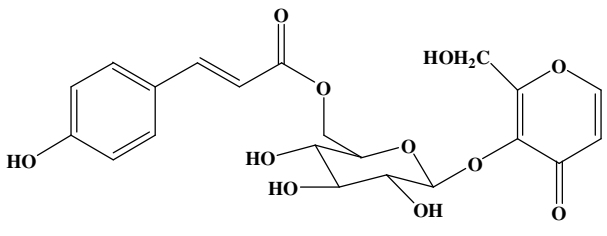
Figure 1-5 (Continued)



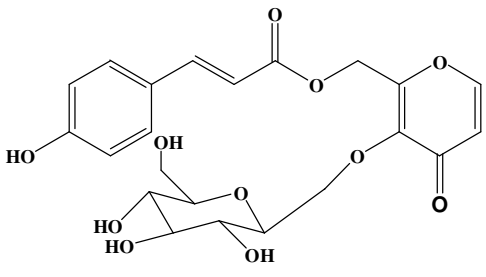
Cyanidin-3-*O*-rutinoside



Pelargonidin-3-*O*-rutinoside

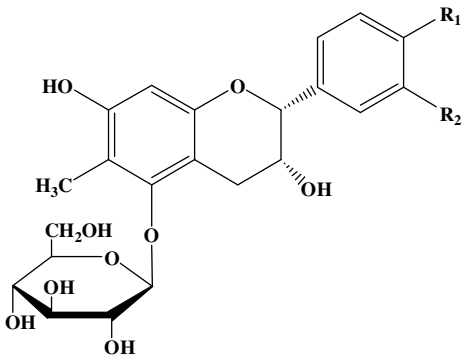


Bockioside A



Bockioside B

Figure 1-5 (Continued)

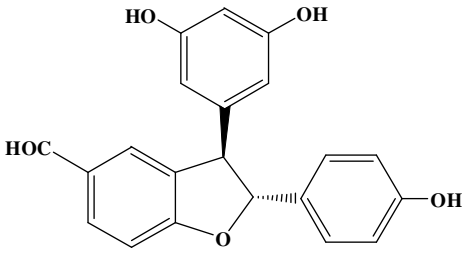


R_1= OH, R_2= OCH_3

(2s, 3s)-5-*O*-β-D-glucopyranosyloxy-6-methyl-3′-methoxy-3,7,3′-Trihydroxyflavan

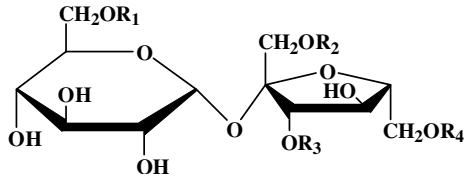
R_1= OCH_3, R_2= OH

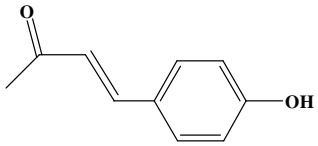
(2s, 3s)-5-*O*-β-D-glucopyranosyloxy-6-methyl-4′-methoxy-3,7,4′-Trihydroxyflavan

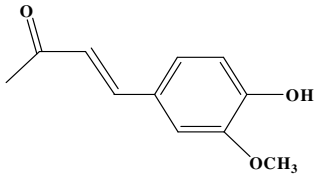


3β-{3′, 5′-dihydroxyphenyl}-2α-{4′′-hydroxyphenyl}-dihydrobenzofuran-5-carbaldehyde

Figure 1-5 (Continued)







(F) **(P)**

$R_1=R_3=H, R_2=P, R_4=F$

1-p-*O*-coumaroyl-6-*O*-feruroyl}-β-D-fructofuranosyl-α-D-glucopyranoside

$R_1=H, R_2=P, R_3=R_4=F$

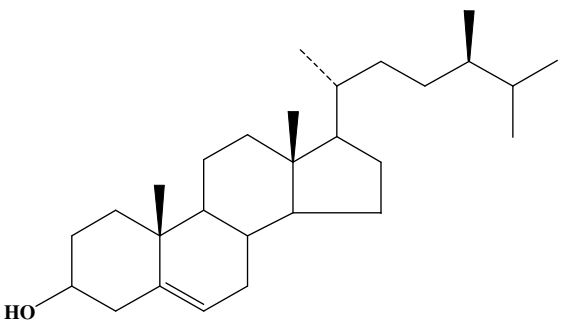
1-p-*O*-coumaroyl-3,6-di-*O*-feruroyl}-β-D-fructofuranosyl-α-D-glucopyranoside

$R_1=CH_3CO, R_2=R_3=H, R_4=F$

6-*O*-feruroyl}-β-D-fructofuranosyl-{6-*O*-acetyl}-α-D-glucopyranoside

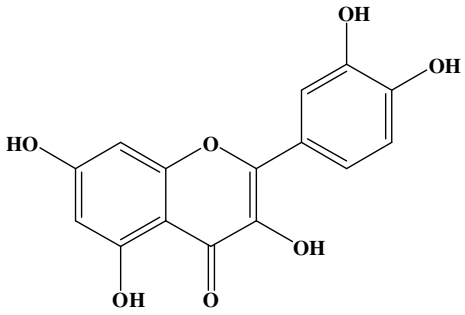
$HOOCCH_2CH(OH)CONHCH(COOH)CH_2CH_2CH_2NHC(NH)NH_2$

N^2-(2-hydroxysuccinoyl) arginine

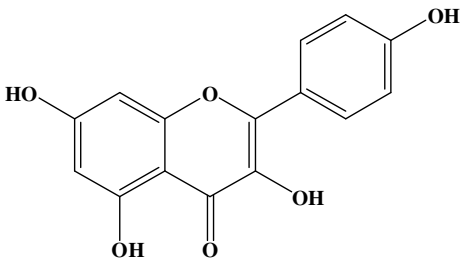


Campesterol

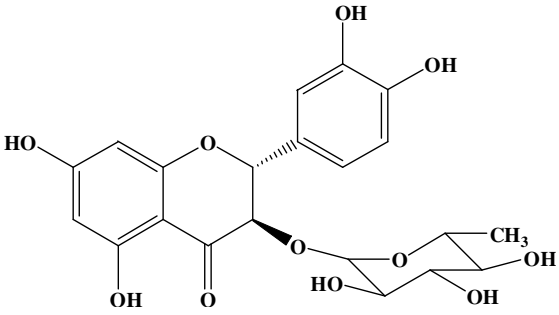
Figure 1-5 (Continued)



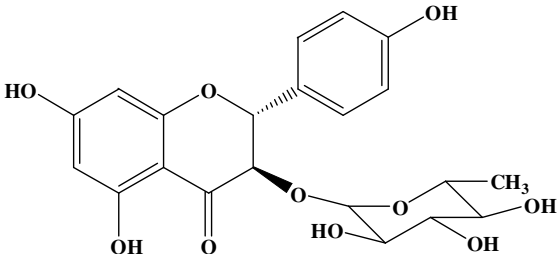
Querceti



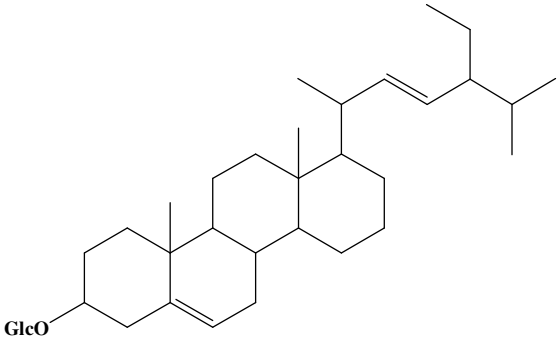
Kaemf



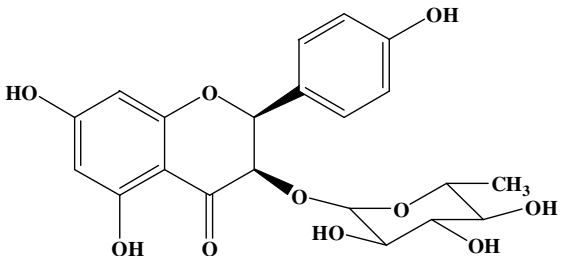
Astilbi



Engel

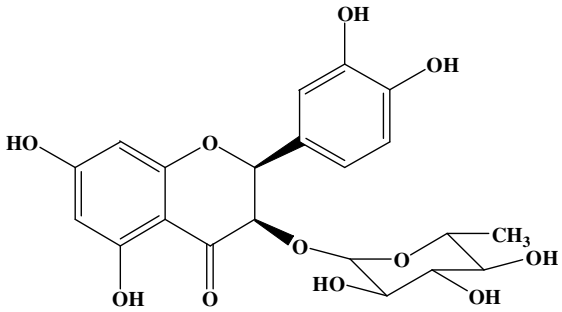


Daucoste

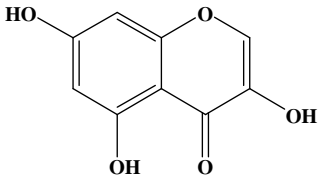


Isoengel

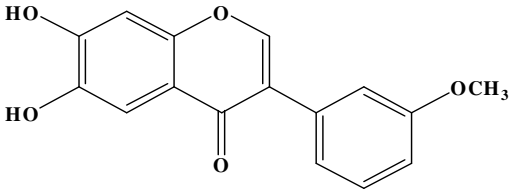
Figure 1-5 (Continued)



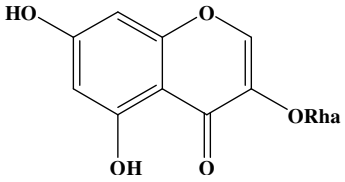
Isoastil



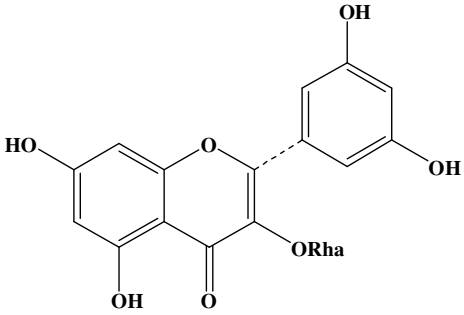
Smiglan



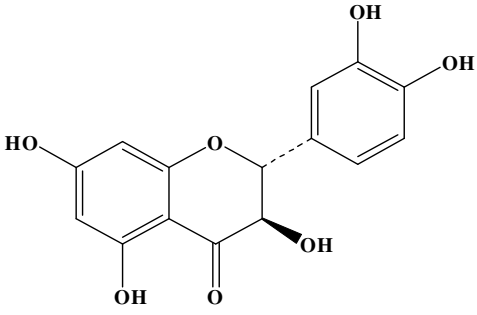
6,7-Dihydroxy-3-methoxyl -



Eurryphi

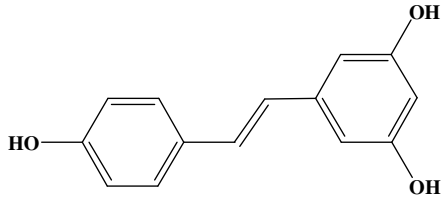


Smitilb
in

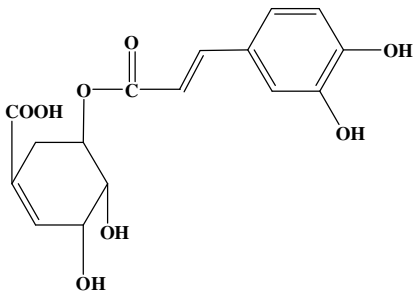


Dihydroque

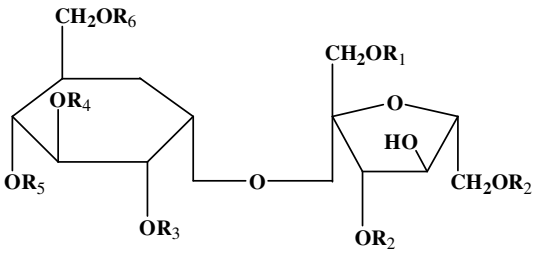
Figure 1-5 (Continued)

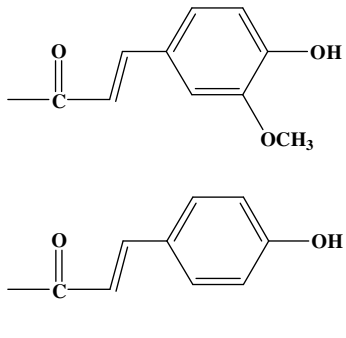


Resverat



5-*O*-

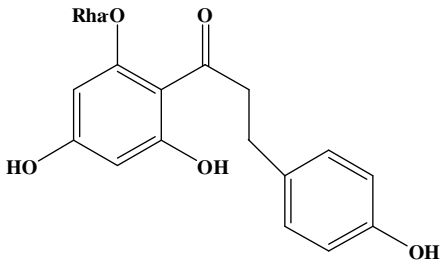




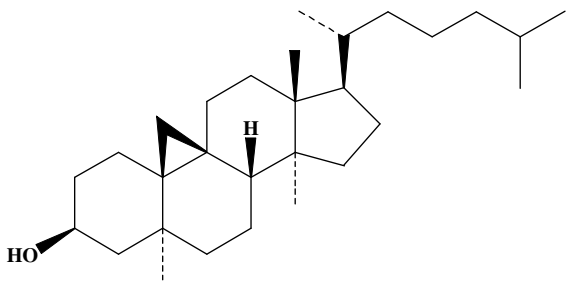
–

	R_1	R_2	R_3	R_4	R_5	R_6
Helonioside	H	X	H	H	H	H
Smilaglaside A	X	X	Ac	H	Ac	Ac
Smilaglaside B	X	X	Ac	H	H	Ac
Smilaglaside C	H	X	Ac	H	Ac	Ac
Smilaglaside D	X′	X	Ac	H	Ac	Ac
Smilaglaside E	X′	X	Ac	H	H	Ac

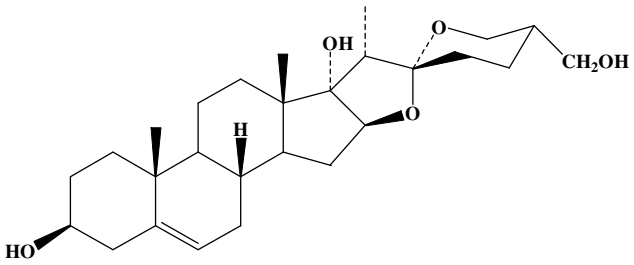
Figure 1-5 (Continued)



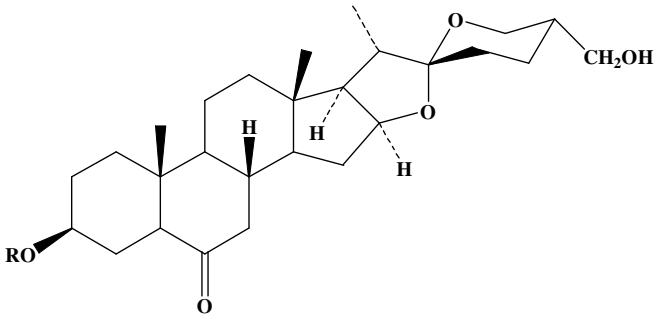
Glycyphyllin or Phloretin-2-



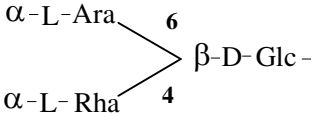
Pollinaster



Spirost-5-ene-3,17,27-



Sieboldin A;



Sieboldin B;

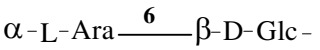
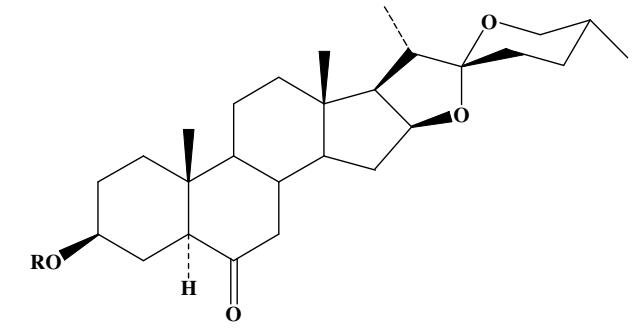
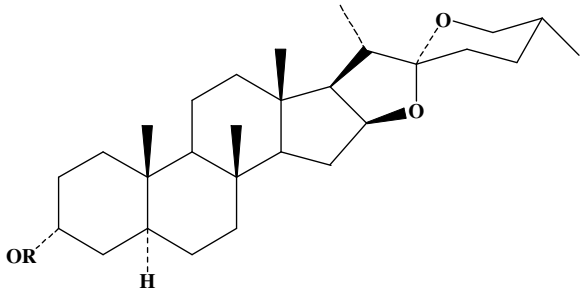


Figure 1-5 (Continued)

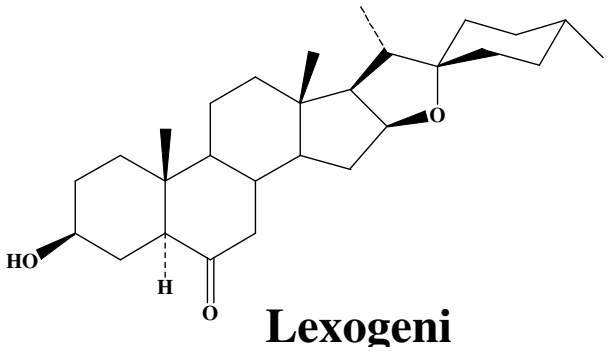


Smilaxin α-L-Ara 6 β-D-Glc-

Smilaxin α-L-Ara 6 β-D-Glc-
β-D-Glc 4

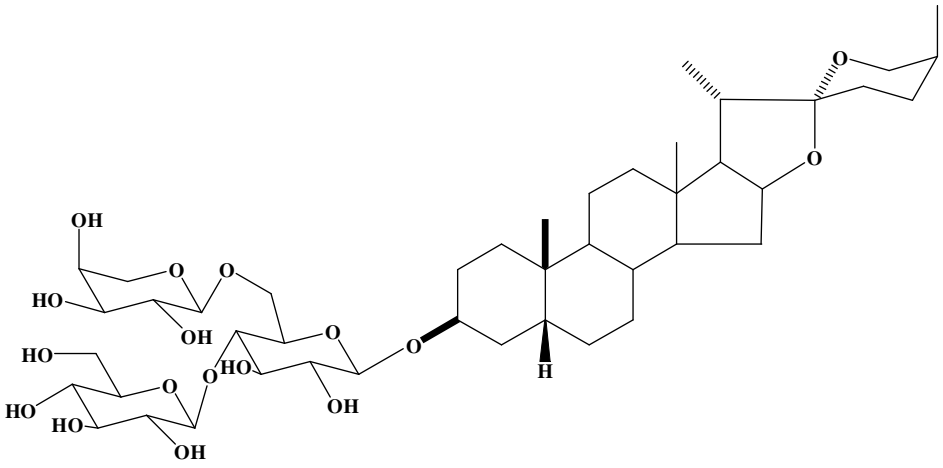


Smilaxin α-L-Ara 6 β-D-Glc-
β-D-Glc 4

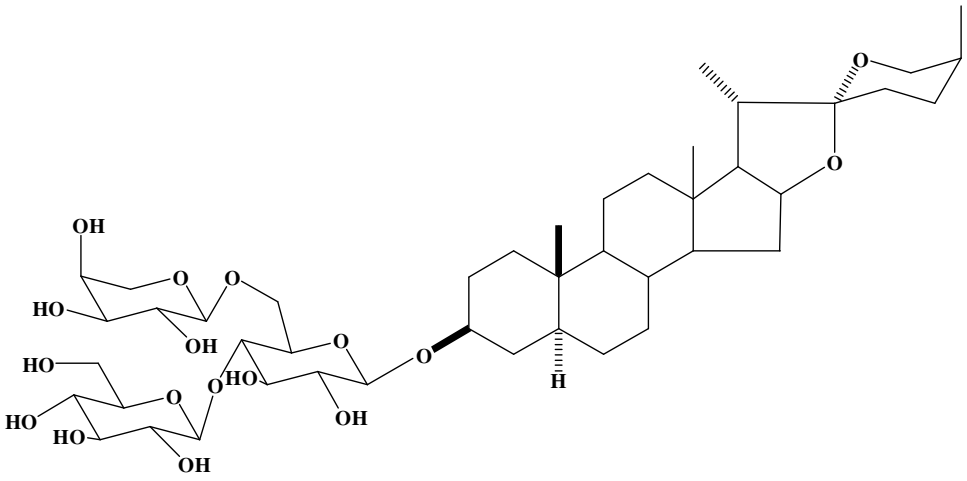


Lexogeni

Figure 1-5 (Continued)

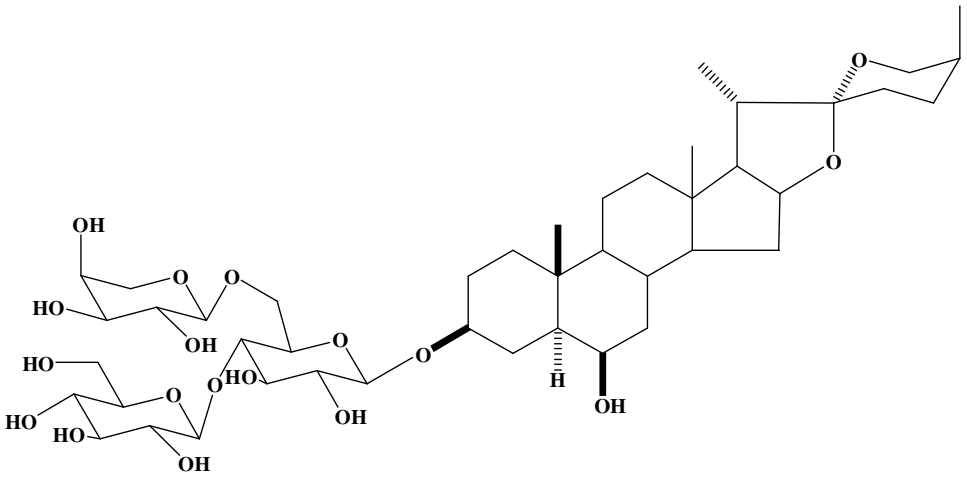


Sarsasapogenin 3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside]

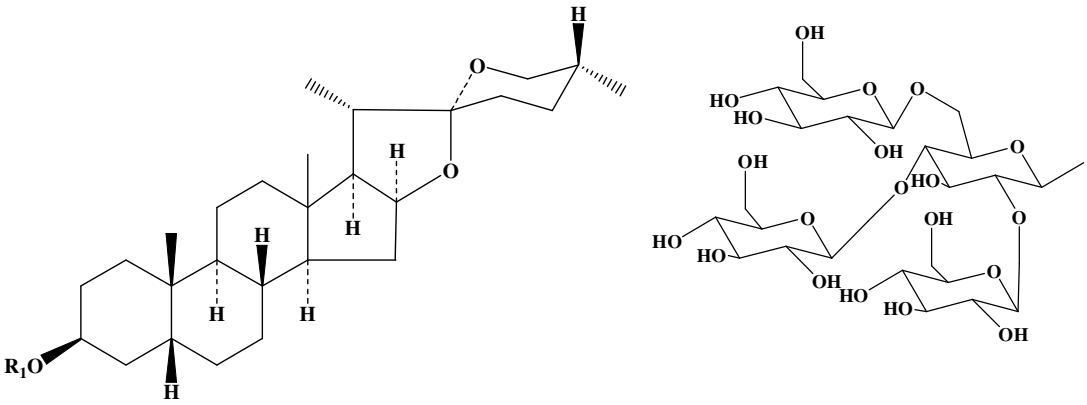


Neotigogenin 3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→6)-β-D- glucopyranoside]

Figure 1-5 (Continued)



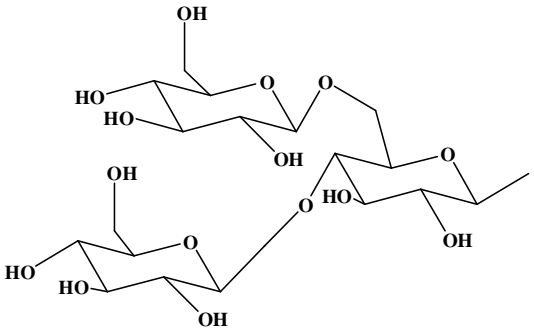
25S-spirostan-6β-ol 3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→6)]-β-D- glucopyranoside



R_1=

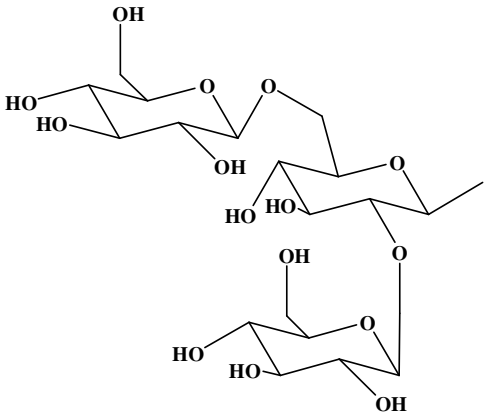
(25R)-5β-spirostan-3β-ol-3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D- glucopyranoside

Figure 1-5 (Continued)



R_1=

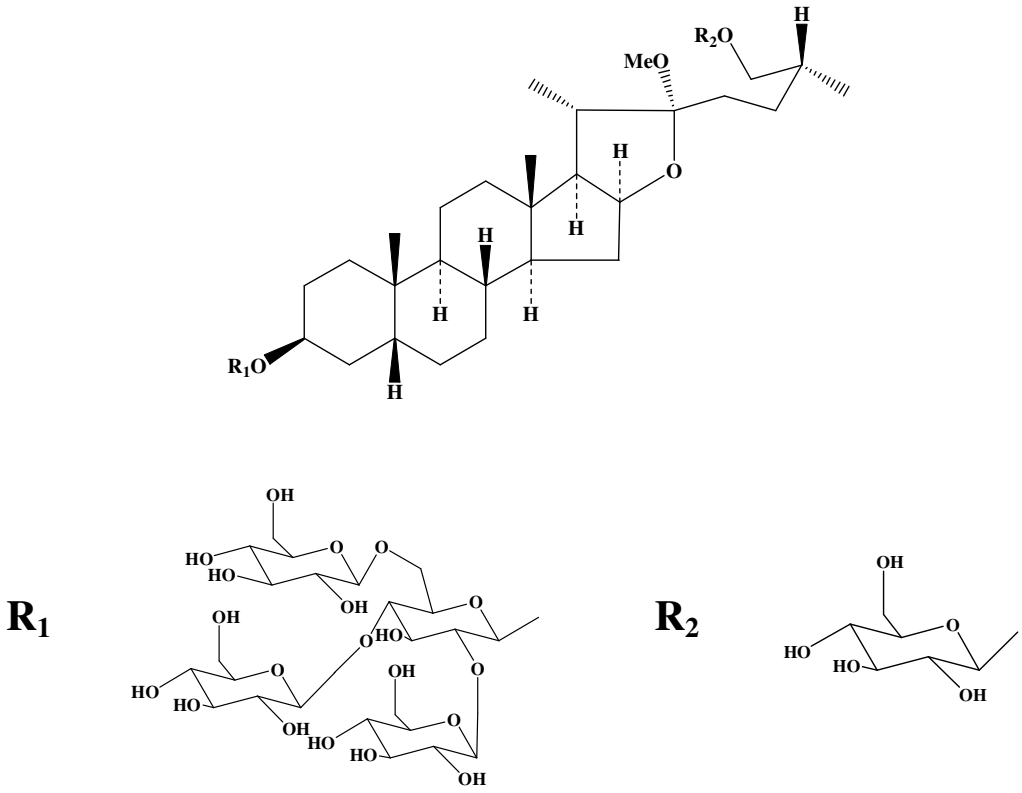
(25R)-5β-spirostan-3β-ol-3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside



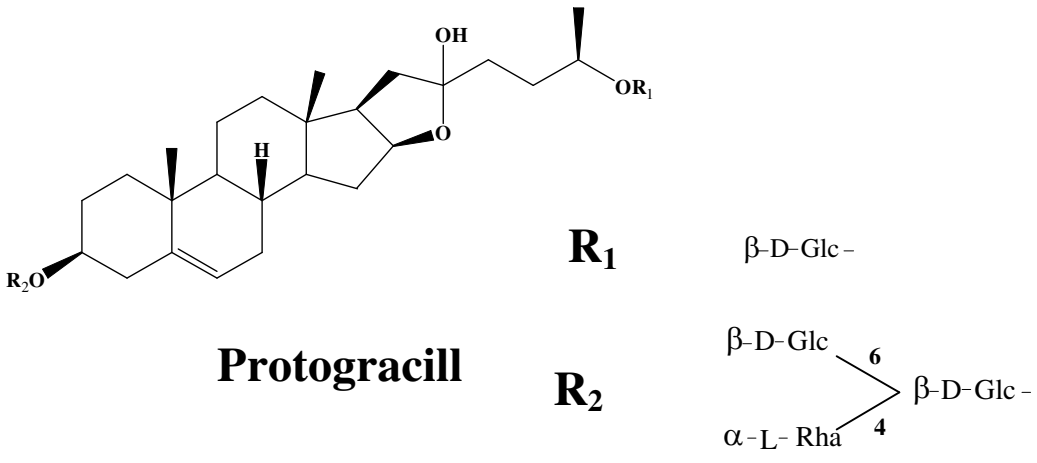
R_1=

(25R)-5β-spirostan-3β-ol-3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside

Figure 1-5 (Continued)



(25R)-3β, 5β, 22α-methoxyfurostann-3β, 26-diol-3-*O*-β-D-glucopyranosyl -(1→6)-[β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-



Protogracill

Figure 1-5 (Continued)

1.5 Objectives

The objectives of the thesis are to study:

1.5.1 The antioxidant activity of the ethanolic extract of *Smilax corbularia.*

1.5.2 The activity of anti-HIV-1 integrase of the ethanolic extract of *Smilax corbularia.*

1.5.3 The isolation and structural elucidation of active compounds possessing the antioxidant and HIV-1 integrase inhibitory effect.