Study on anti-HIV-1 integrase activity of *Albizia procera* (Roxb.) Benth bark

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ชื่อวิทยานิพนธ์ การศึกษาฤทธิ์ต้านเอนไซม์ HIV-1 integrase ของสารสกัดเปลือกต้นทิ้งถ่อน
ผู้เขียน นาย ภัทรพันธ์ ป่านทอง
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บทคัดย่อ

ต้นทิ้งถ่อนเป็นพืชสมุนไพรในตำรับยาอายุวัฒนะ ซึ่งอยู่ในวงศ์ Mimosaceae เปลือกและใบของต้นทิ้งถ่อนเป็นที่นิยมใช้ในยาแผนโบราณถูกนำมาใช้อย่างกว้างขวางในการรักษาบาดแผล และอาการปวดท้อง เนื่องจากพบว่าสารสกัดหยาบจากชั้นเอทานอลของทิ้งถ่อนมีฤทธิ์ต้านเอนไซม์ HIV-1 integrase ได้ดีที่ IC_{50} เท่ากับ 19.5 µg/mL จึงนำสารสกัดหยาบนี้มาแยกสารบริสุทธิ์เพื่อทดสอบฤทธิ์ต้านเอนไซม์ HIV-1 integrase และการศึกษาในระดับโมเลกุลต่อไป และจากการแยกด้วยวิธี partition พบว่าสารสกัดในชั้นเอทิลอะซิเตท แสดงฤทธิ์ต้านเอนไซม์ HIV-1 integrase ได้ดีที่ IC_{50} เท่ากับ 19.1 µg/mL จากการแยกสารด้วยวิธี bioassay-guided isolation พบว่าสารสกัดในชั้นเอทิลอะซิเตทให้สารบริสุทธิ์จำนวนสองสารได้แก่ (+)-catechin (**1**) และ protocatechuic acid (**2**) จากการทดสอบพบว่า (+)-catechin (**1**) แสดงฤทธิ์ต้านเอนไซม์ HIV-1 integrase ได้ดีที่เท่ากับ IC_{50} เท่ากับ 46.3 µM ในขณะที่ protocatechuic acid (**2**) แสดงฤทธิ์ต้านเอนไซม์ HIV-1 integrase ได้ 46.0% ที่ความเข้มข้น 100 µM และ(+)-catechin (**1**) มีปฏิสัมพันธ์กับ Thr66, Gly148 และ Glu152 ในโดเมนหลักของเอนไซม์ integrase ในขณะที่ protocatechuic acid (**2**) มีปฏิสัมพันธ์กับ Thr66, His67, Glu152, Asn155 และ Lys159 อย่างไรก็ตาม (+)-catechin (**1**) แสดงให้เห็นว่ามี binding energy ที่ต่ำกว่า protocatechuic acid (**2**) โดย (+)–catechin (**1**) มีค่า binding energy เท่ากับ -5.16 kcal/mol ในขณะที่ protocatechuic acid (**2**) มีค่าเท่ากับ -4.85 kcal/mol ดังนั้น (+)-catechin (**1**) จึงมีปฏิสัมพันธ์ที่ดีกับเอนไซม์ HIV-1 integrase ทำให้มีฤทธิ์สูงกว่า protocatechuic acid **(2)** รายงานนี้เป็นรายงานครั้งแรกในการศึกษาฤทธิ์ต้านเอนไซม์ HIV-1 integrase ในส่วนเปลือกของต้นทิ้งถ่อน การทดลองที่ได้แสดงให้เห็นว่า เปลือกของต้นทิ้งถ่อนมีศักยภาพในการต้านเอนไซม์ HIV-1 integrase

Thesis Title Study on anti-HIV-1 integrase activity of *Albizia procera* (Roxb.) Benth bark

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Abstract

Albizia procera, tall albizia, is belonging to the Mimosaceae family. It is a medicinal plant in traditional longevity preparations. The bark and leaves of *Albizia procera* were extensively used for the treatment of variety of wounds and considered useful in pregnancy and stomachache. Since the EtOH extract of this plant showed good effect against HIV-1 integrase (IN) with an IC_{50} value of 19.5 µg/mL, its fractions and isolated compounds were therefore tested on anti-HIV-1 IN activity and studied on molecular docking. Ethyl acetate fraction exhibited the most potent effect (IC_{50} = 19.1 µg/mL) against HIV-1 IN. From bioassay-guided isolation, the ethyl acetate fraction was further separated to give two compounds which are (+)-catechin (**1**) and protocatechuic acid (**2**). Of the tested samples, (+)-catechin (**1**) exhibited appreciable activity against HIV-1 IN with an IC_{50} of 46.3 µM, whereas protocatechuic acid (**2**) showed mild activity with 46.0% inhibition at concentration of 100 µM. (+)-Catechin (**1**) could interact with Thr66, Gly148 and Glu152 in the core domain of IN enzyme, whereas protocatechuic acid (**2**) could bind with Thr66, His67, Glu152, Asn155 and Lys159. However (+)-catechin (**1**) showed lower binding energy

(-5.16 kcal/mol) than that of protocatechuic acid (**2**, -4.85 kcal/mol), thereby (+)-catechin (**1**) could have good interaction with HIV-1 IN and possessed higher activity than protocatechuic acid (**2**). This is the first report on anti-HIV-1 IN activity of *Albizia procera* bark. The results may suggest that *Albizia procera* bark has potential as anti-HIV-1 IN agent.

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CHAPTER 1
INTRODUCTION

1.1 Introduction

1.1.1 Global AIDS epidemic and AIDS in Thailand

Acquired immunodeficiency syndrome (AIDS) has been rapidly spreading in several countries and is worldwide public health problem. AIDS epidemic, an estimated 35.3 (32.2–38.8) million people were living with AIDS in 2012. From the previous years, the increasing of more people are getting the antiretroviral therapy for the life-saving. The new HIV infections around 2.3 (1.9–2.7) million, indicating the new HIV infections of HIV-1 from 3.4 (3.1–3.7) million in 2001, a 33% decline in number. Moreover, the number of HIV-1 infection patients deaths is also declining with1.6 (1.4–1.9) million in 2012, which is down in 2005 from 2.3 (2.1–2.6) million (UNAIDS global report, 2013) (Figure 1).

Figure 1 Adults and children who are living with HIV around the world

(UNAIDS Core epidemiological, 2013)

HIV infection leading to AIDS has become the most concerning cause of disability that lost in the Thai population. The situation of AIDS and symptoms of HIV infection in Thailand at last reported that the number of people living with HIV 376,690 people (female: 120,119 people and male: 256,571 people), deaths due to AIDS 98,721 people. The trend lower shift than in the past because the treatment of AIDS patients with antiretroviral drugs makes the patient have improved quality of life (Bureau of Epidemiology, Thailand, 2011). The Thailand's HIV infections go through unsafe sex, especially among the risk groups such as female sex workers (FSWs), men who have sex with men (MSM) and their clients, and injecting drug users (IDUs). However, antiretroviral treatment (ART) access among pregnant women attending antenatal clinics has consistently been above 90 percent for the last six years. This has substantially lower rates mother-to-child transmission of HIV as a significantly transmission route (NationalAIDS committee Thailand, 2012) (Figure 2).

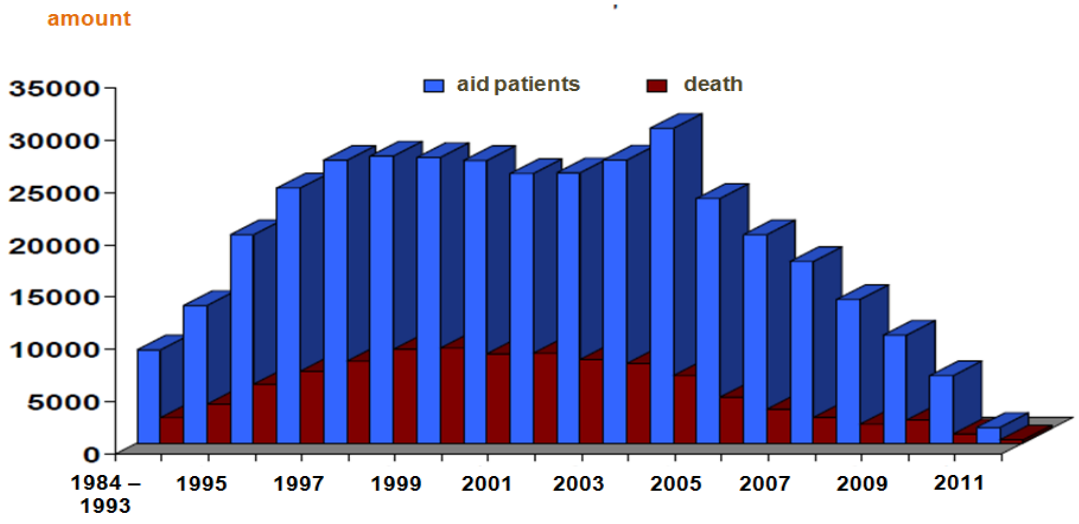


Figure 2 The trend of AIDS and HIV infection with symptoms that are directly attributable to HIV infection. By the years from Setember 1984 – 15 November 2011 (Bureau of Epidemiology, Thailand, 2013)

1.1.2 Rationale and background for this study

Highly active antiretroviral therapy (HAART) is now required for the treatment of an acquired immunodeficiency syndrome (AIDS). Three enzymes that are essential for the HIV-1 life cycle are HIV-1 protease (PR), reverse transcriptase (RT) and integrase (IN). HIV-1 IN has become an appealing target for AIDS treatment since there are only three HIV-1 IN inhibitors named raltegravir, elvitegravir and dolutegravir that are now available in the market. HIV-1 IN functions as a dimer and the integration process is composed of two steps: 3′ processing and 3′ joining (strand transfer) which finally integrates viral DNA into host chromosome (Katz &Skalka, 1994; Lucia, 2007). However, if the challenges are overcome, the number of people on treatment keepsrisingquickly enough and if the effectiveness of HIV prevention efforts keeps improving,theworld can reach the goal of ending the AIDS epidemic.

Albiziaprocera, tall albizia, is belonging to the Mimosaceae family. It is a medicinal plant in traditional longevity preparations, locally known in Thai as Thing thon. The bark and leaves of *Albizia procera* were widely used for the treatment of wounds and be useful in stomachache and pregnancy (Kokila et al., 2013). Since the EtOH extract of this plant showed good effect against HIV-1 IN with an IC_{50} value of 19.5 μg/mL, its fractions and isolated compounds were therefore tested on anti-HIV-1 IN activity and studied on molecular docking.

1.1.3HIV/AIDS basics

AIDS is a severe medical problem. A person whose his/her immune system is too weak to fight with infections is diagnosed as AIDS. The human immunodeficiency virus (HIV) can not alive or reproduce on their own, the infection to the cells of a living organism in order to replicate is needed (make new copies of themselves). HIV is belonging to a retroviruses named lentiviruses. The retroviruses genome is composed of ribonucleic acid (RNA), and each virus has two single chains of RNA. People with good immune systems can be exposed to some viruses and do not have a reaction to them. But people living with HIV can face serious threats from opportunistic infectionscaused by fungus (e.g. candidiasis, cryptococcosis, pneumonia), bacteria (e.g. tuberculosis, bacterial pneumonia), viruses (e.g. herpes simplex, herpes zoster), and protozoan parasites (e.g. toxoplasmosis, cryptosporidiosis). These can have a devastating impact on the mental health and physical health status of people living with HIV/AIDS, even those being treated with antiretroviral therapy.

1.1.4 HIV transmission

The most usual ways for HIV transmission are unprotected sex with an infected partner, sharing needles with HIV-infected person, blood product infection and the infected mother to fetus transmission. The transmission of HIV from an infected person to another could be through semen, blood, breast milk and vaginal secretions, etc. Especially, infectiousness is depended on the HIV-1 infected cells in

relevant body fluid such as blood or genital tract secretions and concentration of HIV-1 (Levy, J.A. 1988).

1.1.5 HIV structure

HIV is belonging to the family *Retroviridae*, subfamily *Lentivirinae*, and genus *Lentivirus* (Chiu et al., 1985; Wain-Hobson et al., 1985; Vogt et al., 1997). The HIV structure follows the pattern of the retrovirus family, with a diameter at 120 nm, which is around 60 times smaller than a red blood cell. It is consisted of two copies of the positive RNA single-strand that codes for the genes of virus which is composed of 2,000 copies of the p24 of viral protein enclosed by a conical capsid. The single-stranded RNA is strongly bound to nucleocapsid proteins (p7) and enzymes essential for the development of the virion such as proteases, reverse transcriptase, integrase and ribonuclease. The viral protein p17 surrounds the capsid ensuring the integrity of the virion (Gangl et al., 2002). For the mature virions are able to infect another host cell (Figure 3).

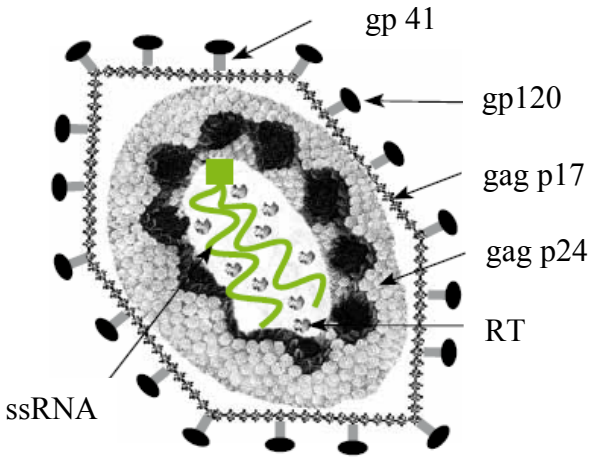


Figure 3 HIV-virus structure (HIV Management in Australasia, 2009)

1.1.6 HIV life cycle

The life cycle begins with viral entry, a multi-step interaction between the HIV envelope and the host target cell surface receptors. In the initial step of HIV entry, the HIV gp120 binds to the host target cell CD4 receptor thereby anchoring HIV to the host cell. HIV binding with CCR5 and CXCR4 is the main coreceptors used by HIV. The viral then fuses with host membranes, the viral capsid into the cell, and the viral released RNA, its genetic material, into the host cell cytoplasm. The HIV releasing the two copies of single-stranded HIV RNA inside the cell. The next step, referred to as reverse transcription, involves the conversion of the single-stranded HIV RNA to double-stranded HIV DNA by the HIV enzyme reverse transcriptase. Next the HIV DNA, migrates inside the host cell nucleus. The HIV integrase enzyme then catalyzes the integration of the HIV DNA into the host cell DNA. Once the HIV DNA has integrated into the host genome, is called a proviral DNA. The cellular enzymes (RNA polymerase) transcribe the proviral DNA into messenger RNA (mRNA) and genomic RNA. The viral mRNA then is exported out of the nucleus into the host cell cytoplasm where cellular enzymes translate the viral mRNA into viral proteins. By the HIV enzyme protease. The Final step multiple components of the HIV are then assembled combine genomic RNA. Outside the cell, then the virus can now move on to infect other cells (Figure 4).

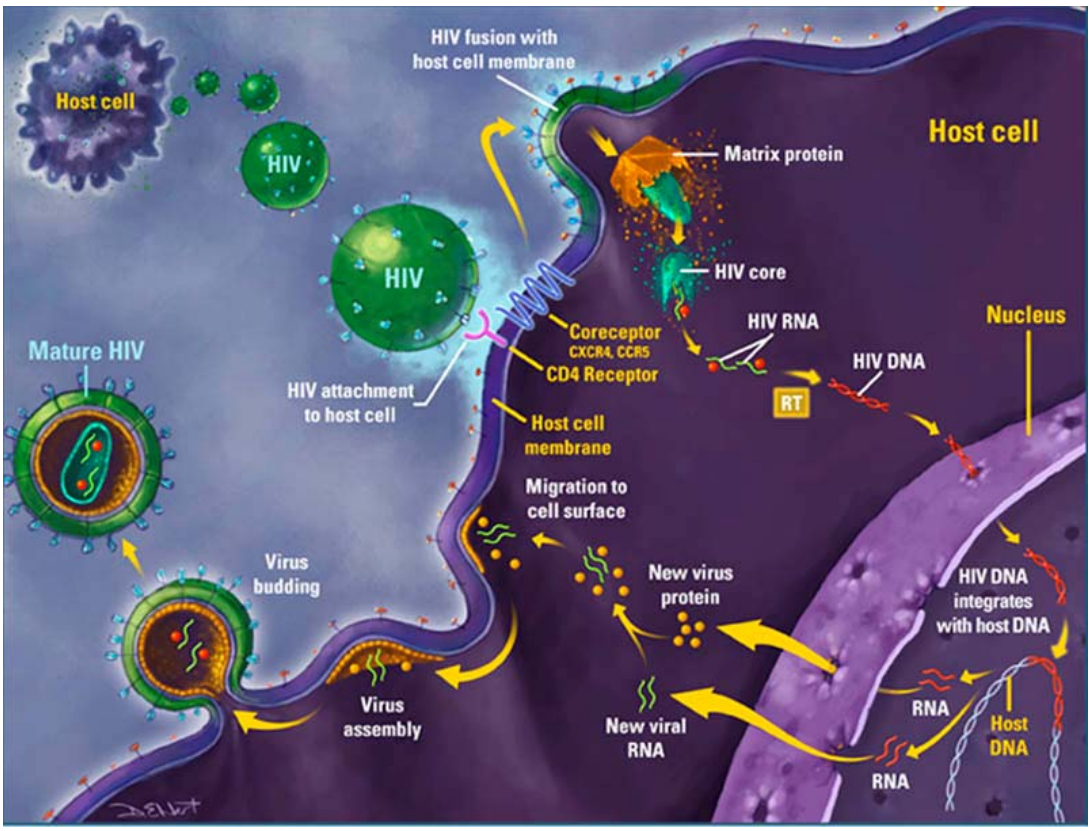


Figure 4 HIV life cycle(HIV web study, 2013)

1.1.7 HIV-1 integrase inhibitors

Raltegravir is the first drug approved to markedly inhibit the HIV-1 integrase enzyme (Hazuda et al., 2000). This drug approved by the Food and Drug Administration (FDA) of the US in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-experienced adult patients who have evidence of viral replication and HIV-1 strains resistant to multiple antiretroviral agents. It received approval by the U.S. FDA on 12 October 2007, the first of a new class of HIV drugs, the integrase inhibitors, to receive such approval. In 27 August 2012, elvitegravir was approval by the U.S. FDA, it is intended for use in initial

therapy of adults with HIV-1 infection (Sax et al., 2012; DeJesus et al., 2012) and Dolutegravir was approved in 12 August 2013 by the U.S. FDA for use in initial (Raffi et al., 2013).

1.1.8 Molecular docking study

In the area of molecular modeling, docking study is a method which estimates the suitable orientation of one molecule to a second one when they are bound to each other to form a stable complex (Lengauer&Rarey, 1996). Knowledge of the orientation may be used to predict the strength of association or binding affinity between two molecules using such as scoring functions. The associations between biologically relevant molecules which are nucleic acid, proteins, lipids and carbohydrates play an important role in signal transduction. Moreover, the relative orientation of the two interacting partners might affect the type of signal produced (e.g., agonismvsantagonism). Thus, docking study is benefecial for predicting both the type of signal produced and strength. Docking is commonly used to predict the binding orientation of small molecule drug candidates to their protein targets for the prediction of the affinity and activity of the small molecule. Therefore, docking is an essential role in the rational design of drugs (Kitchenet al., 2004). Regarding the pharmaceutical and biological significance of molecular docking, the efforts have been directed to improve the methods used for docking prediction.

1.2 Literature reviews

1.2.1 Phytopharmacological properties of *Albizia* species

Albizia species (Family Mimosaceae) are used in traditional medicine for the treatment of wounds and stomach ache. Genus *Albizia* has inferred them as a source of different phytochemistry of natural products (saponins, diterpenoids, triterpenoids, lignans and pyridine glycosides). Plant extracts showed activity against cancers and several other diseases (Kokila et al., 2013). The genus *Albizia* comprises between 100 and 150 species, differential distribution in Asia, Africa and South and Central America (Bown, 1995).

Table 1 Some isolated chemical compounds found in *Albizia* species (Kokila et al., 2013).

Plants	**Part-used**	**Chemical compounds**
A. subdimidiata	Whole plant	Albiziatrioside A and B
A. julibrissin	Flowers	Quercitrin and isoquercitrin
		3, 5, 4′-trihydroxy, 7, 3-dimethoxy-3-*O*-β-D-glucopyranosyl- α-L-xylopyranoside
	Bark	Julibroside J29**,** J30 and J31
A. grandibracteata	Leaves	Grandibracteosides A−C

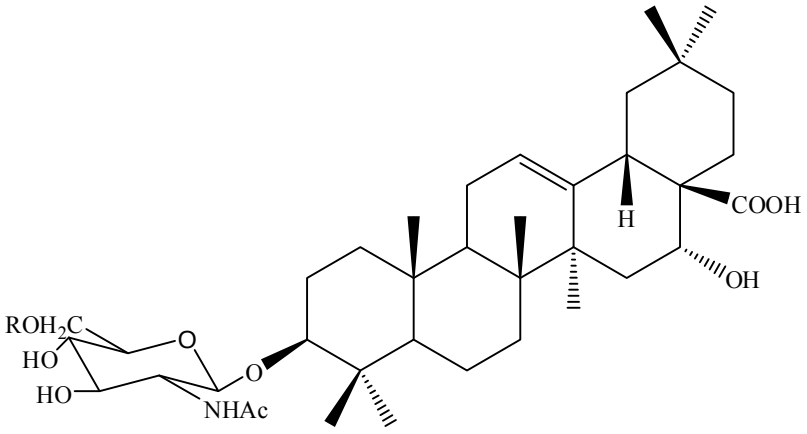
Table 1 (continued)

Plants	Part-used	Chemical compounds
A. procera	Bark	3-O-[β-Dxylopyranosyl-(1→2)-α-L-arabinopyranosyl- (1→6)-2 acetamido-2- deoxy-β-D-glucopyranosyl] echinocystic acid, 5,2′, 4′-trihydroxy-3,7,5′-trimethoxyflavonol-2′-O- β-D-galactopyranosyl- (1→4)-O-β D-glucopyranoside
A. chinensis	Leaves Bark	Kaempferol-3-O-α-L-rhamnopyranoside, Quercetin-3-O-α-L-rhamnopyranoside, Luteolin, Kaempferol, Quercetin Albizosides A-C
A. gummifera	Stem bark	Methylglucuronate 3-O-{β-D-glucopyranosyl(142)-[α-L-arabinopyranosyl(146)]-β-D-glucopyranosyl}-oleanolic acid
	Bark	Vitalboside-A, vitalboside-A, 2′-

Table 1 (continued)

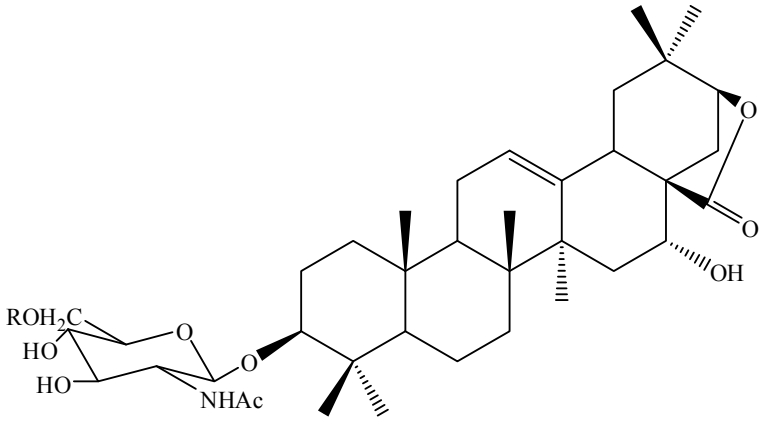
Plants	Part-used	Chemical compounds
A. lebbeck	Leaves	Quercetin, kaempferol, 3-O-α-
	Seeds	Budmunchiamines L1-L3
	Bark	rhamnopyranosyl (1→6)-β-
		glucopyranosyl(1→6)-β-
		galactopyranosides.
		Albiziasaponins A, B and C
A. myriophylla	Stem	Albiziasaponins A-E
	Bark	Albizzine A
A. mollis	Bark	Molliside A-B, Concinnoside A,
		Albiziasaponin A
A. falcataria	Bark	Syringaresinol
A. odoratissima	Root bark	7,8-Dimethoxy-39,49
		methylenedioxyflavone, 7,29,49-
		Trimethoxyflavone

Albizia procera, tall albizia, belongs to the Mimosaceae family, is a large deciduous tree and fast growing tree, attaining usually 60-70 cm diameter and 25 m height and is adaptive to diverse geo-climates. Mature individuals possess tall clear, erect or curved trunk and large branches with spreading crown and stout taproot system. The leaves of the plant are bi-pinnate and are reddish when juvenile. Flowering occurs in June to September in India. Pods with 6-12 seeds are reddish brown produced in large numbers and ripen in 3-5 months after fertilization. Bark is nearly smooth, whitish to grey-green, yellowish-green or brown colour and exfoliates in thin flakes with red undersides. It is a medicinal plant in traditional longevity preparations, locally known in Thai as Thingthon.



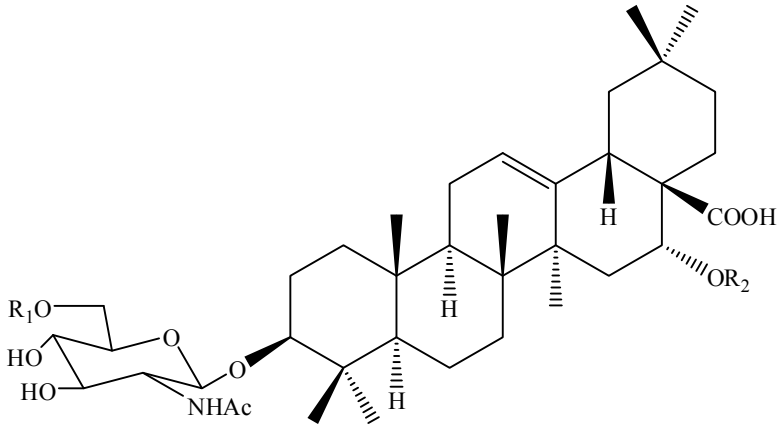
R

1- β-D-Xylp-(1→2)-α-L-Arap-
2- α-L-Arap-(1→2)-β-D-Fucp-
3- α-L-Arap-(1→2)-α-L-Arap-

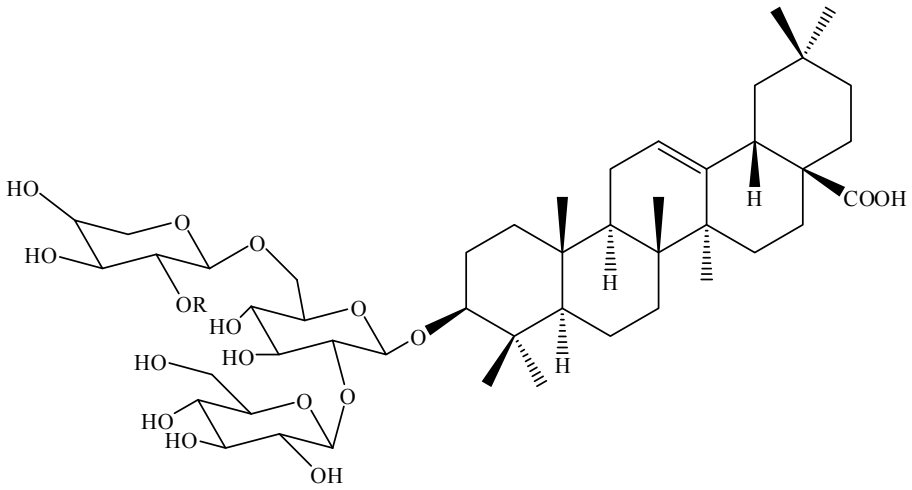


4- β-D-Xylp-(1→2)-α-L-Arap-

Saponins**1–4** were evaluated *in vitro* for their cytotoxic activity against HEPG2 cell line. Saponins**1** and **3** exhibited cytotoxic effect with IC_{50} 9.13 µg/mL and 10.0 µg/mL, respectively (Melek et al., 2007).



R_1	R_2
5- β-D-Xylp-(1→2)-β-D-Galp-	-β-D-Glcp-
6- β-D-Xylp-(1→2)-α-L-Arap-	-β-D-Glcp-
7- α-L-Arap-(1→2)-α-L-Arap-	-β-D-Glcp-
8- β-D-Xylp-(1→2)-α-L-Arap-	H
9- α-L-Arap-(1→2)-α-L-Arap-	H

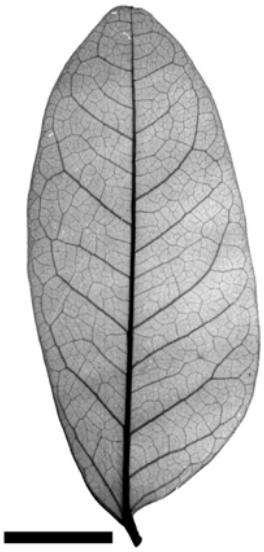


R

10- β-D-Xylp-

11- α-L-Arap-

Glycosides **5-11** were found inactive when assayed by MTT method for their toxicities against the human tumor cell lines A549, HEPG2, MCF7 and HT29. The results revealed the importance of the free hydroxyl group at C-16 of the aglycone for having cytotoxic effects (Miyase et al., 2010).



Leaves Scale bar (10 mm)

Leaves and fruits

Leaves and flowers

Figure 5 *Albiziaprocera* (Roxb.) Benth (Australian tropical rainforest plants, 2010)

Flowers and buds

Seeds

Figure 5(continued)

Cotyledon stage, epigeal germination

Leaves

Figure 5(continued)

Bark

Figure 5(continued)

The leaves and bark of *Albizia procera* were widely used for the treatment of variety of wounds and claimed to be useful in stomachache and pregnancy (Kokilaet al., 2013). All parts of *Albizia procera* are reported to show anti-cancer activity and commonly used in traditional medicines. The roots contain alpha-spinasterol and a saponin that has been reported to possess spermicidal activity at a dilution of 0.008%. The leaves are valued as insecticide and for the treatment of ulcers. The bark contains tannins and reddish gum used as a fish poison. A decoction of the bark is given for rheumatism and hemorrhage. The seeds contain proceranin which is toxic to mice and rats when administered perintoneally and orally.*Albizia procera* afforded of secondary metabolites from bark such as four triterpenoidsaponins (Melek et al., 2007) and threetriterpene glycosides (Miyase et al., 2010).

1.2.2 Compounds isolated from *Albiziaprocera* (Roxb.) Benth bark

Protocatechuic acid (Figure 6) is widely distributed and present in most edible plants used in folk medicine (Liu et al., 2004). It is considered as an active component of some traditional Chinese herbal medicines such as *Cibotiumbarometz*(L.) (Li et al., 2011). Protocatechuic acid is detected in many fruits, such as plums (*Prunusdomestica*L.) (Kayanoet al., 2002); gooseberries (*Ribesuvacrispa* L.)(Kayanoet al., 2002); grapes (*Vitisvinifera*) (Kayanoet al., 2010); and nuts, such as almonds ordinary (*Prunusamygdalus*) (Sang et al., 2002).

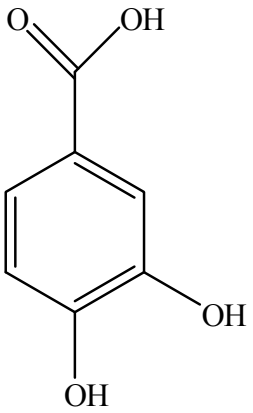


Figure 6 Chemical structure of protocatechuic acid (Kakkar andBais,2014)

Pharmacological activity of protocatechuic acid has demonstrated anticancer activity of generation of free radicals in the human body, influences in phases 1 and 2 of the metabolism of certain carcinogens, directly blocks the binding site of carcinogens with DNA molecules. It exhibits antiulcer activity antiulcer cytoprotective action and strengthening of the gastric mucosa which enhances mucosal defence, antiaging by increases activity of glutathione peroxidase, catalase and decreases the malondialdehyde level. Antifibrotic activity by inhibits the levels of TGF-β1, CTGF andinhibits HSCs proliferation. Antiviral by downregulates the secretion of HBsAg and decreases the release of the HBV DNA from HepG2. Antiatherosclerotic by inhibits monocyte adhesion to TNF-α activated mouse aortic endothelial cells VCAM-1, ICAM-1 expression and reduces NF-Xb binding activity. Antibacterial by decreases lipid oxidation levels. Neurological effect by inhibited the cytotoxicity, apoptotic morphology, reduction of TH expression, and abnormal oligomerization of alpha-synuclein in PC12 cells (Kakkar and Bais, 2014).

Recently, catechins found in green tea has been significant attention, both in the consumer communities and in scientific for the health benefits in several disorders, such as cancer and weight loss. This information has led to the great consumption of green tea by the patient and general population, and to the inclusion of green tea extract as a featured component in many nutritional supplements such as multivitamins. In ancient times, green tea has been used by the Chinese and Japanese populations for decades, and is the most consumed drinking besides water, in Asia. The good effects of green tea are recognised to the polyphenolic components having in green tea, especially the catechins, which is up to 30% of green tea dry weight leaves (Graham, 1992).

Catechins are phytochemical compounds found in high concentrations in a variety of plant-based foods and beverages. Based on their structures, catechins are classified as flavanols and include the following compounds: catechin, epicatechin, epigallocatechin, epicatechingallate, and epigallocatechingallate. High concentrations of catechin can be found in broad beans, red wine, apricots, black grapes and strawberries. Epicatechin concentrations are high in blackberries, cherries, apples, black grapes, broad beans, raspberries, pears, and chocolate. Epicatechingallate, epigallocatechingallate and epigallocatechin are in high concentrations in both green and black tea (Williamson and Manach, 2005)

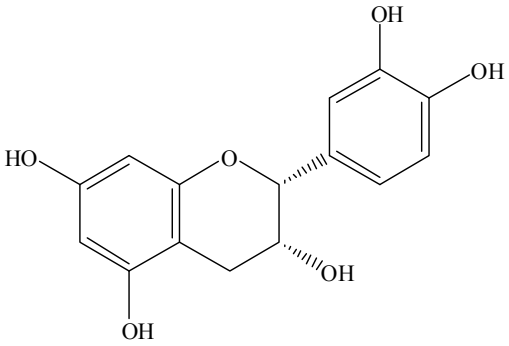
Table 2 Catechin contents of some foods (Nutrient Data Laboratory US Department of Agriculture, 2007)

Sample	Content of epicatechin (mg/100g)	Content of epigallocatechin, epicatechingallate, & epigallocatechin gallate (mg/100g)	Content of catechin (mg/100g)
Apples	6.1	0.6	0.9
Blackberries	4.7	0.8	37.1
Black Grapes	8.7	2.8	10.1
Black Tea (Brewed)	2.1	23.1	1.5
Green Tea (Brewed)	8.3	114.3	2.6
Cherries	7.0	0.4	1.3
Cocoa	26.2	0.00	0.00
Dark Chocolate	41.5	0.00	12.0

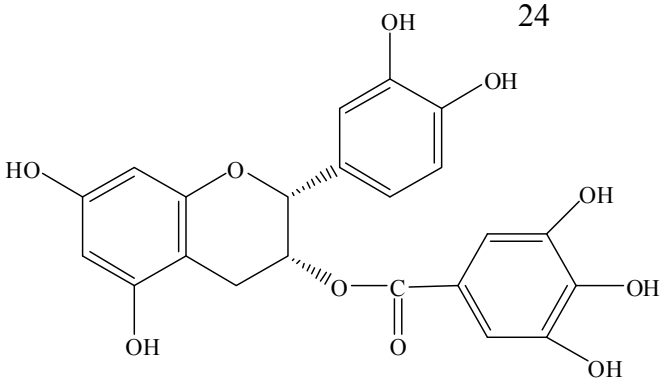
Fava Beans	7.8	4.7	8.2
Milk Chocolate	6.3	0.00	2.1
Pears	3.8	0.8	0.3
Raspberries	4.1	1.0	1.6
Red Table Wine	3.3	0.1	7.0

Green tea and its component catechins are well known for their antioxidant effect, which has led to their evaluation in a number of diseases associated with reactive oxygen species (ROS), such as cardiovascular, neurodegenerative and cancer diseases. Many epidemiological studies and studies in animal have shown that green tea can protect towards several cancers such as those of the breast, skin, lung and prostate (Mukhtar and Ahmad, 2000; Yang et al., 2002). In cardiovascular diseases, green tea consumption has been dealed with a lower incidence of Japanese people for coronary artery disease (Sano et al., 2004). Green tea extract also attenuated blood pressure increases in hypertensive rats, an effect comes from its antioxidant activity (Negishi et al., 2004). Whereas these studies indicate that drinking green tea may protect against drug interactions between green tea, cardiovascular diseases, and cardiovascular therapy, particularly in the Western part, where the use of cardiovascular drugs is widespread (Izzo et al., 2005). For neurodegenerative diseases, green tea protected against ethanol-induced oxidative stress in mice, and prevented serum protein and lipids from oxidative damage, produced by ethanol and enhanced by aging (Luczaj et al., 2004). For weight loss and obesity, many studies have revealed that consumption of green tea may protect against obesity-related disorders such as hypertension, diabetes and artherosclerosis.

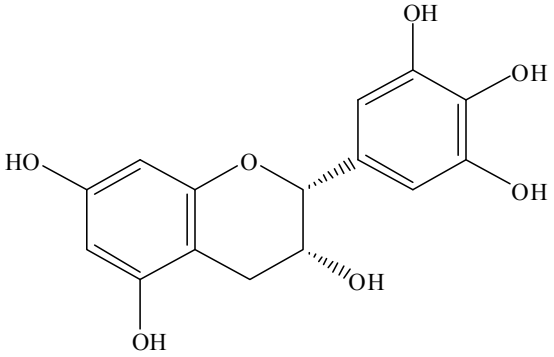
Interestingly, Kao and co-workers showed that EGCG (50–100 mg/kg), but not other green tea catechins, dramatically reduced or prevented an increase in body weight in obese and lean Zucker rats. According to the American Cancer Society, the consumption of green tea has been associated with a risk reduction of skin, stomach, esophagus, pancreas, colon, bladder, lung, breast cancer and prostate in an experimental models. Unfortunately, the results from human research do not currently support the findings from these cell and animal studies. Therefore, consumption of tea solely for cancer prevention is not recommended. For bone Density, in a recent investigation with elderly women (70-85 years old), green and black tea consumption was associated with an increase rate of hip bone mineral density (Devine et al., 2007). Followed by microbial diseases, green tea has been well-known to protect dental caries for centuries. Recently, EGCG has received significant attention for its action against HIV infection and multidrug-resistant *Staphylococcus aureus* infections (Nance and Shearer, 2003; Stapleton et al., 2004). Kawai et al. (2003) showed that EGCG prevents the attachment of the HIV-1 virion, gp120, to the CD4 molecules on T-helper cells, which would preventing the HIV-1 infection in the first step. EGCG has also been exhibited to inhibit HIV-1 replication by inhibiting HIV reverse transcriptase and by interfering the binding of the HIV envelope, therefore the virus particle unable to fuse with the host cell (Figure 7).



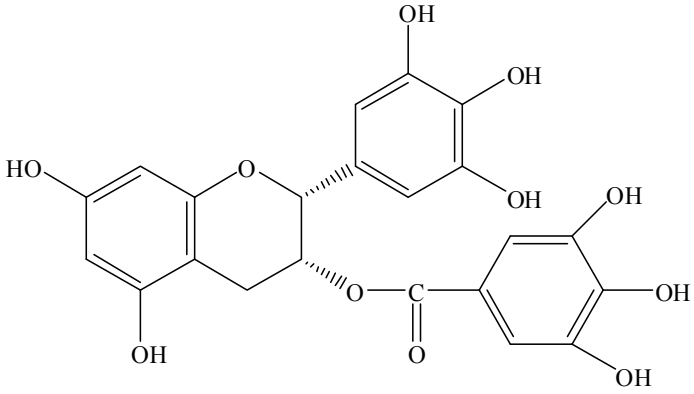
(–) epicatechin (EC)



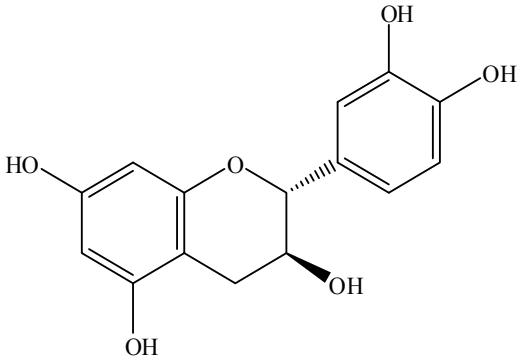
(–) epicatechingallate (ECG)



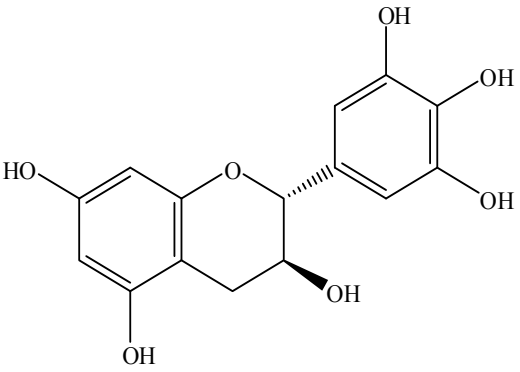
(–) epigallocatechin (EGC)



(–) epigallocatechingallate (EGCG)



(+) catechin



(+) gallocatechin (GC)

Figure 7 Structures of polyphenoliccatechins presenting in green tea.

(Zaveri, 2005)

CHAPTER 2

EXPERIMENTAL

2.1 General

2.1.1 Instruments

Equipments	Company, Country
Balance	Explorer, OHAUS Corp, USA
Hot air oven	Memmert, Germany
IR spectrophotometer	JASCO IR-810, Japan Spectroscopic, Japan
Mass spectrometer	MAT95 XL MS, Thermofinigan
Microplate reader	Biotek Power-x, BioTek Instruments, Inc, USA
Micropipette	Socorex, Switzerland; Pipetman, France
Rotary evaporator	Aspirator A-3S, EYELA, Japan
TLC cabinet	CN-6, Vilber Lourmat, France
TLC-plate silica gel GF_{254}	Merck, Germany
TLC-plate RP-18 F_{254s}	Merck, Germany
UV-VIS spectrophotometer	Genesis-6, Thermo scientific, USA
Water bath	Memmert, Germany

2.1.2 Chemicals

Chemicals	Company, Country
Acetic acid, glacial	Lab-scan Asia Co., Ltd., Bangkok, Thailand.
Anisaldehyde	Fluka, Switzerland
Chloroform, analytical grade	Lab-scan Asia Co., Ltd., Bangkok, Thailand.
Dichloromethane, analytical grade	Lab-scan Asia Co., Ltd., Bangkok, Thailand.
Ethanol (95%v/v)	Lab-scan Asia Co., Ltd., Bangkok, Thailand.
Ethyl acetate, analytical grade	Lab-scan Asia Co., Ltd., Bangkok, Thailand.
Hexane, analytical grade	Lab-scan Asia Co., Ltd., Bangkok, Thailand.
TLC-plate silica gel GF_{254}	Merck, Germany
TLC-plate RP-18 F_{254s}	Merck, Germany
Methanol	Lab-scan Asia Co., Ltd., Bangkok, Thailand.
Silica gel 60 (SiO_2 60, 230-400 mesh)	Merck, Germany
Sulfuric acid	J.T. Baker, USA

2.3 Plant materials

The *Albizia procera* bark was collected from the Suan Ya Thai Thongnoppakhun herbal garden in Angthong province in 2011 and were identified by a Thai traditional doctor, Mr. Sraupsin Thongnoppakhun, and the voucher specimen number is SKP 115011601, were stored at room temperature. The sample was kept at the Faculty of Pharmaceutical Sciences, Department of Pharmacognosy and Pharmaceutical Botany, Prince of Songkla University, Thailand.

2.4 Isolation of compounds from *Albizia procera* bark extract

Three kilograms dried weight of *Albizia procera* were cleaned, cut up into small pieces and ground to powder. The powder (3 kg) dried weight of plant which showed the highest inhibitory activity on HIV-IN were ground and macerated in 8 L of ethanol for five times at room temperature. The EtOH extract was evaporated and partitioned between hexane and water, and then partitioned with water and chloroform. After that, the water layer was partitioned with EtOAc. All partition was evaporated *in vacuo* to afford residues of hexane, chloroform, EtOAc and water fractions, respectively (Figure 8).

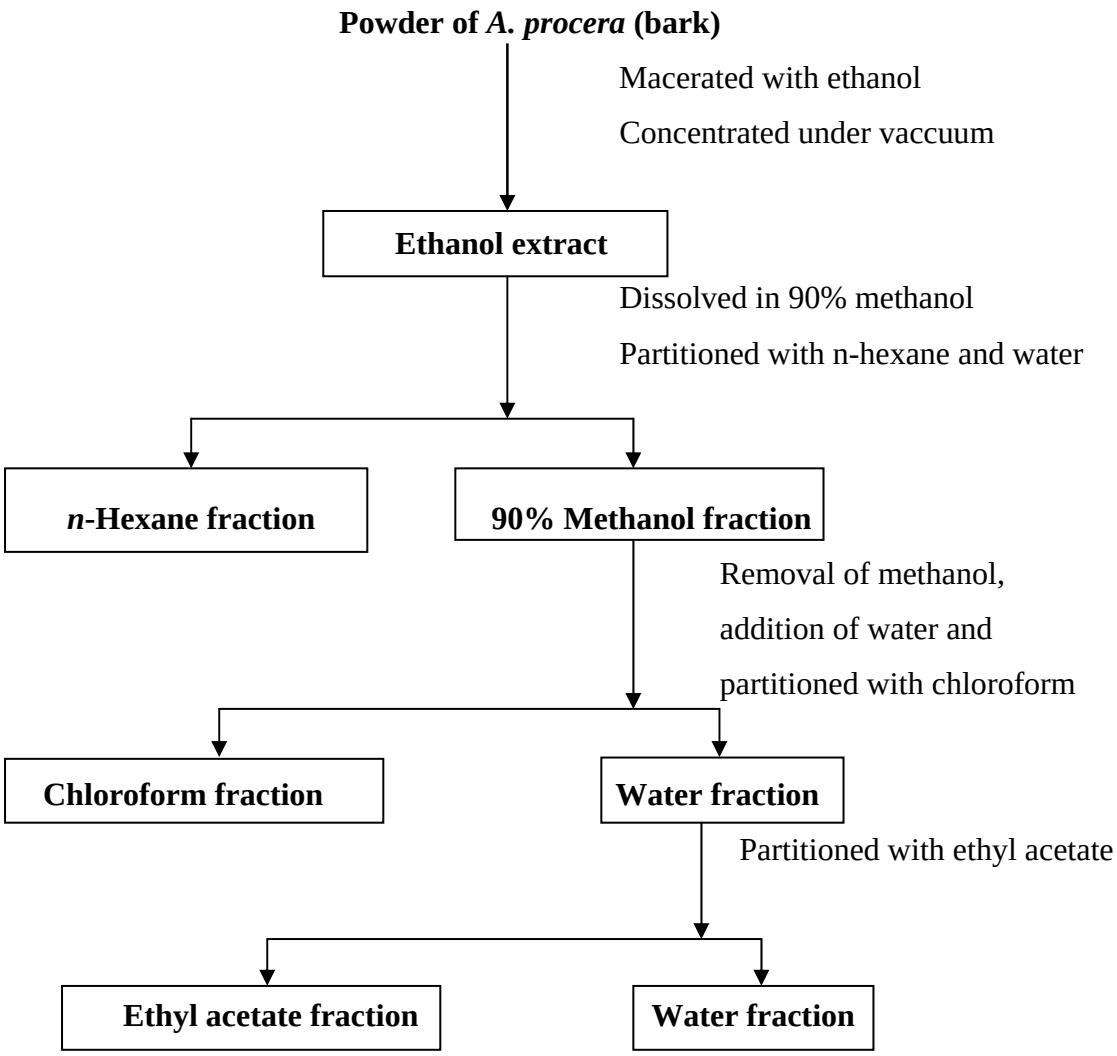


Figure 8 Flow chart of separation and partition of *A. procera* (bark)

2.5 Purification of compounds

Fractions of plant extract which showed the highest inhibitory activity on HIV-1-IN were purified by using chromatography technique such as classical column chromatography (silica gel, sephadex LH-20) and preparative thin layer chromatography (PTLC). After that, compounds were tested for purification by using

thin layer chromatography (TLC), and the structures were interpreted using spectroscopy techniques.

2.6 Structure elucidation

For the interpretation of spectral data and elucidation the structures of compounds, we used ultraviolet visible spectroscopy (UV-Vis Spectroscopy), mass spectroscopy (MS), nuclear magnetic resonance spectroscopy (NMR), and infrared spectroscopy (IR).

2.7 Anti-HIV-1 IN assay

Anti-HIV-1 IN effect was detected following the previous method (Tewtrakul et al., 2001). Briefly, 45 µL of a mixture, composed of IN buffer 12 µL [containing 3-(*N*-morpholino) propane sulfonic acid 150 mM, (MOPS) pH 7.2, 5 mM dithiothritol (DTT), 75 mM $MnCl_2$, glycerol 25% and BSA (bovine serum albumin) 500 µg/mL], digoxigenin-labelled target DNA (5 pmol/mL) 1 µL and distilled water 32 µL, was added into a 96-well plate. Subsequently, sample solution (6 µL) and 1/5 dilution of integrase enzyme (9 µL) was added to each well and incubated for 80 min at 37°C. The wells were washed 4 times with PBS, and the alkaline phosphatase (AP) labelled anti-digoxigenin antibody (500 mU/mL) 100 µL was then added to the wells and incubated for 1 h at 37°C. The microplate was washed again with washing buffer containing 0.05% Tween 20 (in PBS) 4 times and with PBS 4 times. Afterthat, AP

buffer (150 µL) containing Tris-HCl 100 mM (pH 9.5), 5 mM $MgCl_2$, 100 mM NaCl and 10 mM *p*-nitrophenyl phosphate was added to the well and incubated for 1 h at 37°C. At final, the well-plate was determined with a plate reader at a 405 nm. A control is composed of a reaction mixture, DMSO 50% and an IN enzyme, whereas a blank was that of the buffer-E containing 20 mM MOPS (pH 7.2), 1 mM ethylenediamine tetraacetate disodium salt (EDTA. 2Na), 400 mM potassium glutamate, 0.1% Nonidet-P 40 (NP-40), DTT 1 mM, 20% glycerol and urea 4 M without the IN enzyme (Figure 9). Suramin (a polyanionic HIV-1 IN inhibitor) was used as a positive control. The % inhibition on HIV-1 IN was calculated as follows:

% Inhibition on HIV-1 IN = [(OD control - OD sample)/ OD control] x 100

The OD is an absorbance which is detected from each well.

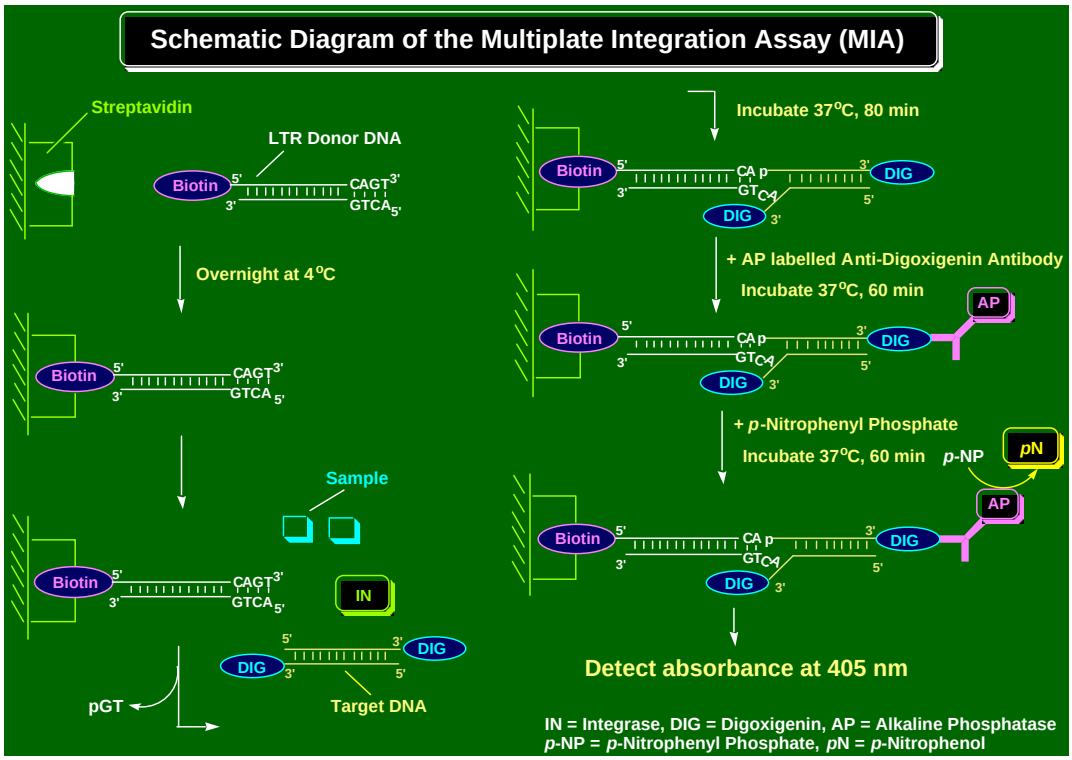


Figure 9 The multiplate integration assay (MIA)

2.8 Molecular docking method

2.8.1 Ligand preparation

The three dimensional structure of pure compounds were generated using Hyperchem professional 8.0. Energy minimization of each compound was performed using the PM3 semi-empirical method. Subsequently, geometry optimization was done for each compound using energy 0.05 kcal/mol conjugated gradientsalgorithm. Before docking, Gasteiger charges were assigned to each compound.

2.8.2 HIV-1 IN preparation

The HIV-1 IN crystal structure of the core domain, residues CYS56–GLN209, complexed with inhibitor 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)-propenone (5-CITEP) in the active site was obtained from Protein Data Bank (PDB code 1QS4). Only Chain A which co-crystallized with 5-CITEP was selected, whereas chains B and C were deleted. All the molecules of water and 5-CITEP were deleted while a Mg^{2+} ion at the active site was present. The loop of amino acid residues 141-144 (missing residues) were incorporated and polar hydrogen atoms were added to this chain (Vajragupta et al., 2005). The second Mg^{2+} ion was placed in the same relative position according to the two metal structure of the Prototype Foamy Virus integrase (PDB code 3OYA), a high structural homolog to HIV-1 IN (Krishnan & Engelman, 2012).

2.8.3 Molecular docking

Molecular docking was used with the AutoDock program version 4.2. Before docking, the grid boxes were prepared for IN structures using AutoGrid 4.0. The center of the grid boxes were set on the location of the co-crystallized inhibitor. The grid dimensions were set to 60 x 60 x 60 Å in each dimension, with a grid spacing of 0.375 Å which is large enough for the free rotation of the ligand.

The docking calculations were using of the Lamarckian genetic algorithm (LGA). The optimized docking parameters were set as follows: population size was 150, the number of GA run was 100, the maximum number of generation in the genetic algorithm was increased to 100,000 and the maximum number of energy evaluations was increased to 2,500,000 per run. Other docking parameters were set at their default.

A cluster analysis was used to group similar conformation, 100 independent conformations of each compound with the root mean square deviation (RMSD) differ less than 2 Å were grouped together. The best docked conformations were the greatest number of conformations in the cluster and the lowest binding energy (Healy et al., 2009). Amino acids within 6 Å of the ligand in the HIV-1 IN active site were selected for H-bond interactions analysis using the H-bond monitor in the program of DS Viewer Pro.

2.9 Statistical analysis

The IC_{50} values are expressed as four determinations of mean ± S.E.M. and the Microsoft Excel programme was used.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Isolation and structure elucidation

The aqueous and EtOH extracts of *Albizia procera* (bark) were screened for their inhibitory effects against HIV-1 IN using the multiplate integration assay (MIA). The EtOH fraction and water fraction showed anti-HIV-1 IN effect with IC_{50} values of 19.5 μg/mL and 5.9 μg/mL respectively.

Three kilograms dried weight of *Albizia procera* were cleaned, cut up into small pieces and ground to powder. The powder bark materials (3 kg) were subjected to maceration in 8 L of ethanol for five times at room temperature. The solvent were removed under reduced pressure to give the crude product 202.2 g of EtOH extract and then partitioned between 90 % methanol and hexane, removed of methanol, added of water and partitioned with chloroform. After that the water layer was partitioned with ethyl acetate. Each partition was evaporated to dryness *in vacuo* to give residues of hexane (7.0 g), chloroform (0.6 g), ethyl acetate (14.4 g) and water fractions (98.4 g) respectively (Figure 10).

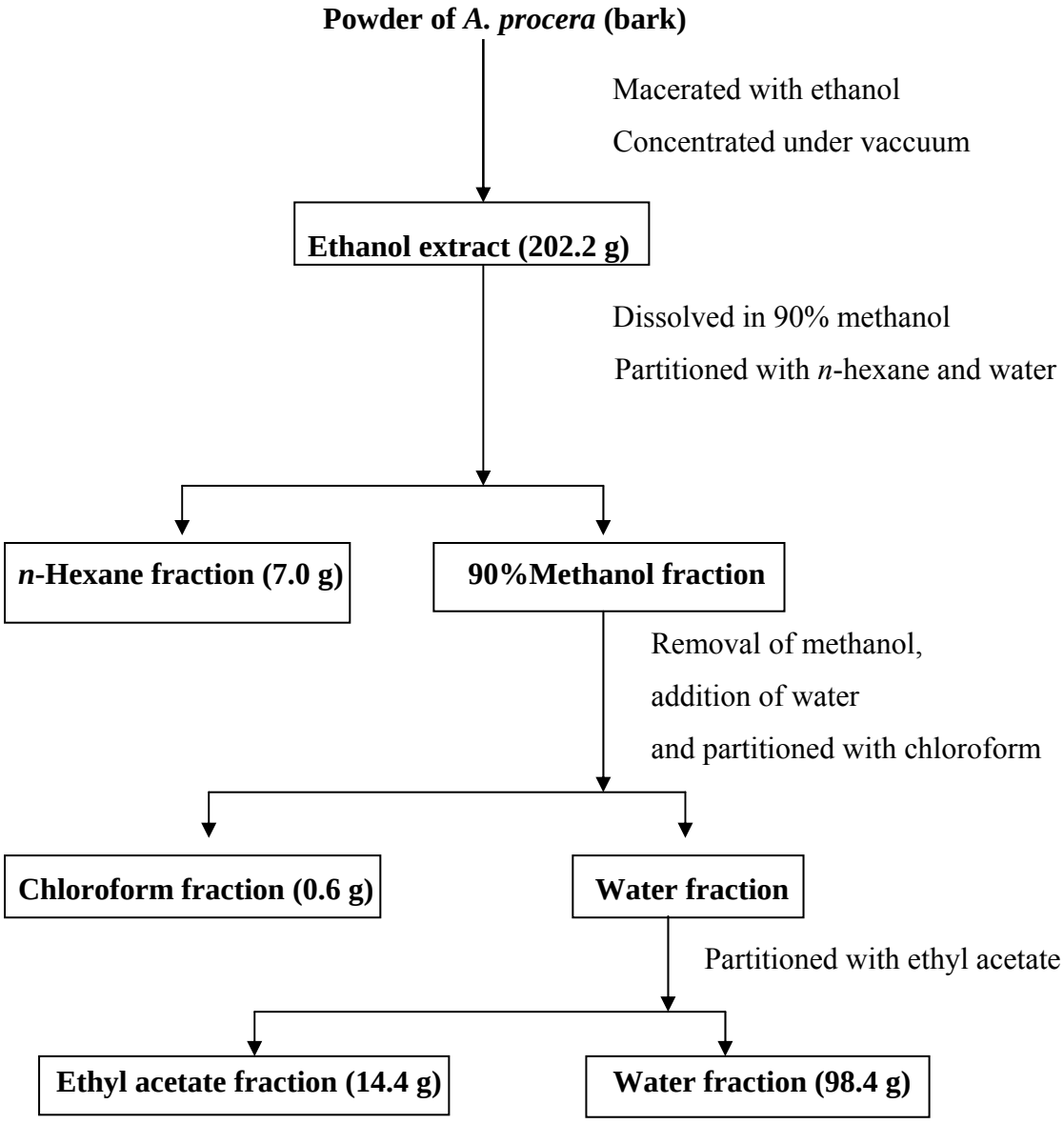


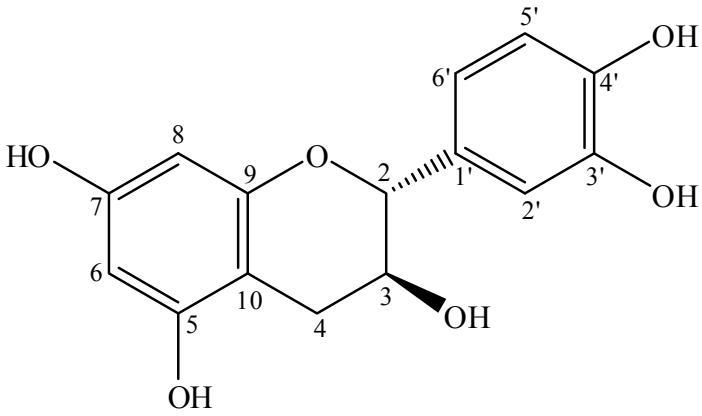
Figure 10 The extraction procedure of *Albizia procera* bark

3.2 Isolation of compounds from ethyl acetate fraction

The fractions were dried under reduced pressure and then redissolved in 50% DMSO for bioassay. These fractions were prepared in the concentration of 3-100 μg/mL. The ethyl acetate fraction (14.4 g), which showed good separation on thin layer chromatography (TLC) and exhibited marked anti-HIV-1 IN activity (IC_{50} = 19.1 μg/mL), was further separated by silica gel column chromatography using EtOAc:MeOH:H_2O (98:1:1 to 96:2:2) to afford 113 fractions (F1-F113). Fractions F4-F5 (0.74 g) was purified on silica gel (20 g) using $CHCl_3$:MeOH (8:2) to 100% MeOH to give 33 subfractions (F1a-F33a). F6a-F8a (0.32 g) was purified by column chromatography on Sephadex LH-20 using 100% MeOH to obtain F1b-F9b. F3b was chromatographed on Sephadex LH-20 using 100% MeOH to afford (+)-catechin (**1**) (white solid, 13.3 mg, 0.09% w/w). Fraction F6-F13 (0.21 g) was purified by column chromatography on silica gel using $CHCl_3$:MeOH (9:1) to give F1d-F7d. F6d-F7d (15 mg) was chromatographed on Sephadex LH-20 using 100% MeOH to give protocatechuic acid (**2**) (white solid, 10.8 mg, 0.07% w/w).

3.3 Structure elucidation of the isolated compounds

The structures of compounds **1-2** were elucidated using spectroscopic techniques and compared with reported spectral data (Devis et al., 1996).



Identifications of compound 1

(+)-Catechin (**1**): colorless crystalline solid**,** the UV spectrum showed maximum absorption bands at 289, 375 nm which revealed the presence of conjugated system.

The IR spectrum revealed absorption bands for O-H group (3326 cm^{-1}), C=C group (1609 cm^{-1}) and C-O group (1142 cm^{-1}).

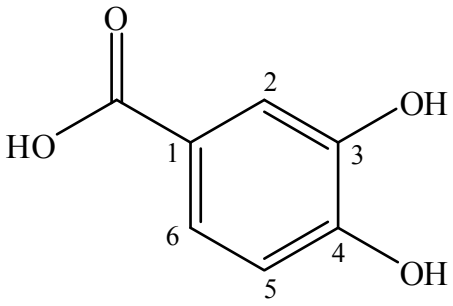
The ^{13}C-NMR spectral data were recorded in CD_3OD showed the existence of 15 signals for 15 carbon atoms in the molecule. This compound suggested the presence of seven quaternary aromatic carbons at δ 100.8, 132.2, 146.2, 146.2, 156.9, 157.6, 157.8, seven methine aromatic carbons at δ 68.2, 82.9, 95.5, 96.3, 115.2, 116.1, 120.0 and a signal of methylene aromatic carbons at 28.5.

The ^{1}H-NMR spectral data were recorded in CD_3OD showed four pyran ring protons at δ 2.49 (1H, dd, *J*=16.5, 8.0 Hz, H-4b), 2.80 (1H, dd, *J*=16.5, 5.5 Hz, H-4a), 3.96 (1H, m, H-3), 4.55 (1H, d, *J*=8.0 Hz, H-2) and five aromatic protons at 5.84 (1H, d, *J*=2.0 Hz, H-8), 5.92 (1H, d, *J*=2.0 Hz, H-6), 6.71 (1H, d, *J*=8.0 Hz, H-5′), 6.75 (1H, dd, *J*=8.0, 2.0 Hz, H-6′) and 6.83 (1H, d, *J*=2.0 Hz, H-2′).

Thus, the structure of compound **1** was determined to be (+)-catechin. The data was confirmed by comparison with spectral analysis data reported in the literature (Devis et al., 1996).

Table 3 Spectral data of compound **1** (CD_3OD, 500 MHz for ^1H NMR, CD_3OD, 125 MHz for ^{13}C NMR) comparing with the reference compound **R**

Position	Type of C	δ_C/ppm		δ_H/ppm	
		compound **1**	**R**	compound **1**	**R**
2	CH	82.9	82.7	4.55 (d, *J*=8.0)	4.56 (d, *J*=7.8)
3	CH (OH)	68.2	68.3	3.96 (m)	3.99 (ddd, *J*=8.4, 7.8, 5.5)
4	CH_2	28.5	28.8	H-4a, 2.80 (dd, *J*=16.5, 5.5) H-4b, 2.49 (dd, *J*=16.5, 8.0)	H-4a, 2.91 (dd, *J*=16.1, 5.5) H-4b, 2.53 (dd, *J*=16.1, 8.4)
6	CH	96.3	96.1	5.92 (d, *J*=2.0)	6.02 (d, *J*=2.3)
8	CH	95.5	95.5	5.84 (d, *J*=2.0)	5.88 (d, *J*=2.3)
5	C (OH)	157.6	157.2	-	-
7	C (OH)	157.8	157.7	-	-
9	C	156.9	156.9	-	-
10	C	100.8	100.6	-	-
1′	C	132.2	132.2	-	-
2′	CH	115.2	11 5.2	6.83 (d, *J*=2.0)	6.89 (d, *J*=1.9)
3′	C (OH)	146.2	145.6	-	-
4′	C (OH)	146.2	145.7	-	-
5′	CH	120.0	11 5.7	6.71 (d, *J*=8.0)	6.79 (d, *J*=8.1)
6′	CH	116.1	120.0	6.75 (dd, *J*=8.0, 2.0)	6.75 (dd, *J*=8.0, 1.9)



Identifications of compound 2

Protocatechuic acid (**2**): white solids, the UV spectrum showed maximum absorption bands at 299 nm which revealed the presence of conjugated system.

The IR spectrum showed characteristic bands patterns for O-H group (3436 cm^{-1}) and C-O group (1611 cm^{-1}).

The ^{13}C-NMR spectral data were recorded in CD_3OD showed 7 signals for 7 carbons. This compound presented a signal of quaternary aromatic carbon at 127.2, three methine aromatic carbons at δ 115.8, 117.9, 123.9, two signals characteristic of phenolic carbons at 146.1, 151.4 and a signal of carboxylic acid carbon at 170.9.

The ^{1}H-NMR spectral data were recorded in CD_3OD showed trisubstituted benzene protons at δ 6.78 (1H, d, *J*=8.0 Hz, H-5), 7.42 (1H, dd, *J*=8.0, 2.0 Hz, H-6) and 7.43 (1H, d, *J*=2.0 Hz, H-2).

Thus, the structure of compound **2** was identified to be protocatechuic acid. The data was confirmed by comparison with spectral data reported in the literature (Lee et al., 2010).

Table 4 Spectral data of compound **2** (CD_3OD; 500 MHz for ^1H NMR, CD_3OD, 125 MHz for ^{13}C NMR) comparing with the reference (CD_3OD; 300 MHz for ^1H NMR, CD_3OD, 75.5 MHz for ^{13}C NMR) compound **R**

Position	Type of C	δ_C/ppm		δ_H/ppm	
		compound **2**	**R**	compound **2**	**R**
1	C	127.2	127.5	-	-
2	CH	117.9	117.8	7.43 (d, *J*=2.0)	7.42 (d, *J*=2.1)
3	C (OH)	146.1	145.6	-	-
4	C (OH)	151.4	150.0	-	-
5	CH	115.8	115.4	6.78 (d, *J*=8.0)	6.74 (d, *J*=8.4)
6	CH	123.1	123.4	7.42 (dd, *J*=8.0, 2.0)	7.38 (dd, *J*=8.4, 2.1)
7	COOH	170.9	173.4	-	-

3.4 Effect of fractions and isolated compounds of *Albizia procera* on anti-HIV-1IN activity

Since the EtOH extract of *Albizia procera* possessed potent anti-HIV-1 IN effect (IC_{50} = 19.5 μg/mL), the hexane, chloroform, ethyl acetate and water fractions of *Albizia procera* bark were then determined for anti-HIV-1 IN activity. Among the tested samples, the ethyl acetate fraction exhibited the most potent inhibitory effect with an IC_{50} value of 19.1 μg/mL, followed by water fraction (IC_{50} = 21.3 μg/mL), hexane and chloroform fractions (IC_{50} > 100 μg/mL), respectively (Table 5). From bioassay-guided fractionation, two compounds were isolated from the ethyl acetate fraction of *Albizia procera* which are (+)-catechin (**1**) and protocatechuic acid (**2**), respectively. Of the tested samples, (+)-catechin (**1**) exhibited marked effect towards HIV-1 IN with an IC_{50} of 46.3 μM, whereas protocatechuic acid (**2**) showed mild activity (IC_{50} >100 μM) with 46.0% inhibition at concentration of 100 μM (Table 6). This is the first result on anti-HIV-1 IN effect of *Albizia procera* bark. These results may suggest that *Albizia procera* bark has potential for treating AIDS patients.

Table 5 Anti-HIV-1 IN effect of EtOH extract and fractions from *Albizia procera* bark

Sample	% Inhibition at various concentrations (μg/mL)					IC_{50} (μg/mL)
	1	3	10	30	100	
EtOH ext.	-	-	26.6±3.2	70.2±1.8	93.5±0.4	19.5
Hexane fr.	-	-	-	-	27.1±4.7	>100
$CHCl_3$ fr.	-	-	-	-	34.4±1.8	>100
EtOAc fr.	-	-	29.6±3.0	65.4±2.7	98.1±0.8	19.1
H_2O fr.	-	-	22.8±3.3	65.0±1.3	99.3±0.9	21.3
Suramin (Positive control)	20.6 ±4.6	29.0±2.9	60.4±1.7	82.4±1.3	83.1±0.7	7.0 μM (10.0 μg/mL)

Table 6 % Inhibition and IC_{50} values of isolated compounds from ethyl acetate fraction of *Albizia procera* bark against HIV-1 IN activity

Compound	% Inhibition at various concentrations (μM)				IC_{50} (μM)
	3	10	30	100	
(+)-Catechin	-	31.2±0.6	34.2±0.9	66.2±1.4	46.3±0.5
Protocatechuic acid	-	31.9±2.4	36.4±1.9	46.0±1.1	>100
Suramin (Positive control)	29.0±2.9	60.4±1.7	82.4±1.3	83.1±0.7	7.0±0.8

3.5 Molecular docking study of compounds 1 and 2

From the molecular docking study, it was shown that (+)-catechin (**1**) interact with Thr66, Gly148 and Glu152 in the core domain of IN enzyme (Figure 11, Table 7), whereas protocatechuic acid (**2**) could bind with Thr66, His67, Glu152, Asn155 and Lys159 (Figure 12, Table 7). The result showed that these two compounds bind with the Glu152 which is one of the amino acid residue in the catalytic triad of HIV-1 IN core domain. However (+)-catechin (**1**) showed lower binding energy (-5.16 kcal/mol) than that of protocatechuic acid (**2**, -4.85 kcal/mol), thereby (+)-catechin (**1**) could have good interaction with HIV-1 IN and possessed higher effect than protocatechuic acid (**2**).

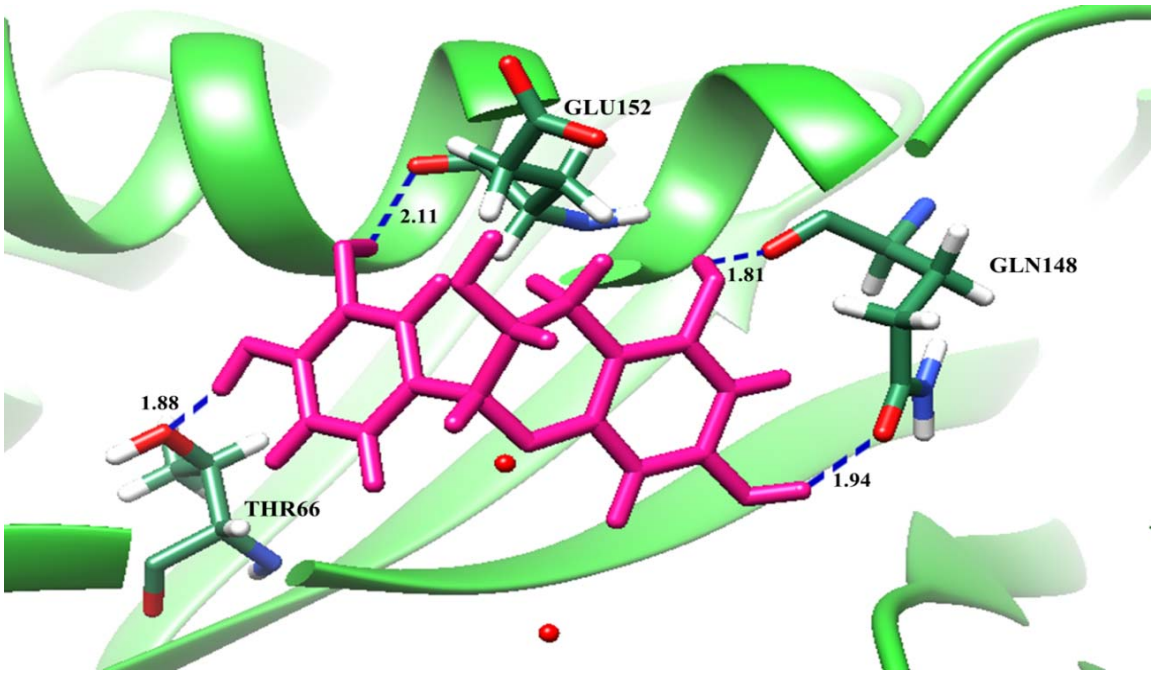


Figure 11 Molecular docking of (+)-catechin (**1**) with HIV-1 IN. The blue dash lines are H-bond interactions and represent bond length in angstrom (Å). Mg^{2+} ions are as red balls. The ribbon model shows the backbone of HIV-1 IN catalytic domain with the interacting amino acid residues shown as stick models and colored by element. (+)-Catechin is displayed pink stick model.

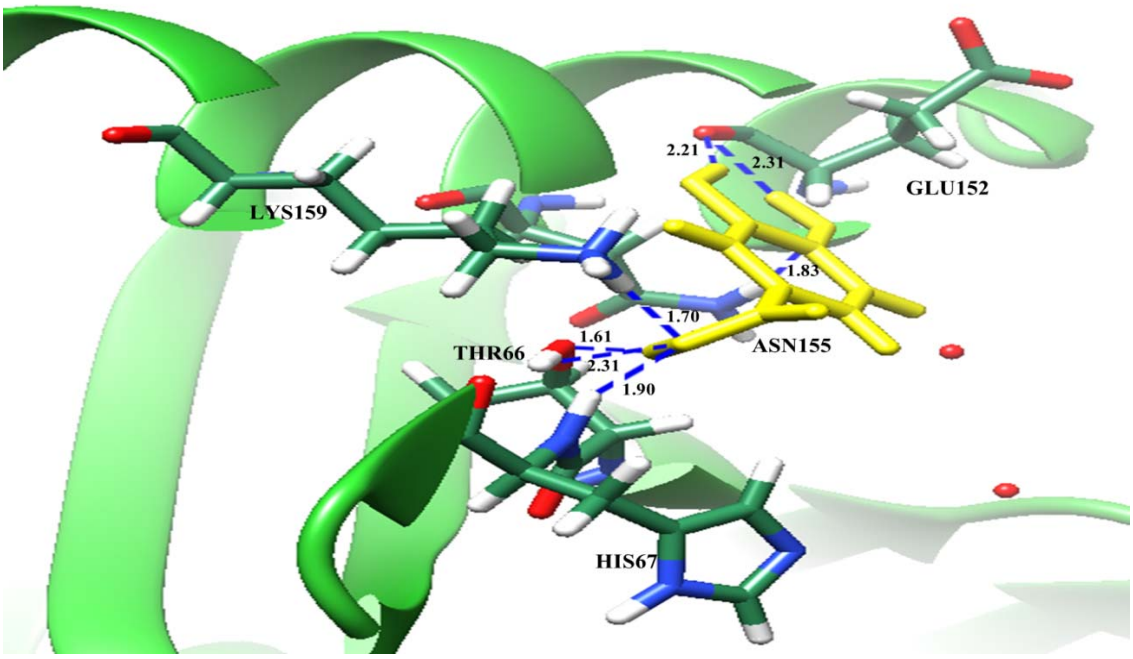
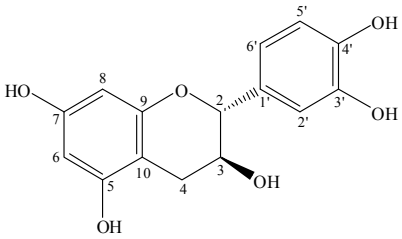
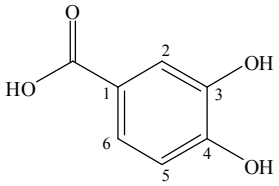


Figure 12 Molecular docking of protocatechuic acid (**2**) with HIV-1 IN. The blue dash lines are H-bond interactions and represent bond length in angstrom (Å). Mg^{2+} ions are exhibited as red balls. The ribbon model shows the backbone of HIV-1 IN catalytic domain with the interacting amino acid residues shown as stick models and colored by element. Protocatechuic acid is represented as yellow stick model.

Table 7 Anti-HIV 1 IN effect and molecular docking study of pure compounds from *Albizia procera* bark

Compounds	Anti-HIV-1-IN activity (IC_{50}; µM)	Binding energy (kcal/mol)	Amino acid residues with form H-bonding for each compound (distance : Å)						
			ASP64	THR66	HIS67	GLN148	GLU152	ASN155	LYS159
(+)-Catechin (**1**) 	46.3	-5.16 C =1 F = 33	-	OG1---4'-H (1.88)	-	O---5-H (1.81) OE1---7-H (1.94)	O---3'-H (2.11)	-	-
Protocatechuic acid (**2**) 	>100	-4.85 C = 2 F = 47	-	OG1---7-H (1.61) HG1---7-O (2.31)	HN---7-O (1.90)	-	O---3-H (2.21) O---4-H (2.31)	HD21---4-O (1.83)	HZ3---7-O (1.70)

Regarding the compounds isolated from *Albizia procera* bark, triterpenoid saponins with N-acetyl sugar were reported to exhibit cytotoxic effect against HEPG2 cell line (Melek, et al., 2007). Echinocystic acid bisglycosides, the triterepenoid glycoside derivatives, isolated from this plant bark also possessed anti-cancer activity against HEPG2, HT-29 and MCF-7 cells (Miyase et al., 2010). (+)-Catechin has been reported to exhibit anti-bacterial effect against *Helicobactor pylori* (Mabe et al., 1999). Catechins isolated from green tea was also had anti-viral activity on influenza virus (Song, et al., 2005). Protocatechuic acid has been shown to have anti-inflammatory and analgesic properties in rats and mice (Lende et al., 2011). This compound also inhibited cancer cell metastasis involving down regulation of NF-κB pathway (Lin et al., 2011).

CHAPTER 4

CONCLUSION

The EtOH extract of *A. procera* (bark) exhibited potent activity against HIV-1 IN (IC_{50} = 19.5 μg/mL). This extract was then further partitioned to four fractions of hexane (7.0 g), chloroform (0.6 g), ethyl acetate (14.4 g) and water fractions (98.4 g) which were investigated for their inhibitory activity against HIV-1 IN using the MIA assay. Among the tested fractions, the ethyl acetate fraction exhibited the most potent inhibitory activity with an IC_{50} value of 19.1 μg/mL, followed by water, chloroform and hexane fractions with IC_{50} values of 21.3, > 100 and > 100 μg/mL, respectively. These results provide the basic guideline for traditional use of *A. procera* bark for AIDS treatment.

Two compounds which are (+)-catechin (**1**) and protocatechuic acid (**2**) were isolated from the EtOAc fraction of *Albizia procera*. (+)-Catechin (**1**) exhibited the highest activity against HIV-1 IN with an IC_{50} of 46.3 μM, whereas protocatechuic acid (**2**) possessed mild effect (IC_{50} >100 μM). The result showed that these two compounds bind with the Glu152 which is one of the amino acid residue in the catalytic triad of HIV-1 IN core domain. However (+)-catechin (**1**) showed lower binding energy (-5.16 kcal/mol) than that of protocatechuic acid (**2**, -4.85 kcal/mol), thereby (+)-catechin (**1**) could have good interaction with HIV-1 IN and possessed higher activity than protocatechuic acid (**2**).

This is the first report on anti-HIV-1 IN activity of *Albizia procera* bark. These results may suggest that *Albizia procera* bark has potential as anti-HIV-1 IN agent which may support to use this plant for treating HIV infection.

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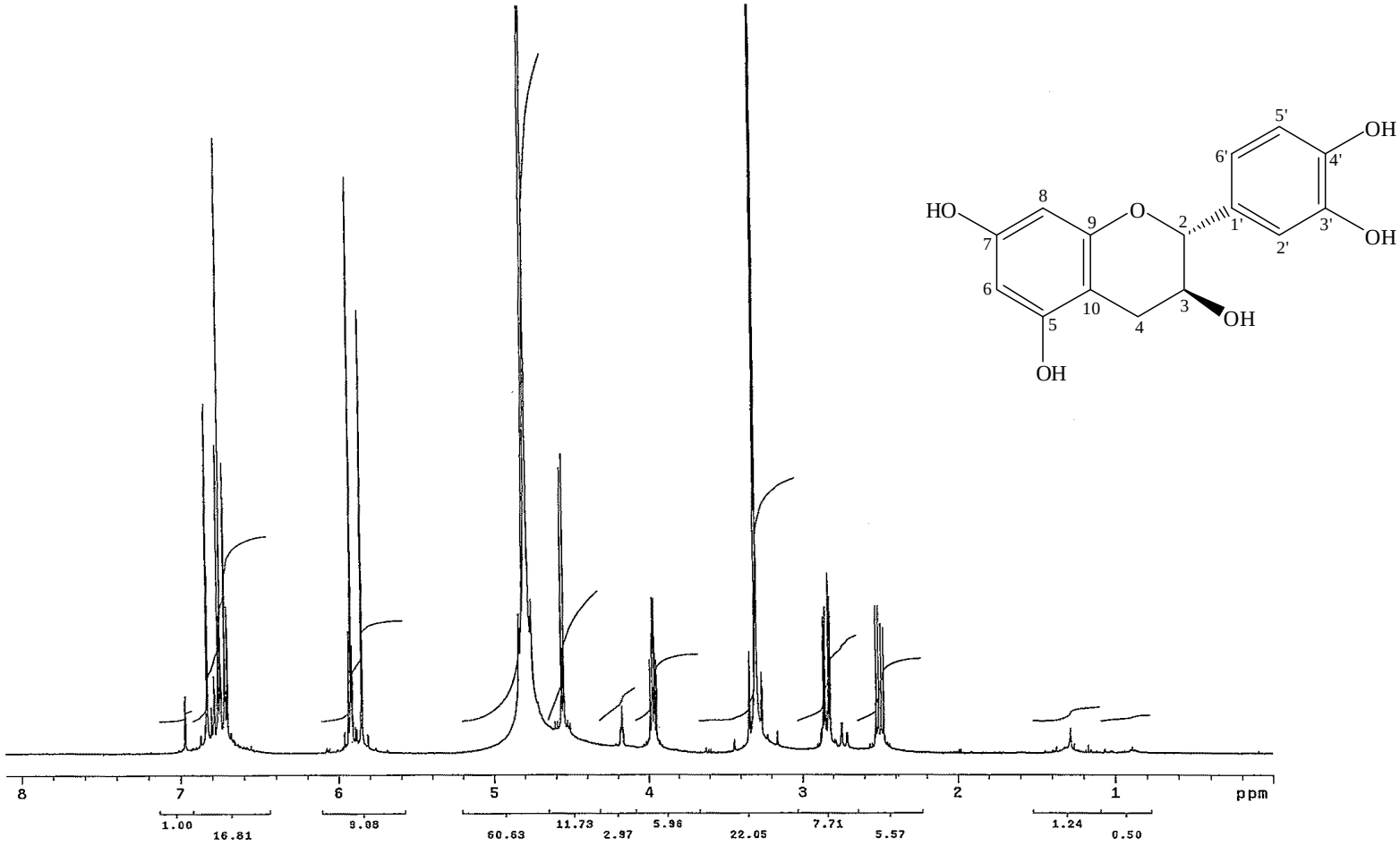
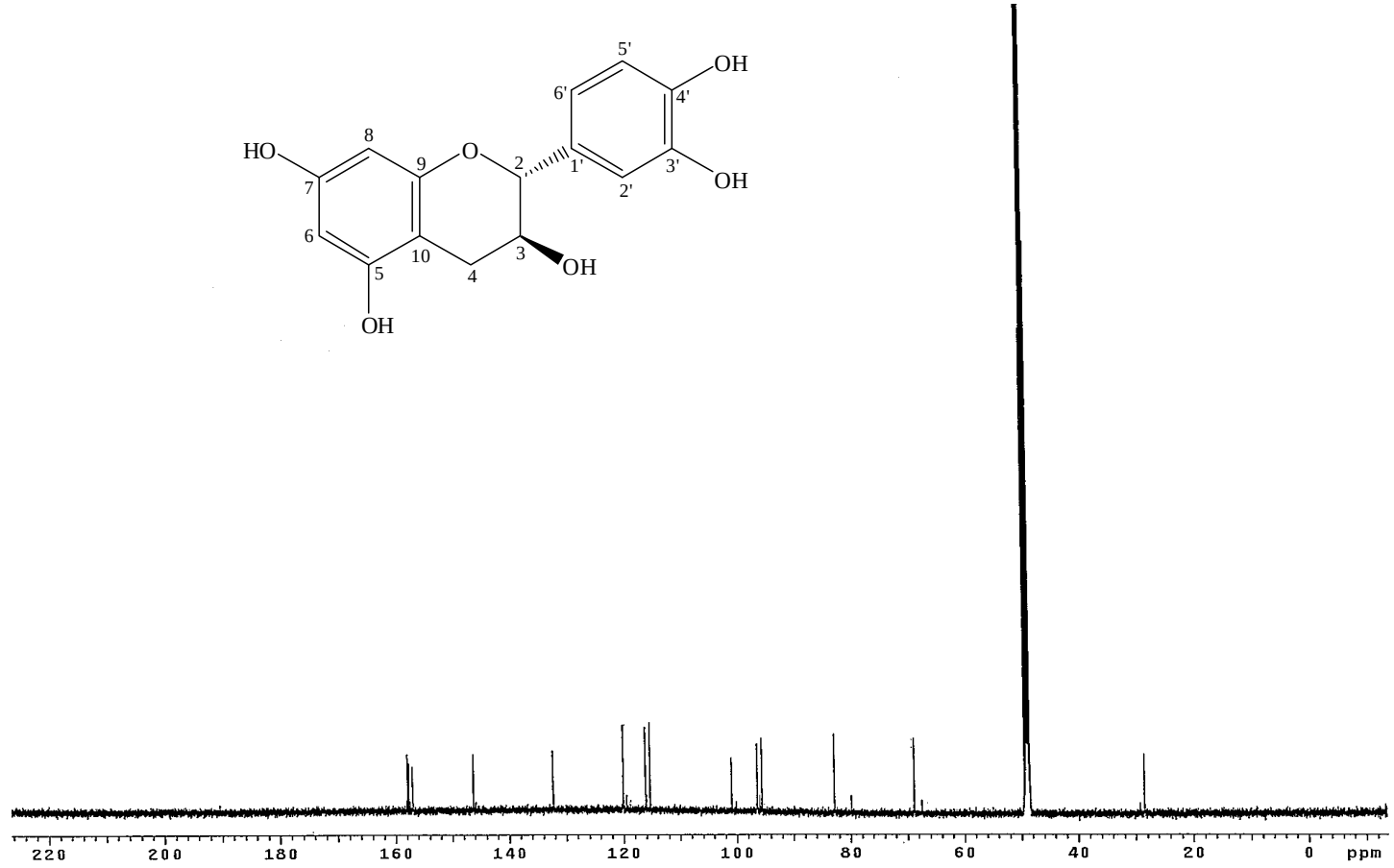
APPENDIX



^{1}H NMR spectrum of compound **1** (CD_3OD, 500 MHz)



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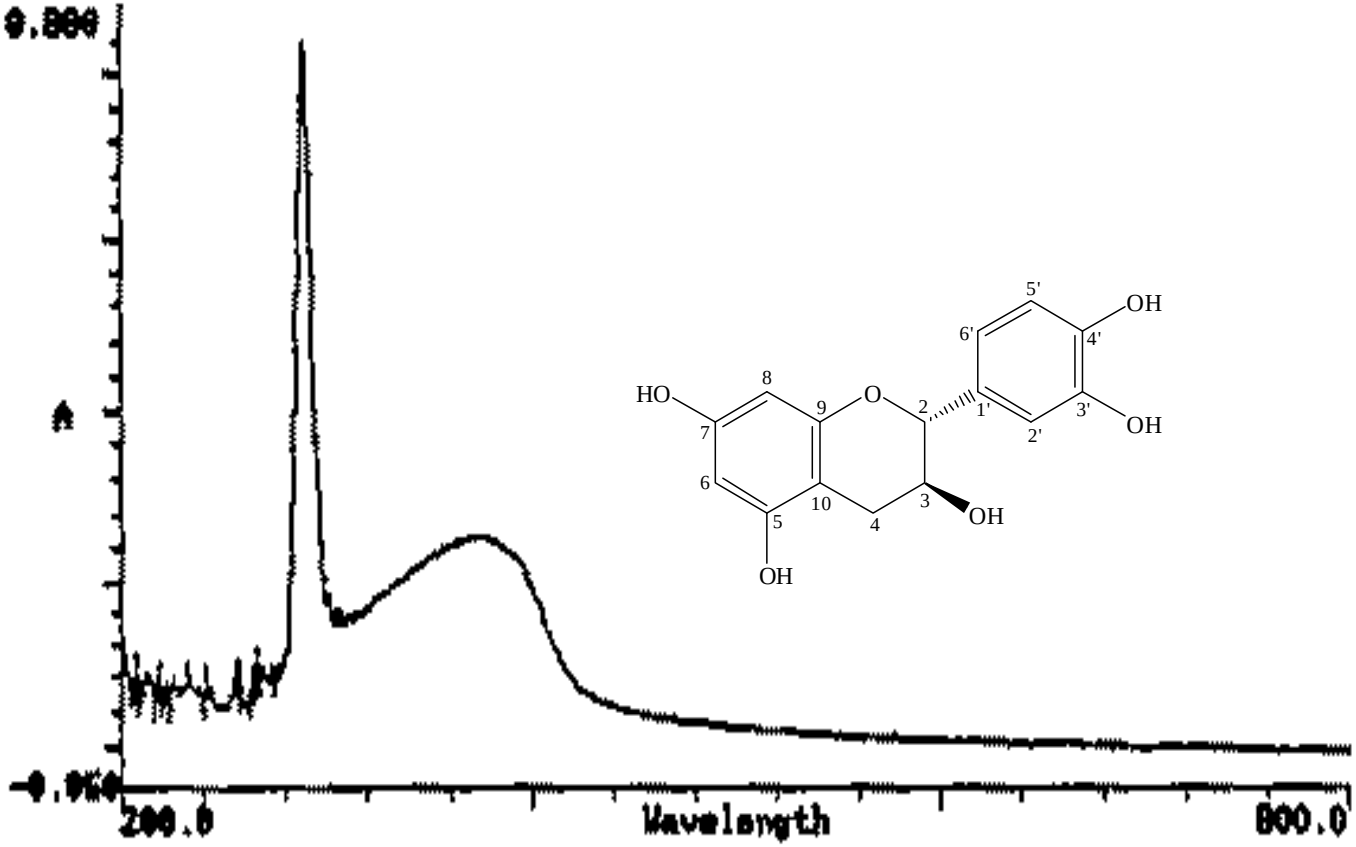

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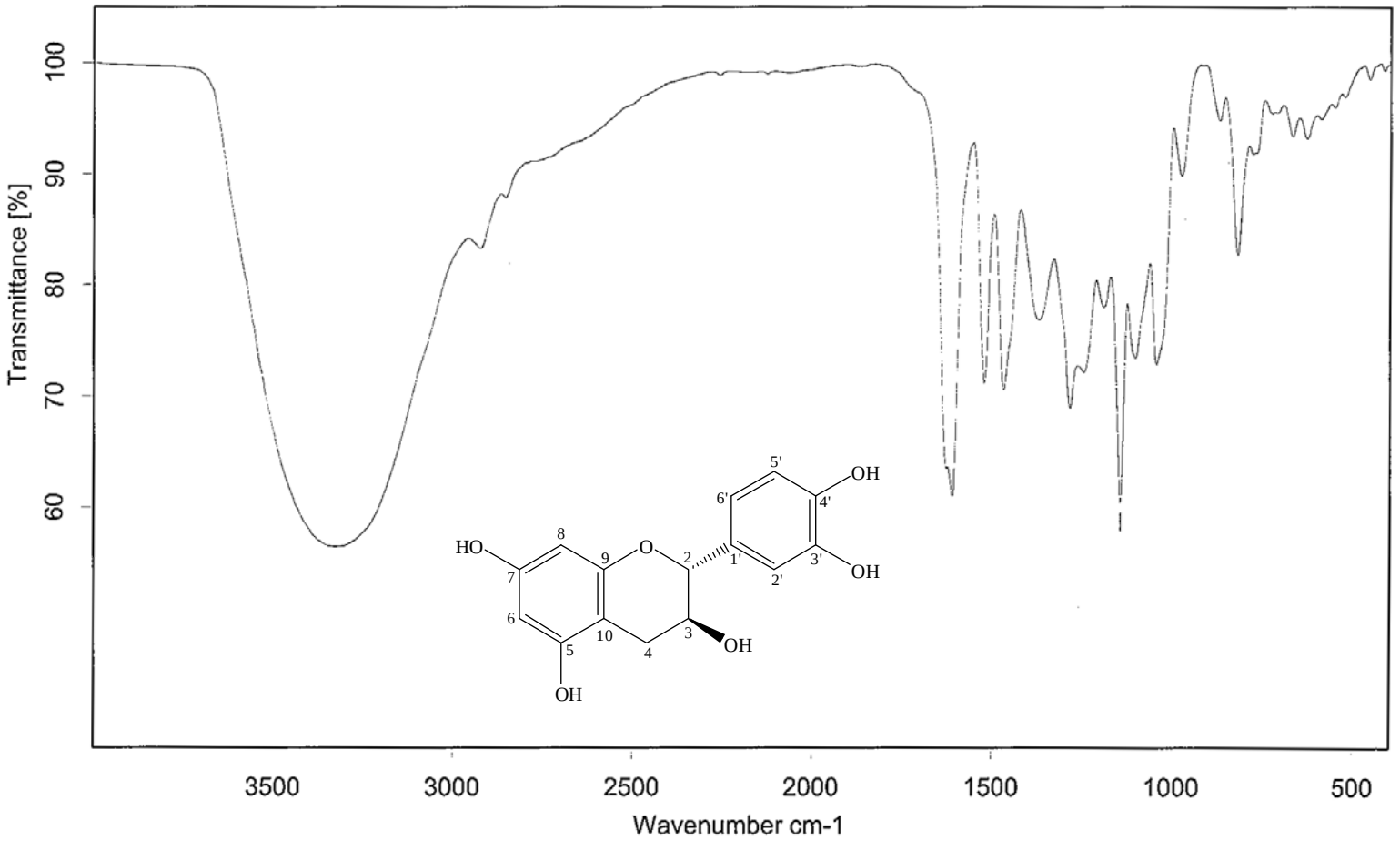

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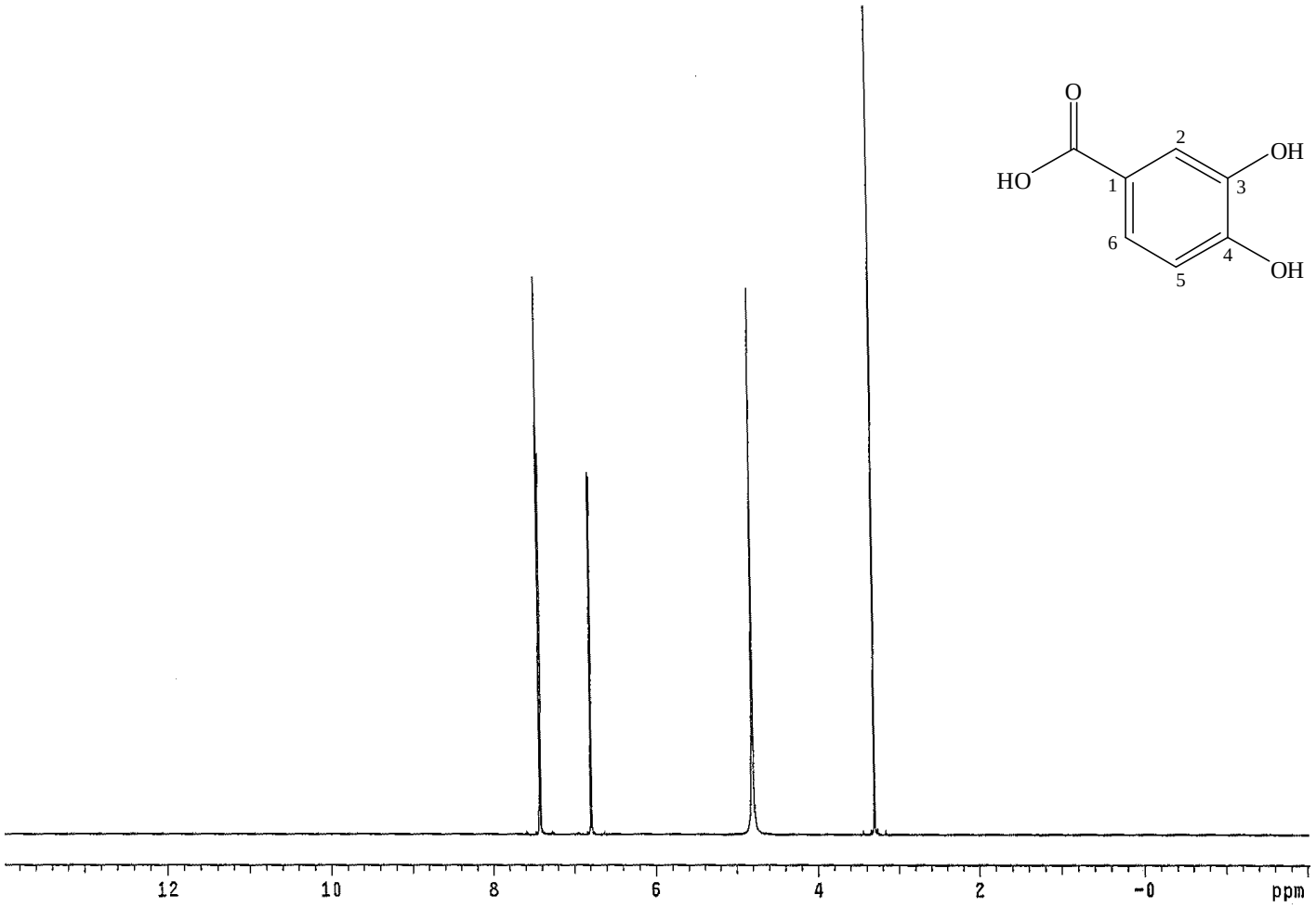

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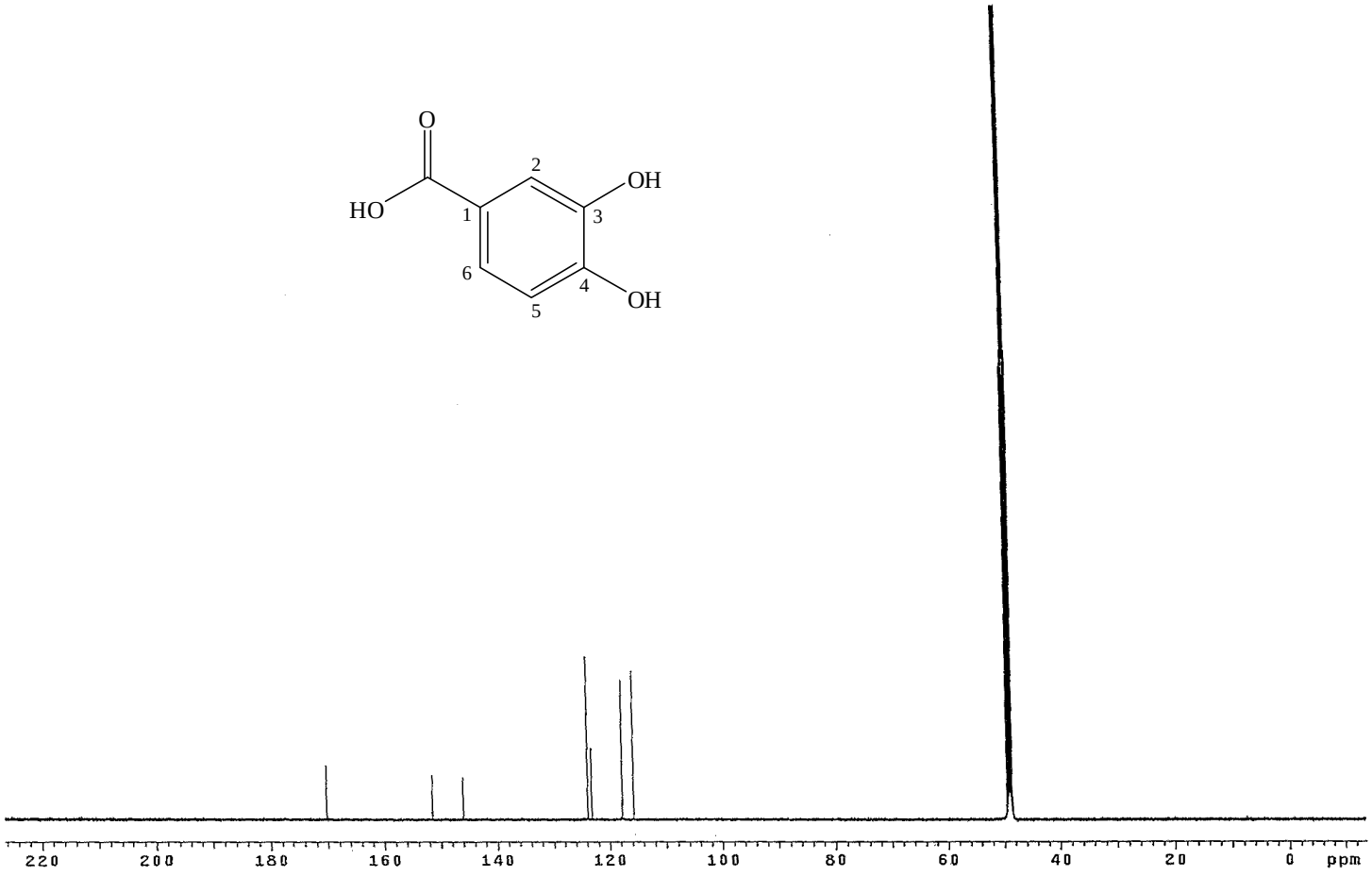

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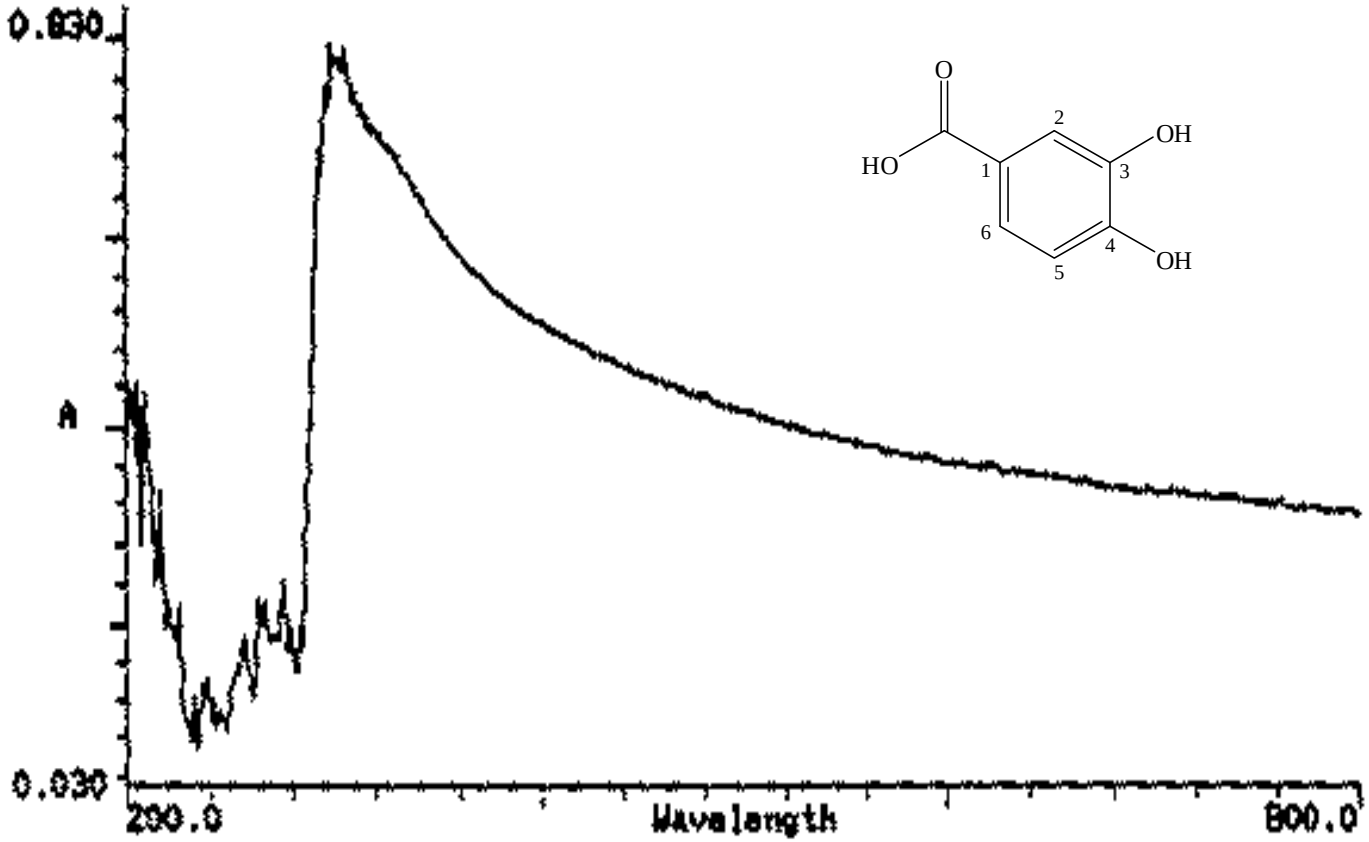

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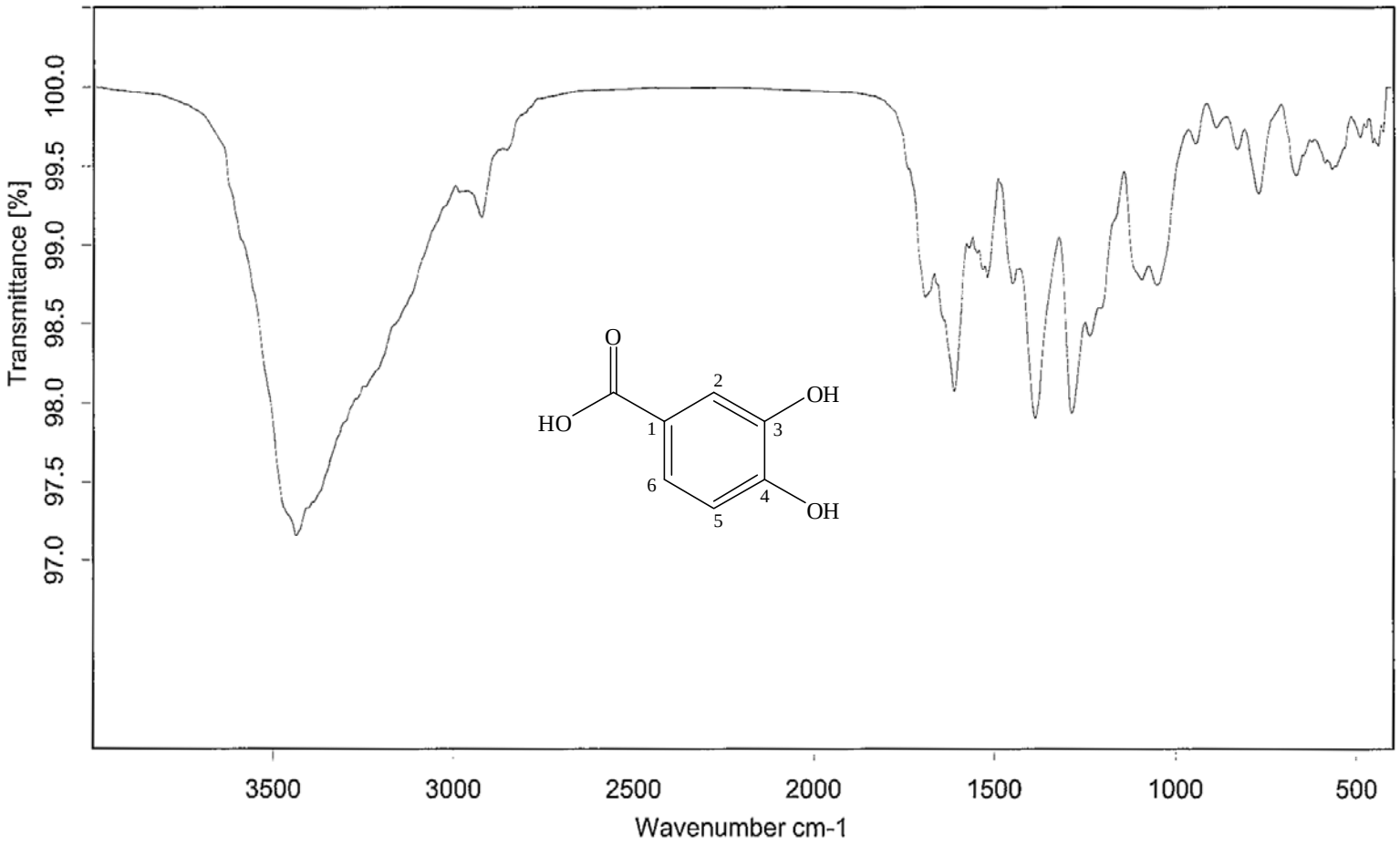

IR spectrum of compound **2** (KBr)