CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF SOME ACANTHACEOUS PLANTS

WASANA PRAPALERT

DOCTOR OF PHILOSOPHY

IN PHARMACY

GRADUATE SCHOOL

CHIANG MAI UNIVERSITY

JULY 2014

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A THESIS SUBMITTED TO CHIANG MAI UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACY

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THIS THESIS HAS BEEN APPROVED TO BE A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACY

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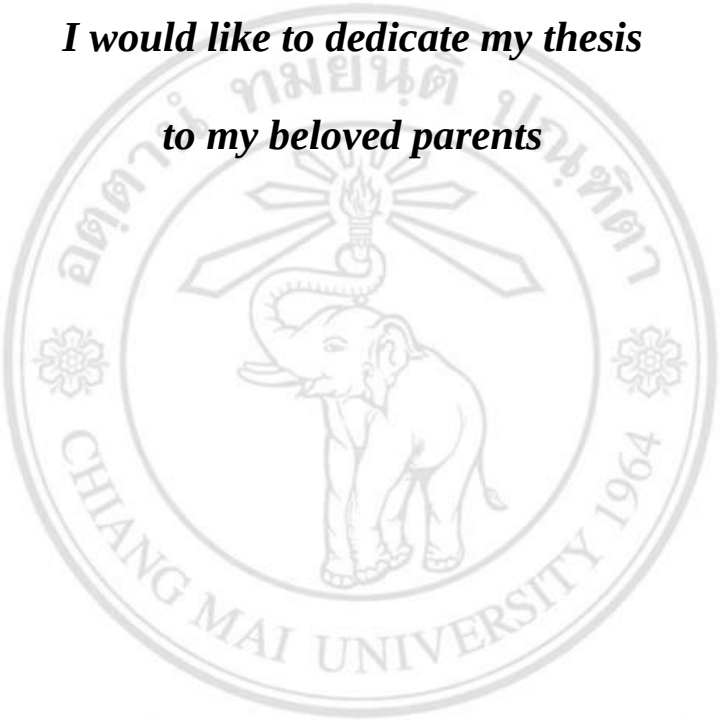
17 July 2014

I would like to dedicate my thesis

to my beloved parents



Acknowledgement

Firstly, I would like to express my sincere thanks to my advisor and co-advisors, Asst. Prof. Dr. Sunee Chansakaow, Asst. Prof. Dr. Dammrong Santiarworn amd Assoc. Prof. Dr. Saisunee Liawruangrath, for their kind guidance, encouragement, and helpful comments during my Ph. D. work. I would like to deep express my sincere gratitude to my excellent co-advisors, Assoc. Prof. Dr. Boonsom Liawruangrath and Prof. Stephen G. Pyne for their kind and patient suggestions, encouragement and continuous guidance throughout my thesis work. I would like to sincerely thank Dr. Theeraphan Machan and Asst. Prof. Dr. Aphiwat Teerawutgulrag, the examination committee, for their kind suggestions and corrections.

Secondly, I am special thank to all of my friends, all members of the Boonsom and the Pyne group for their friendship, helps and encouragements. I also wish to thank all of my colleagues for their friendship, understanding and helps. I would like to thank all the technical staffs at the Faculty of pharmacy, Chiang Mai University for their kind helps. I would like to thank University of Wollongong for facilities. I am grateful to thank Dr. Wilford Lie for his kind training, assistance and suggestions on the NMR instruments. I also wish to thank all the technician staffs from the mass spectrometry group, especially Dr. John Korth, for their kind training and providing the mass spectral measurements.

I gratefully acknowledge to Chiang Mai Rajabhat University, the Graduate school of Chiang Mai University, the Faculty of Pharmacy, Chiang Mai University and the Commission on Higher Education for their partial financial support.

Finally, I wish to express my deepest gratitude to my parents, my sister, and my husband for their love and encouragement throughout my Ph. D. work.

Wasana Prapalert

หัวข้อวิทยานิพนธ์ องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของพืชวงศ์อะแคนทาซีอีบางตัว

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บทคัดย่อ

วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อศึกษาฤทธิ์ทางชีวภาพ ได้แก่ ฤทธิ์การต้านออกซิเดชัน ฤทธิ์การต้านแบคทีเรีย และฤทธิ์การต้านมะเร็ง รวมถึงการค้นหาสารผลิตภัณฑ์เมทาบอไลต์ทุติยภูมิชนิดใหม่ จากพืชวงศ์อะแคนทาซีอี 4 ชนิด ได้แก่ ดาดตะกั่ว หว้าชะอำ สังกรณี และ อังกาบ

จากการศึกษาฤทธิ์การต้านออกซิเดชันของสารสกัดหยาบเมทานอลจากดาดตะกั่ว หว้าชะอำ สังกรณี และ อังกาบ พบว่ามีฤทธิ์ต้านออกซิเดชันต่ออนุมูลอิสระดีพีพีเอชสูง โดยมีค่า IC_{50} เท่ากับ 53 176 73 และ 18 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ การตรวจสอบฤทธิ์การต้านแบคทีเรียเบื้องต้นใช้วิธี Agar disc diffusion method พบว่าสารสกัดหยาบจากเมทานอลของหว้าชะอำมีฤทธิ์ต้านเชื้อแบคทีเรียบาซิลัส ซับทิลิสที่สภาวะพีเอช 6 และ พีเอช 7.2 โดยมีขนาดโซนใสของการยับยั้งเท่ากับ 8.0 และ 10.0 มิลลิเมตร ตามลำดับ สารสกัดของอังกาบแสดงฤทธิ์ต้านเชื้อบาซิลลัส ซับทิลิส ที่สภาวะพีเอช 7.2 และ พีเอช 8 รวมทั้งมีฤทธิ์ต้านเชื้อบาซิลลัส ซีเรียส ที่สภาวะพีเอช 6 โดยมีขนาดโซนใสของการยับยั้งเท่ากับ 7.0 9.0 และ 7.0 มิลลิเมตร ตามลำดับ แต่อย่างไรก็ตาม จากการศึกษาพบว่าสารสกัดหยาบเมทานอลของดาดตะกั่วและสังกรณีไม่แสดงฤทธิ์ต้านเชื้อจุลินทรีย์ที่ใช้ในการทดสอบ นอกจากนี้ การทดสอบฤทธิ์การต้านมะเร็งของสารสกัดจากพืชทั้ง 4 ชนิดที่มีความเข้มข้น 50 ไมโครกรัมต่อมิลลิลิตร พบว่าไม่แสดงฤทธิ์ต้านการเจริญของเซลล์มะเร็ง KB (มะเร็งช่องปาก) MCF-7 (มะเร็งทรวงอก) และ NCI-H187 (มะเร็งปอด)

การตรวจสอบพฤษเคมีเบื้องต้นพบสารกลุ่มซาโปนินและสเตอรอยด์ในสารสกัดทั้ง 4 ชนิด มีการตรวจพบสารกลุ่มแทนนินในสารสกัดจากอังกาบ สังกรณี และ ดาดตะกั่ว ขณะที่ตรวจพบสารกลุ่มฟลาโวนอยด์ในสารสกัดจากหว้าชะอำเท่านั้น อย่างไรก็ตาม สารสกัดทุกชนิดให้ผลลบต่อการตรวจสอบสารกลุ่มแอลคาลอยด์และแอนทราควิโนน

การศึกษาครั้งนี้สามารถแยกสารประกอบแอลคาลอยด์ชนิดใหม่ได้ชนิดหนึ่งชื่อว่า 1,6-benzoxazocine-5-one ในรูปของอนุพันธ์บิวทิล อะเซทัล พร้อมด้วยสารประกอบ peristrophine ได้จากการสกัดแยกด้วยตัวทำละลายบิวทานอล และ เอทิลอะซิเตตของสารสกัดหยาบเมทานอลจากหว้าชะอำ นอกจากนี้ยังสามารถแยกสารประกอบเทอร์พีน 2 ชนิด คือ lutein และ lutein-3′-methyl ether ได้จากการสกัดแยกด้วยตัวทำละลายเอทิลอะซิเตต ซึ่งเป็นการค้นพบสารจากหว้าชะอำเป็นครั้งแรก นอกจากนี้สามารถแยกสารกลุ่มฟีนิลเอทนอยด์กลัยโคไซด์ซึ่งเป็นคู่ไอโซเมอร์กันได้แก่ parvifloroside A และ parvifloroside B ได้เป็นครั้งแรกจากสารสกัดหยาบเมทานอลของสังกรณี โครงสร้างของสารที่แยกได้เหล่านี้ได้รับการยืนยันโครงสร้างโดยใช้เทคนิค 1D และ 2D นิวเคลียร์แมกเนติกเรโซแนนซ์สเปคโทรสโกปี ได้แก่ ^{1}H และ ^{13}C NMR รวมทั้ง COSY HMBC HSQC และ NOESY

Thesis Title	Chemical Constituents and Biological Activities of Some Acanthaceous Plants	
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ABSTRACT

The goal of this study was to evaluate the biological activities, including antioxidant, antibacterial and anticancer activities of some Acanthaceous plants and search for their novel secondary metabolites. Four plant species belonging to Acanthaceae, *Hemigraphis colorata* Blume, *Peristrophe lanceolaria* (Roxb.) Nees, *Barleria strigosa* Willd. and *Barleria cristata* Linn., were used for this investigation.

The antioxidant activity of the crude methanol extracts of *H. colorata*, *P. lanceolaria*, *B. strigosa* and *B. cristata* exhibited strong antioxidant activity against the DPPH radical with IC_{50} values of 53, 176, 73 and 18 μg/mL, respectively. The antimicrobial activity screening using the agar disc diffusion method showed that the methanol extract of *P. lanceolaria* exhibited antibacterial activity against *B. subtilis* at pH 6 and pH 7.2 with the inhibition zones of 8.0 and 10.0 mm, respectively. *B. cristata* extract showed antibacterial activity against *B. subtilis* at pH 7.2, *B. subtilis* at pH 8 and *B. cereus* at pH 6 with the inhibition zones of 7.0, 9.0 and 7.0 mm, respectively. However, *H. colorata* and *B. strigosa* extracts were inactive to all the test organisms. The cytotoxicity testing of these extracts showed no activity against KB (oral cavity

cancer), MCF-7 (breast cancer) and NCI-H187 (small cell lung cancer) cell lines at the concentration of 50 μg/mL.

The screening for phytochemical constituents showed that there are saponins and steroids present in all extracts, tannins are found in *B. cristata, B. strigosa* and *H. colorata*, whereas only flavonoids are present in *P. lanceolaria*. However, all the extracts showed negative test for alkaloids and antraquinones.

A new 1,6-benzoxazocine-5-one alkaloid has been isolated as its butyl acetal derivative along with peristrophine from the *n*-butanol and ethyl acetate fractions of the methanol extract of *Peristrophe lanceolaria*. Moreover, two new tetraterpenoids, lutein and lutein-3′-methyl ether, have also been isolated from the ethyl acetate fraction. These compounds were obtained and determined for the first time from the *P. lanceolaria*. Addition, two new phenyl ethanoid glycosides, Parvifloroside A and Parvifloroside B, have been isolated for the first time from the ethyl acetate fraction of the methanol extract of *B. strigosa*. The structures of these compounds were elucidated based on their 1D and 2D nuclear magnetic resonance (NMR) spectroscopic data including ^{1}H and ^{13}C NMR, COSY, HMBC, HSQC and NOESY correlations.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
$([\alpha]_D^{25}$	Specific rotation
A_b	Absorbance of blank
A_c	Absorbance of control
anti-HSV-2	Anti-herpes simplex virus type 2
AR	Analytical reagent
A_s	Absorbance of sample
br. s	Broad singlet
BSAQ	Aqueous extract of *B. strigosa*
BSBU	Butanol extract of *B. strigosa*
BSET	Ethyl acetate extract of *B. strigosa*
BSHE	Hexane extract of *B. strigosa*
BuOH	Butanol
ºC	Degrees celsius
C18	Octadecyl carbon chain
CAM	Cerium-ammonium-molybdate
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
cfu	Colony forming unit
cm	Centimeter
cm^{-1}	Wave number
^{13}C NMR	Carbon 13 nuclear magnetic resonance
COSY	Correlation spectroscopy
CSA	Camphor sulfonic acid
d	Doublet
dd	Doublet of doublet
DMSO	Dimethyl sulfoxide
2D NMR	Two dimension nuclear magnetic resonance
DPPH	2,2-Diphenyl-1-picrylhydrazyl

dt	Doublet of triplet
EtOAc	Ethyl acetate
FU_C	Fluorescence unit of untreated cell
FU_T	Fluorescence unit of cell treated with test compound
g	Gram
GCMS	Gas chromatography mass spectra
^{1}H NMR	Proton nuclear magnetic resonance
HE	Hexane
HL-60 cells	Human promyelocytic leukemia cells
HMBC	Heteronuclear multiple bond correlation spectroscopy
HR-ASAP-MS	High resolution atmospheric solids analysis probe mass spectra
HSQC	Heteronuclear single quantum correlation spectroscopy
Hz	Hertz
IC_{50}	50% Inhibition concentration
IR	Infrared
J	Coupling constant
KB	Human oral cavity cacinoma
kg	Kilogram
KI	potassium iodide
L	Liter
LR-ESI-MS	Low resolution electrospray ionization mass spectra
M	Molar
m	Multiplet
m/z	Mass to charge ratio
MCF-7	Human breast adenocarcinoma
MeOH	Methanol
MeOH-d_4	Deuterated methanol
mg	Milligram
MHz	Megahertz
mL	Milliliter
mm	Millimeter

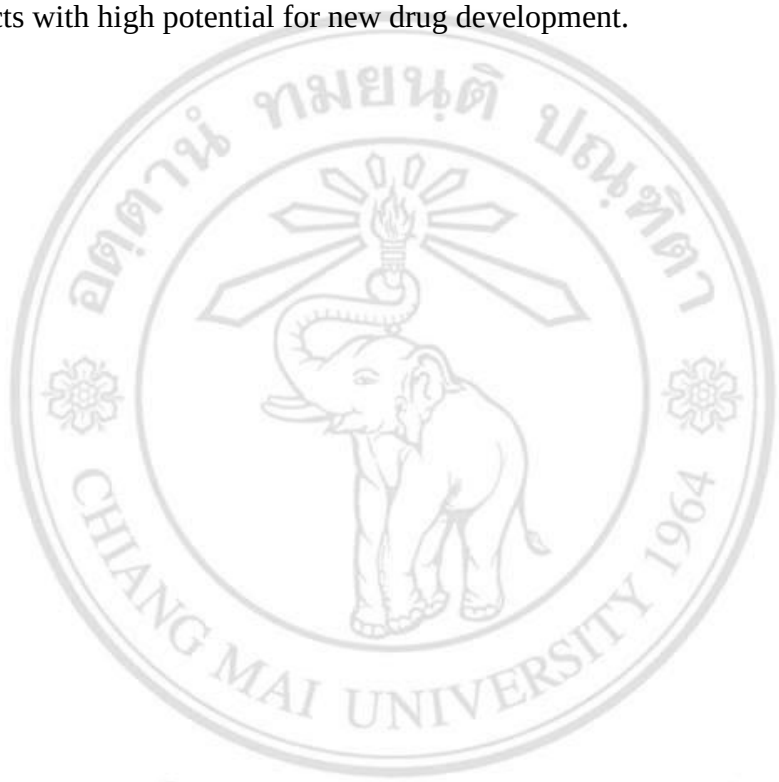
NCI-H187	Human small cell lung carcinoma
nm	Nanometer
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser effect spectroscopy
O/N	Over night
ORAC	Oxygen radical antioxidant capacity
PDE	phosphodiesterase
PDE5	Phosphodiesterase type 5
Pet	Petroleum ether
PLAQ	Aqueous extract of *P. lanceolaria*
PLBU	Butanol extract of *P. lanceolaria*
PLET	Ethyl acetate extract of *P. lanceolaria*
PLHE	Hexane extract of *P. lanceolaria*
ppm	Part per million
REMA	Resazurin microplate assay
ROO•	Peroxyl radical
RP-C18	Reverse phase - octadecyl carbon chain bonded silica gel
s	Singlet
t	Triplet
T	Temperature
TLC	Thin layer chromatography
T-lymphoblast	Thymus lymphoblast, a t-lymphocyte that has become larger after being stimulated by an antigen
TMS	Tetramethylsilane
TPA	12-*O*-tetradecanoylphorbol-13-acetate
UV	Ultraviolet
w/v	Weight by volumn
δ	Chemical shift
µg	Microgram
μL	Microliter
μm	Micrometer

ข้อความแห่งการริเริ่ม

การแยกสารประกอบชนิดใหม่ที่มีฤทธิ์ทางชีวภาพจากพืชวงศ์อะแคนทาซีอี 4 ชนิด ได้แก่ ดาดตะกั่ว หว้าชะอำ สังกรณี และ อังกาบ ด้วยวัตถุประสงค์เพื่อค้นหาสารผลิตภัณฑ์ธรรมชาติที่มีประสิทธิภาพสูงซึ่งจะนำไปสู่การพัฒนายาชนิดใหม่ต่อไป

STATEMENT OF ORIGINALITY

Isolation of novel compounds with biological activities from four acanthaceous plants, *Hemigraphis colorata* Blume, *Baleria strigosa* Willd., *Peristrophe lanceolaria* (Roxb.) Nees, and *Baleria cristata* Linn., are proposed with the expectation to discover natural products with high potential for new drug development.



CHAPTER 1

Introduction

1.1 Statement and significance of the problem

During the past few decades, threats by newly arising diseases and the resistance to existing drugs have increased. Therefore, innovative research and development for new drugs, characterized by excellent efficacy and minimized side-effects, are urgently needed to combat these diseases and threats. Attention has been particularly focused on natural products, especially those from plants, as an alternative medicine. Several important natural products have become widely used for treating major diseases. There are several Acanthaceous plants, which are found in many districts in Thailand, which are expected to contain natural products with high potential for new drug development. While several studies have been reported on the chemical constituents and the pharmacological activities of *Acanthus ilicifolius* Linn. and *Barleria lupulina* Lindl., there are only a few articles dealing with the active constituents of *Baleria cristata* Linn. and *Barleria strigosa* Willd. There are very few literature reports on the chemical constituents and biological activities of *Hemigraphis colorata* Blume and *Peristrophe lanceolaria* (Roxb.) Nees. There is only one review which described the food pigments of *P. lanceolaria* [Zheng, 1992]. Moreover, with respect to these plants, few reports have been reported on their biological activities. Consequently, four medicinal plants in this family, *B. cristata*, *B. strigosa*, *H. colorata*, and *P. lanceolaria*, have been chosen for this investigation. The study of these plants, particularly based on the careful screening for the active constituents, will make it possible to succeed in novel drug discovery.

1.2 Research aims

The major aims of this work were as follows:

1. To search for biological activities of some Thai Acanthaceous plants.

2. To purify, isolate and identify the chemical constituents from two selected plants which showed biological activities.

CHAPTER 2

Literature review

2.1 The Acanthaceous Plants

Rank	Scientific name	Common name
Kingdom	Plantae	Plants
Subkingdom	Tracheobionta	Vascular plants
Superdivision	Spermatophyta	Seed plants
Tuboflorae	Magnoliophyta	Flowering plants
Class	Magnoliopsida	Dicotyledons
Subclass	Asteridae	-
Oder	Scrophulariales	-
Family	Acanthaceae	Acanthus family

The Acanthaceae consist of herbs or shrubs, climbers, sometimes small trees, and rare, aquatic herbs (*Cardentha*) [Singh, 2004 ; Spechiger *et al*., 2004 ; Simson, 2006], comprising about 229 genera and about 3,450 species [Simson, 2006]. Major genera are *Justicia*, *Beloprone*, *Barleria*, *Strobilanthus*, *Ruellia*, *Dicliptera*, *Thunbergia*, and *Adhatoda* which comprise about 400, 300, 240, 230, 190, 140, 140 and 20 species, respectively [Singh, 2004]. Some of them, *A. ilicifolius* (Ngueak-pla-mo) *B. prionitis* (Ang-kap-nuu) and *B. lupulina* (Sa-let-pangpon), showed pharmacological activities, including, anti-diabetic, anti-HSV-2 and anti-inflammatory activity [Maregesi *et al*., 2007]. *A. ilicifolius* is widely distributed in Southeast Asia and has been traditionally used as a Chinese medicine for treating inflammation and hepatitis [Wu *et al*., 2003]. *B. prionitis* has been used as an Indian traditional medicine. Its roots have been used to treat whooping cough and asthma [Bhatia *et al*., 2014] and its leaves have been used for rheumatism [Singh *et al*., 2002] and toothache relief [Jeyaprakash *et al*., 2011]. *B. lupulina* is a small shrub, distributed in Southeast Asia, and has been externally used as

a Thai traditional medicine for treating inflammation due to insect bite, herpes simplex and herpes zoster [Kanchanapoom *et al*., 2001].

2.2 Plants Selected for This Research

2.2.1 *Hemigraphis colorata* (Blume) Hallier f.

Synonym : *Hemigraphis alternata* (Burm.f) T. Anderson

Genus : *Hemigraphis*

Common name : Red ivy, Red flame

Thai name : Data ta kua [priyadi *et al.*, 2010]

Botanical feature : *H. colorata* is an erect herb with tiny light-violet flowers (1 to 1.5 centimeters diameter, five lobed, bell shaped). The leaves are opposite, blades dull, 4.5 to 8 centimeters long, and 3 to 4.5 centimeters wide, dark green above and dark red underneath [Backer et al., 1965] (Figure 2.1).

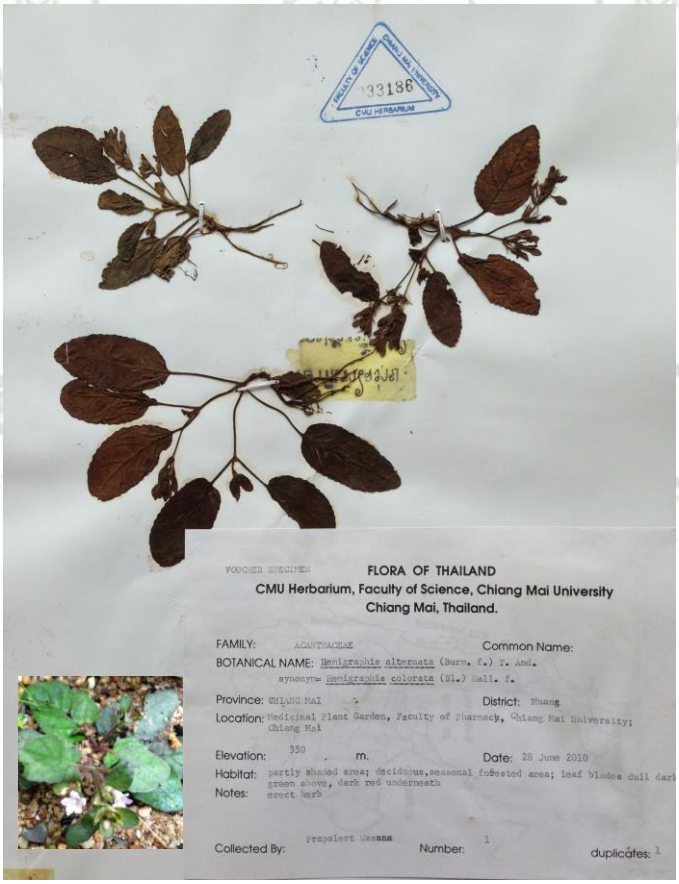


Figure 2.1 *Hemigraphis colorata*

Ethanobotany : The boiled whole plant has been used in Thai traditional medicine to prevent miscarriage and cure skin disease. The leaves have also been used in the treatment for anemia, tuberculosis, dysentery and hemorrhoids [Priyadi *et al.*, 2010]. It has been claimed in an Indian folk medicine that the plant has very good wound healing activity and can be used to cure anemia [Jayaprakasan *et al.*, 2014]. Subramoniam (2001) reported the first study on the excision wound healing, and anti-inflammatory properties of the leaves suspension and paste which were studied in mice. *H. colorata* leaves paste, when applied topically, promoted wound healing in mice but oral administration was ineffective. The leaves paste, when topically applied on mice, showed the faster wound contraction and epithelialisation than the leaves suspension, but the leaves suspension and paste did not show anti-inflammatory activity. Gayathri *et. al*. (2012) reported that the ethyl acetate fraction, which was fractionated by solvent extraction from the hexane extract of *H. colorata*, showed anti-diabetes activity in rats. In 2013, Priya [Priya, 2013] published a review on the anti-bacterial, anti-diabetic, wound healing, anti-oxidant and miscellaneous activities of *H. colorata* including its phytochemistry. In addition, the latest report from Jayaprakasan *et al.* (2014) reported that Ayurvedic herbal preparations containing extracts of three herbs, including *H. colorata* water extract have high activities against *Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa*.

Chemical constituents: *H. colorata* has been screened for its phytochemical constituents which included, terpenoids, steroids and sterols, saponins, coumarin, phenols, flavonoids, tannins, alkaloids, proteins, xanthoproteins, carbohydrates, oils and carboxylic acids. A list of these phytochemical constituents is shown in Table 2.1.

Table 2.1 Phytochemical constituents found in *H. colorata*

Chemical groups	References
Terpenoids	Jayaprakasan *et al.* (2014)
Steroid and Sterols	Saravanan *et al*. (2010) Gayathri *et al*. (2012) Anitha *et al.* (2012) Asha *et al*. (2013-14)
Saponins	Jayaprakasan *et al.* (2014) Anitha *et al.* (2012)
Coumarins	Gayathri *et al*. (2012) Anitha *et al.* (2012)
Phenols	Jayaprakasan *et al.* (2014) Anitha *et al.* (2012) Asha *et al*. (2013-14)
Flavonoids	Jayaprakasan *et al.* (2014) Anitha *et al.* (2012) Asha *et al*. (2013-14)
Tannins	Saravanan *et al*. (2010) Anitha *et al.* (2012) Asha *et al*. (2013-14)
Alkaloids	Saravanan *et al*. (2010) Anitha *et al.* (2012) Asha *et al*. (2013-14)
Proteins	Saravanan *et al*. (2010) Anitha *et al.* (2012) Asha *et al*. (2013-14)
Xanthoproteins	Saravanan *et al*. (2010)

Table 2.1 (Continued)

Chemical groups	References
Carbohydrates	Anitha *et al.* (2012) Asha *et al*. (2013-14)
Oils	Asha *et al*. (2013-14)
Carboxylic acids	Anitha *et al.* (2012)

2.2.2 *Peristrophe lanceolaria* (Roxb.) Nees

Genus : *Peristrophe*

Common name : -

Thai name : Waa cha am

Botanical feature : *P. lanceolaria* is an erect herb which is widely spread in China, Myanmar, India, Laos, Vietnam, and Thailand [Flora of China Editorial committee, 2011]. It is 75 cm tall, with two-lipped pink flowers. The leaves are blades, dark green above and light green below [Clarke, 1885] (Figure 2.2).

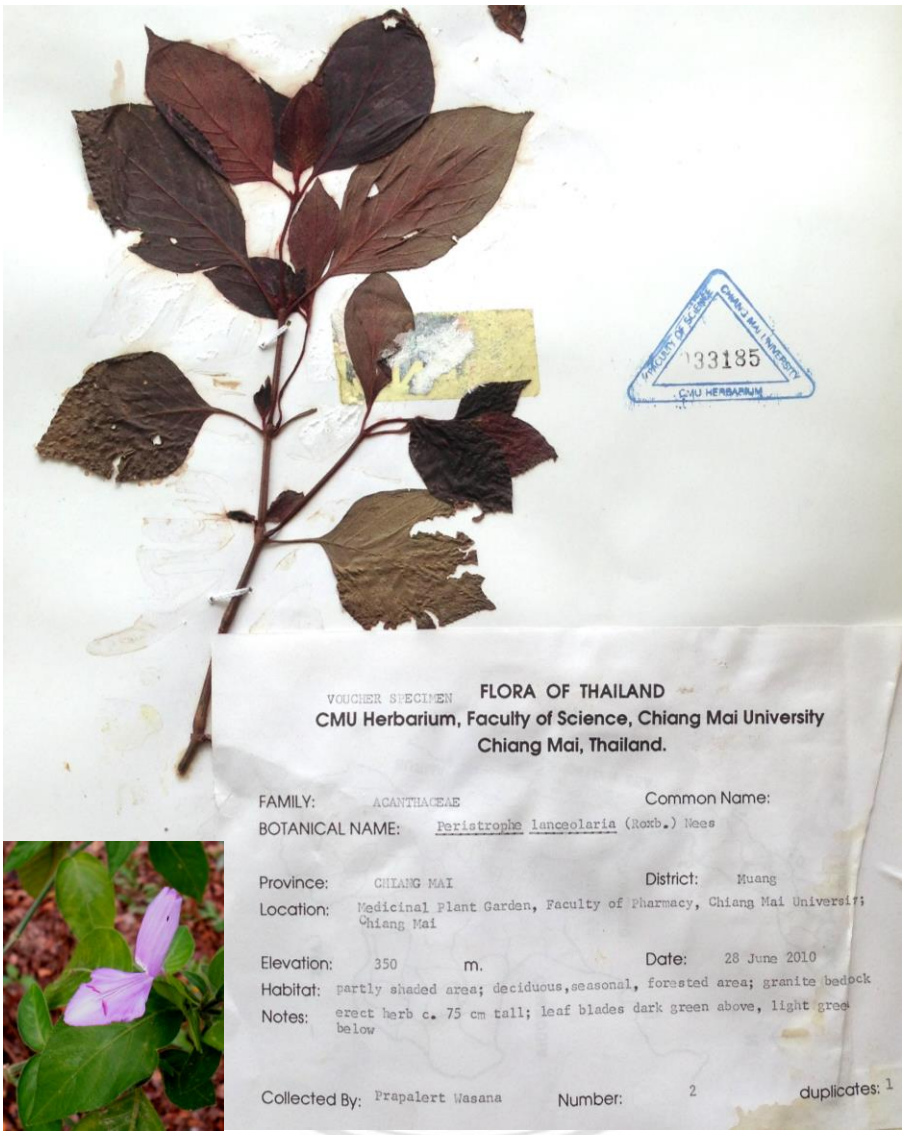


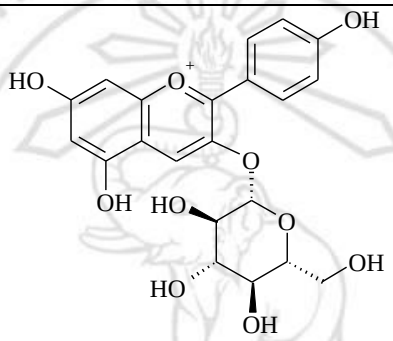
Figure 2.2 *Peristrophe lanceolaria* (Roxb.) Nees

Ethanobotany : The alcoholic root extract has been used as a Thai traditional medicine by applying it on a child's tongue for curing glossitis. The leaves of this plant have been used by the Akha people as a traditional medicine for treating seriously infected wounds, smallpox, rashes, and fever [Inta *et al*., 2008]. In 2010, Charoenchai and colleagues reported that the CH_2Cl_2 extract of *P. lanceolaria* showed cytotoxic activity against T-lymphoblast (acute lymphoblastic leukemia) cell line with an IC_{50} value of 35.9 μg/mL. It could scavenge TPA-induced superoxide anion radical formation in differentiated HL-60 cells with an inhibition rate of 51% at 40 μg/mL. Two hundred μg/mL methanol extract was able to inhibit superoxide anion radical with an inhibition rate of 51%. Moreover, both the CH_2Cl_2 and methanol extracts also showed

antiplasmodial activity and antioxidant activity on Oxygen radical antioxidant capacity assay (ORAC assay), oxygen radical absorbance capacity against ROO•, with ORAC units of 0.6 and 1.2, respectively [Charoenchai *et al.*, 2010].

Chemical constituents : The fresh branches and leaves of *P. lanceolaria* was found to contain a pigment named lanceolin red (4-8%) which consisted of an anthocyanin, pelargonidin-3-β-glucoside (Table 2.2), as the main component [Wei *et al.*, 1989 ; Zheng *et al.*, 1992].

Table 2.2 Compounds isolated from *P. lanceolaria*

Chemical groups	Compounds	References
Anthocyanin	 Pelargonidin-3-β-glucoside or Callistephin (2*R*,5*S*)-2-[5,7-dihydroxy-2-(4-hydroxyphenyl)chromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	- Wei *et al.*, (1989) - Zheng *et al.*, (1992)

2.2.3 *Barleria strigosa* Willd.

Genus : *Barleria*

Common name : Philippine violet

Thai name : Sang ko ra ni, Ya hua nak [Smitinand, 2001]

Botanical feature : *Barleria strigosa* is a deciduous herb native to tropical regions of Asia. It is a tall shrub with blue flowers in dense spikes and dry bracts. Leaves are blades, 14 cm or longer, dark green above and light green underneath [Clarke, 1884] (Figure 2.3).

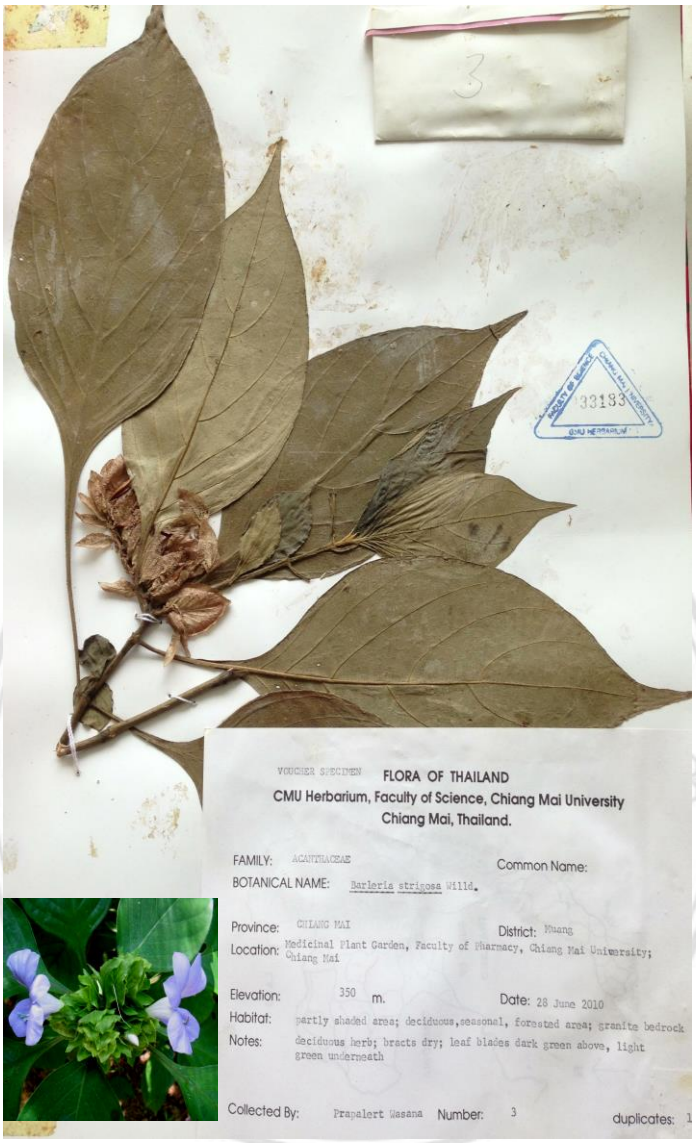


Figure 2.3 *Baleria strigosa* Willd.

Ethanobotany : *B. strigosa* has been used as a Thai traditional medicine for treating influenza, fever, and toxicity of poisons [Kanchanapoom *et al*., 2004]. The root was boiled and used as a restorative [Khuankaew *et al*., 2014]. The whole plant was also used as an aphrodisiac and a neurotonic agent, which was expected to contain a PDE (phosphodiesterase) inhibitor, but the ethanol extract of this plant showed PDE and PDE5 inhibitory activities below 20% [Temkitthawon *et al*., 2008 ; Temkitthawon *et al*., 2011]. The plant has also been used as an Indian medicinal plant for treating skin diseases, pimples, ulcer, leucoderma, and toothache [Yonzone *et al*., 2012 ; Kandi *et al*., 2013]. The root of *B. strigosa* has been used as an Indian medicine to relieve coughing, bile secretion and gastric troubles [Das *et al*., 2003;

Soudahmini *et al*., 2005], or used as an antidote for snake bite [Alagesaboopathi, 2013]. The root juice extract (crushed together with *Piper nigrum*) has been used for treating tuberculosis by the Kotia Hills tribes of the Vizianagaram district in India [Babu *et al*., 2010].

Chemical constituents: The whole plant was found to contain β– and γ–sitosterol [Daniel, 2006; Ganguly *et al*., 1969]. The leaves yielded apiginin, vanillic acid, *p*-hydroxy benzoic acid and *p*-coumaric acid [Daniel, 2006]. It was also found to contain strigoside (4-hydroxyphenylethyl 4-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside), an iridoid (10-*O*-*trans*-coumaryl-eranthemoside), verbascoside, isoverbascoside, decaffeoyl verbascoside, (+)-lyoniresinol 3α-*O*-β-D-glucoside, apigenin 7-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucoside, 7-*O*-acetyl-8-epi-loganic acid and (3*R*)-1-octen-3-ol-3-*O*-β-D-xylosyl-(1→6)-β-D-glucoside [Kanchanapoom *et al*., 2004]. The chemical components from *B. strigosa* which were previously reported are summarized in Table 2.3.

Table 2.3 Compounds isolated from *B. strigosa*

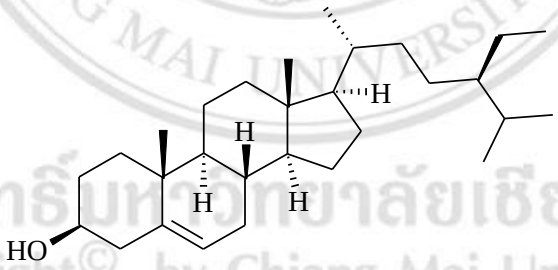
Chemical groups	**Compounds**	**References**
Phytosterols	 β–sitosterol 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	- Daniel (2006) - Ganguly (1969)

Table 2.3 (Continued)

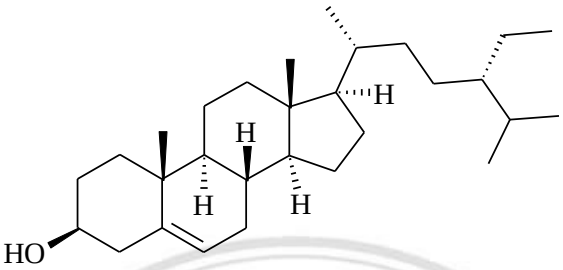
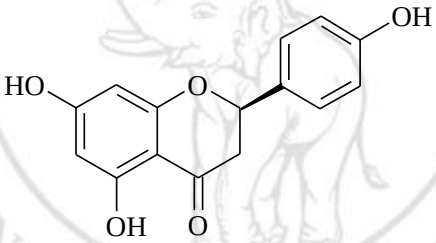
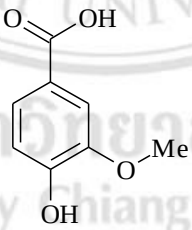
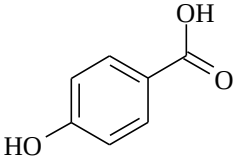
Chemical groups	Compounds	References
Phytosterols	 γ–sitosterol (3*S*)-17-[(5*S*)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	- Daniel (2006) - Ganguly (1969)
Flavonoids	 apiginin	- Daniel (2006)
Phenolic acid	 vanillic acid 4-hydroxy-3-methoxybenzoic acid	- Daniel (2006)
Phenolic acid	 *p*-hydroxybenzoic acid 4-hydroxybenzoic acid	- Daniel (2006)

Table 2.3 (Continued)

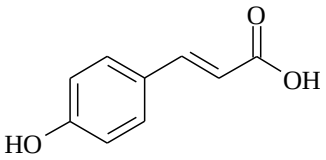
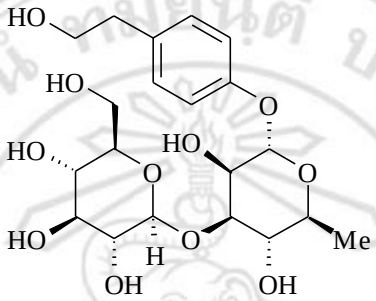
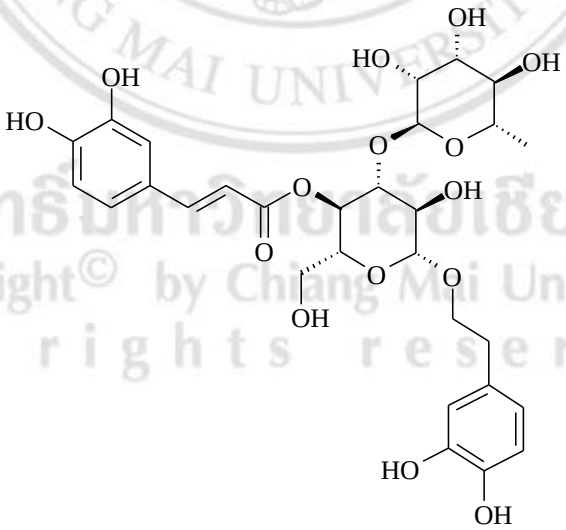
Chemical groups	Compounds	References
Phenolic acid	 *p*-coumaric acid (*E*)-3-(4-hydroxyphenyl)-2-propenoic acid	- Daniel (2006)
Phenyl ethanoid glycosides	 strigoside (4-hydroxyphenylethyl 4-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside)	- Kanchanapoom *et al*. (2004)
Phenyl ethanoid and phenyl propanoid glycosides	 (10-*O-trans*-coumaryl-eranthemoside), verbascoside	- Kanchanapoom *et al*. (2004)

Table 2.3 (Continued)

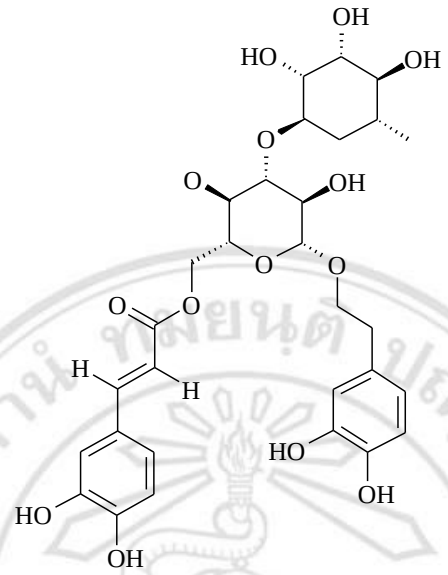
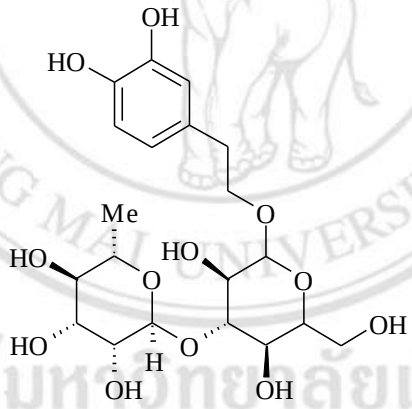
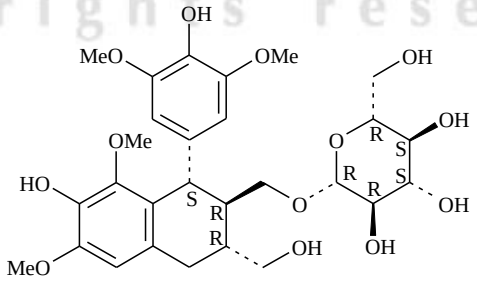
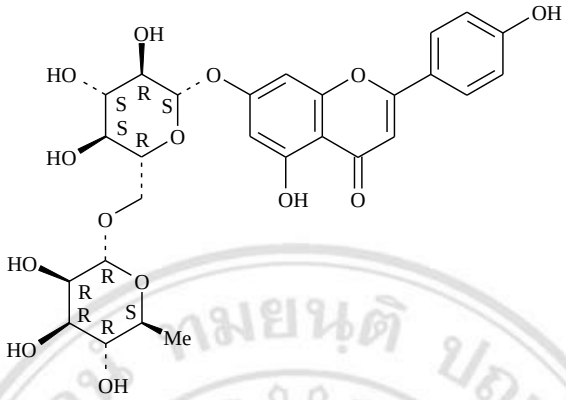
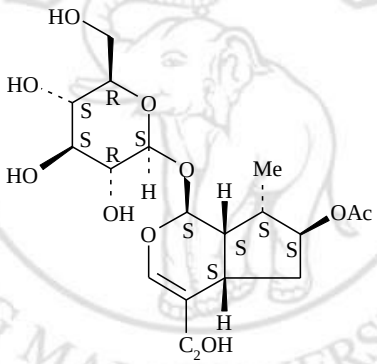
Chemical groups	Compounds	References
Phenyl ethanoid and phenyl propanoid glycosides	 isoverbascoside	- Kanchanapoom *et al*. (2004)
Phenyl ethanoid glycosides	 decaffeoyl verbascoside	- Kanchanapoom *et al*. (2004)
Lignan glycosides	 (+)-lyoniresinol 3α-*O*-β-D-glucoside[3]	- Kanchanapoom *et al*. (2004)

Table 2.3 (Continued)

Chemical groups	Compounds	References
Flavonoid glycosides	 apigenin 7-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucoside[3]	- Kanchanapoom *et al*. (2004)
Iridoids glycosides	 7-*O*-acetyl-8-*epi*-loganic acid	- Kanchanapoom *et al*. (2004)

2.2.4 *Barleria cristata* Linn.

Genus : *Barleria*

Common name : Crested Philippine violet

Thai name : Ang kap [Smitinand, 2001]

Botanical feature : *Barleria cristata*, one of the flowering plant species in the family Acanthaceae, is a shrub with 60 -100 cm tall. The leaves are blades or elliptic to narrowly ovate elliptic-oblong, acute or acuminate, 7.5–10 cm long with yellow-hairy on the upper surface. They are dark green above and pale light green underneath. The flowers are about 5 cm long, funnel-shaped in purple-blue or white color [Clark, 1884].

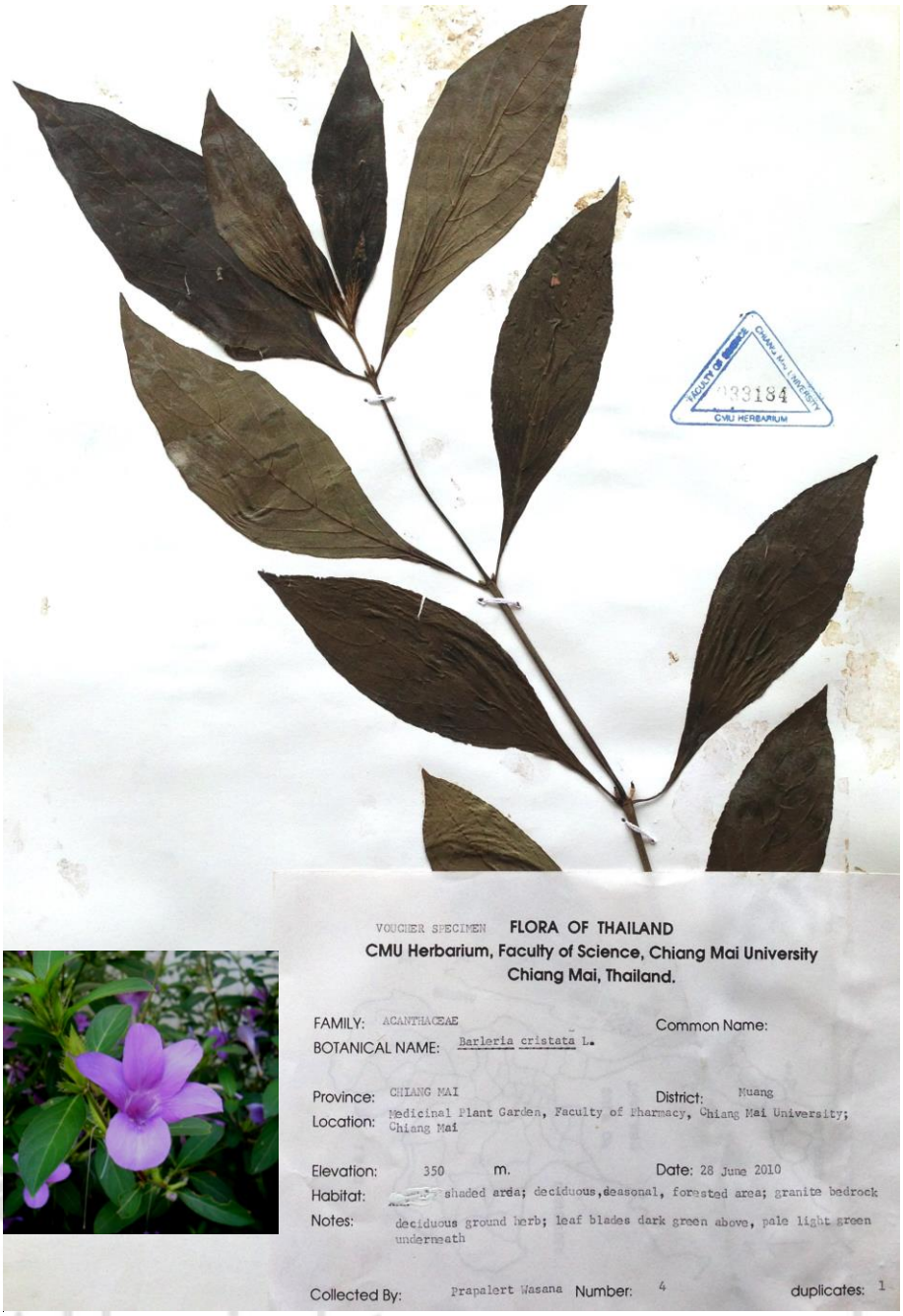


Figure 2.4 *Baleria cristata* Linn.

Ethanobotany : *B. cristata* has been widely used as an Indian traditional medicine. The leaf juice was used as an antidote to snake poison by applying the juice at the site of the snake bite [Bhatia *et al*., 2014]. The warmed leaves and roots paste were used for treating swelling and rheumatism [Jain *et al.*, 2005]. The root paste was also taken orally to treat diarrhea in children [Jeyaprakash *et al*., 2011]. The methanol extract of *B. cristata* showed antioxidant activity using inhibition of

superoxide anion radical formation by xanthine/xanthine oxidase and ORAC assay [Charoenchai *et al*., 2010].

Chemical constituents: There were a few articles studying the chemical constituents of *B. cristata*. The hexane extract from the aerial part of *B. cristata* gave α-amyrin, β-sitosterol, and stigmasterol-3-*O*-D-glucoside (Table 2.2). Acetylbarlerin (6,8-*O*-diacetylshanzhiside methyl ester, shanzhiside methyl ester, apigenin and apigenin-7-*O*-glucoside were isolated from the ethyl acetate extract of *B. cristata* [El-Emary *et al*., 1990]. The violet flower plants were found to contain apigenin, naringenin, apigenin glucuronide and malvindin 3,5-diglucoside [Subramanian *et al*., 1972]. *B. cristata* with white flowers yielded apigenin 7-glucuronide [Subramanian *et al*., 1972]. Scie (2006) reported that the roots of *B. cristata* contained anthraquinones (barlacristone and cristabarlone) and iridoids. The leaves yielded flavones, luteolin, 7-*O*-methyl luteolin, and phenolic acids such as *p*-hydroxy benzoic, vanillic, syringic, and *p*-coumaric acids. Flowers were found to contain β-sitosterol, quercetin, apigenin, naringenin, and malvidin. However, El-Mawla (2005) have studied on the callus cultures of *B. cristata* and found that it contained desrhamnosyl acteoside (β-[(3',4'-dihydroxyphenyl)-ethyl]-(4''-*O*-caffeoyl)-β-D-glucoside), acteoside (β-[(3',4'-dihydroxyphenyl)-ethyl]-(3''-*O*-L-rhamnosyl)-(4''-*O*-caffeoyl)-β-D-glucoside) and poliumoside (β-[(3',4'-dihydroxyphenyl)-ethyl]-(3'',6''-*O*-L-dirhamnosyl)-(4''-*O*-caffeoyl)-β-D-glucoside). The chemical components from *B. cristata* which were previously reported are summarized in Table 2.4.

Table 2.4 Compounds isolated from *B. cristata*

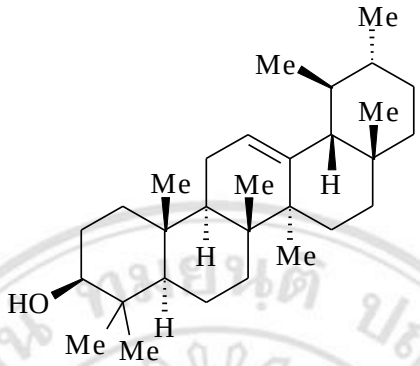
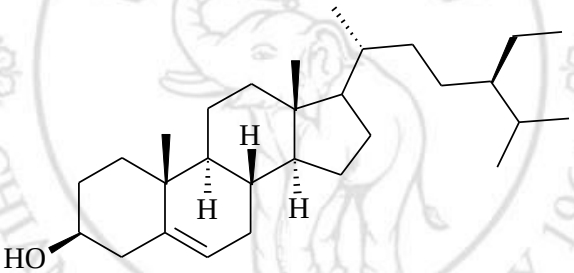
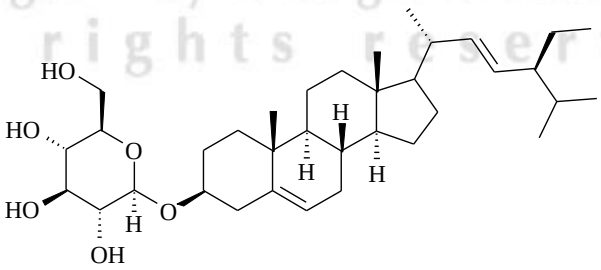
Chemical groups	Compounds	References
Triterpene	 α-amyrin (3β)-Urs-12-en-3-ol	- El-Emary *et al*.(1990)
Phytosterols	 β-sitosterol 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol.	- El-Emary *et al*. (1990) - Scie (2006)
Steroid glycosides	 stigmasterol-3-*O*-D-glucoside (3β,22*E*)-Stigmasta-5,22-dien-3-yl β-D-glucopyranoside	- El-Emary *et al*. (1990)

Table 2.4 (Continued)

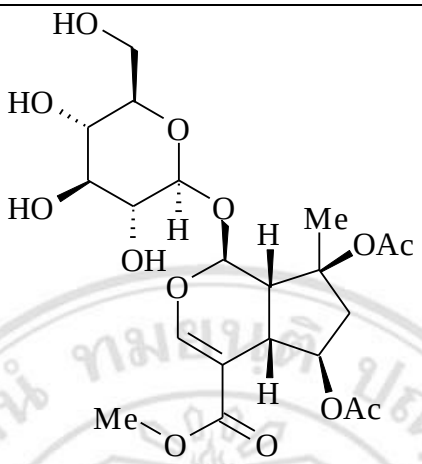
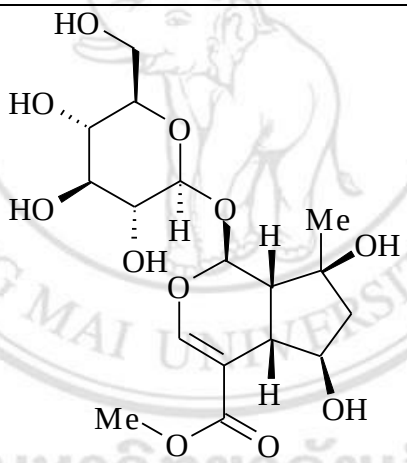
Chemical groups	Compounds	References
Irridoid glycosides	 Acetyl barlerin (6,8-*O*-diacetylshanzhiside methyl ester)	- El-Emary *et al*., (1990)
	 shanzhiside methyl ester, methyl (1*S*,5*R*,7*S*)-5,7-dihydroxy-7-methyl-1-[(2*R*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4alpha,5,6,7alpha-tetrahydro-1*H*-cyclopenta[c]pyran-4-carboxylate	

Table 2.4 (Continued)

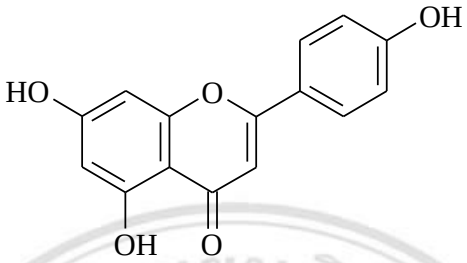
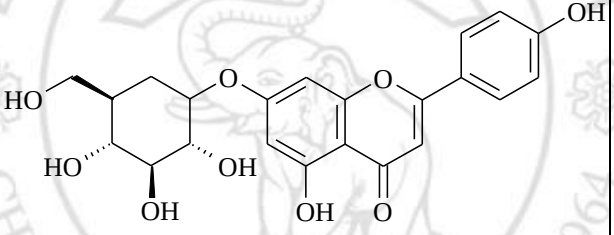
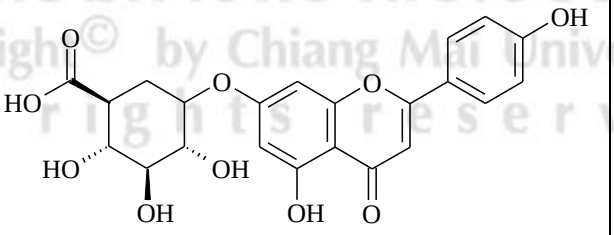
Chemical groups	Compounds	References
Flavones	 apigenin 5,7-Dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one	- El-Emary *et al*., (1990) - Subramanian *et al*. (1972) - Scie (2006)
Flavonoid glycosides	 apigenin-7-*O*-glucoside 5-hydroxy-2-(4-hydroxyphenyl)-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4, 5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	- El-Emary *et al*. (1990)
Flavonoid glycosides	 apigenin 7-glucuronide (2*S*,3*S*,4*S*,5*R*,6*S*)-3,4,5-trihydroxy-6-[5-hydroxy-2-(4-hydroxyphenyl)-4-oxochromen-7-yl]oxyoxane-2-carboxylic acid	- Subramanian *et al*. (1972)

Table 2.4 (Continued)

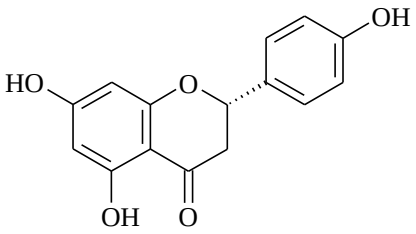
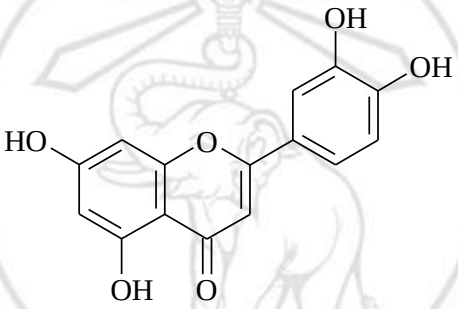
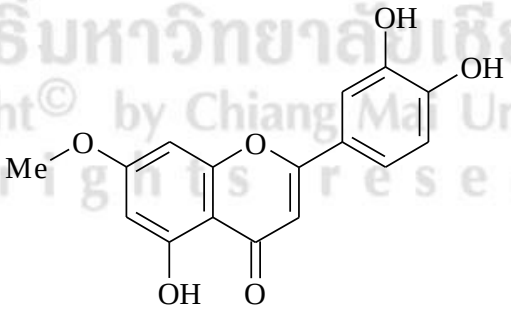
Chemical groups	Compounds	References
Flavanones	 naringenin 5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	- Subramanian *et al*. (1972) - Scie (2006)
Flavones	 luteolin 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-chromenone	- Scie (2006)
	 7-*O*-methyl luteolin 2-(3,4-Dihydroxyphenyl)-5-hydroxy-7-methoxy-4*H*-1-benzopyran-4-one	

Table 2.4 (Continued)

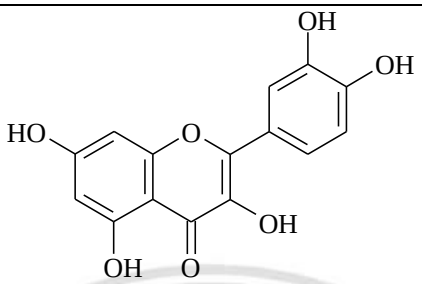
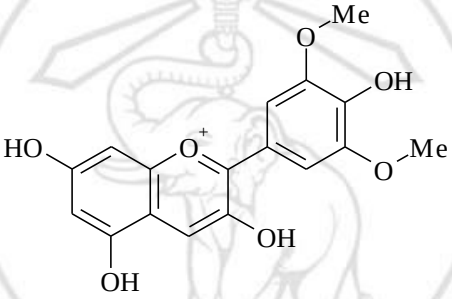
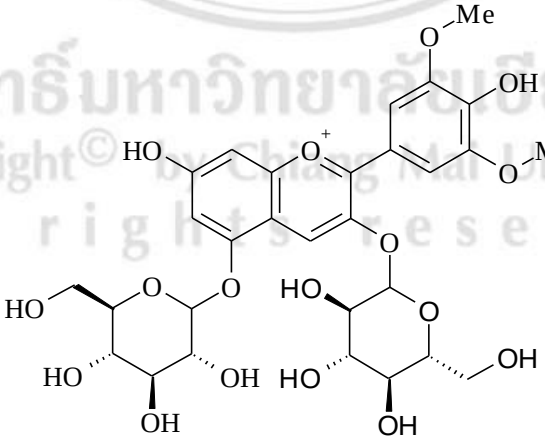
Chemical groups	Compounds	References
Flavonols	 quercetin 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one	- Scie (2006)
Anthocyanidins	 malvidin 3,5,7-trihydroxy-2-(4-hydroxy- 3,5-dimethoxyphenyl)chromenium	- Scie (2006)
Anthocyanin	 Malvin malvindin 3,5-diglucoside	- Subramanian *et al*. (1972)

Table 2.4 (Continued)

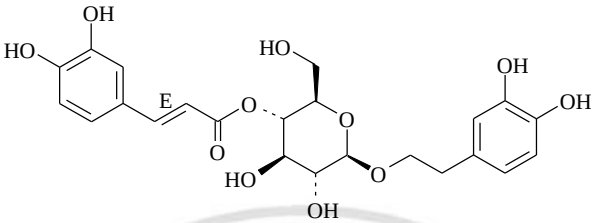
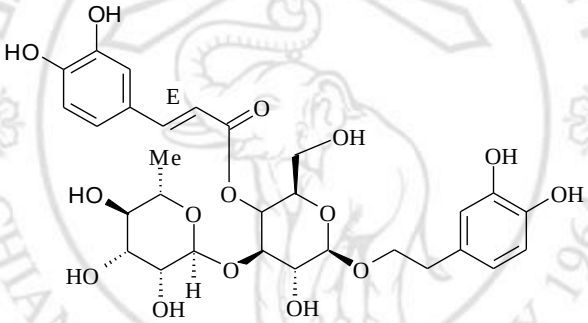
Chemical groups	Compounds	References
Phenyl ethanoid and phenyl propanoid Glycosides	 desrhamnosyl acteoside (β-[(3',4'-dihydroxyphenyl)-ethyl]-(4''-*O*-caffeoyl)-β-D-glucoside)	- El-Mawla (2005)
	 acteoside (2*R*,3*R*,4*R*,5*R*,6*R*)-6-[2-(3,4-dihydroxyphenyl)ethoxy]-5-hydroxy-2-(hydroxymethyl)-4-{[(2*S*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-3-yl (2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoate	

Table 2.4 (Continued)

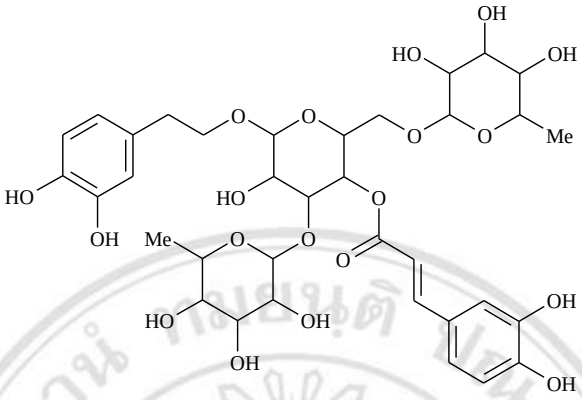
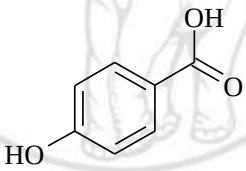
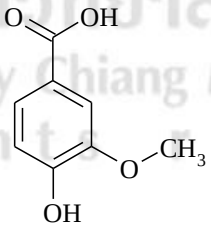
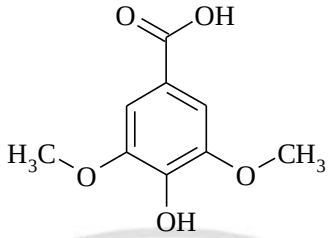
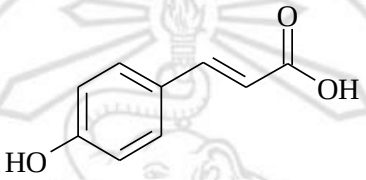
Chemical groups	Compounds	References
Phenyl ethanoid and phenyl propanoid Glycosides	 poliumoside (β-[(3',4'-dihydroxyphenyl)-ethyl]-(3",6"-*O*-L-dirhamnosyl)-(4"-*O*-caffeoyl)-β-D-glucoside)	- El-Mawla (2005)
Phenolic acids	 *p*-hydroxy benzoic acid 4-Hydroxybenzoic acid	- Scie (2006)
	 vanillic acid 4-Hydroxy-3-methoxybenzoic acid	

Table 2.4 (Continued)

Chemical groups	Compounds	References
Phenolic acids	 syringic acid 4-hydroxy-3,5-dimethoxybenzoic acid	- Scie (2006)
	 *p*-coumaric acid (*E*)-3-(4-hydroxyphenyl)-2-propenoic acid	

CHAPTER 3

Experimental

3.1 Source and Authentication of the Plant Materials

The aerial parts of *H. colorata*, *P. lanceolaria*, *B. strigosa*, and *B. cristata* were collected from the Medicinal Plant Garden, Faculty of Pharmacy, Chiang Mai University, Thailand. The voucher specimens were deposited at the herbarium of the Department of Biology, Faculty of Science, Chiang Mai University. The voucher specimen numbers *of H. colorata*, *P. lanceolaria*, *B. strigosa*, and *B. cristata* are Prapalert W.1, Prapalert W.2, Prapalert W.3 and Prapalert W.4, respectively. Plant materials were identified by Mr. James F. Maxwell from the Department of Biology, Chiang Mai University.

3.2 General Techniques

3.2.1 Chemicals

AR grade solvents including petroleum ether, dichloromethane, ethyl acetate, and methanol (Merck, Germany; Sigma-Aldrich, Swizerland; Fisher Scientific, United Kingdom; Ajax Finechem, Australia) were used for column chromatography. AR grade and NMR grade solvents were used for mass and NMR spectrometry, respectively. All commercial grade organic solvents such as n-hexane, dichloromethane, chloroform, ethyl acetate, butanol, and methanol that were used during the extraction and some chromatographic processes were distilled prior to use.

3.2.2 Chromatography

3.2.2.1 Column chromatography

Normal phase column chromatography was carried out using silica gel (40-630 μm, Merck). Hexane or petroleum ether, ethyl acetate and methanol were used as mobile phases with gradient elution.

Reverse phase column chromatography was carried out on octadecyl carbon chain (C18)-bonded silica. Methanol and water were used as mobile phases with gradient and isocratic elutions.

3.2.2.2 Thin layer chromatography

Normal phase TLC analysis were performed on a precoated silica gel aluminum-backed plate (60 GF_{254} silica gel, Merck), and the spots were first viewed under UV light at 254 nm and 365 nm, then stained with cerium-ammonium-molybdate (CAM) TLC stain, followed by heating or stained with Dragendorff's reagent.

Reverse phase TLC were carried out on a precoated C18-bonded silica aluminium-backed plate (RP-18 F254s, 20x20 cm, 0.25 mm thickness, Merck). Fifty percent of methanol solution was used as a mobile phase. The plates were first viewed under UV light at 254 and 365 nm then the colorless components were visualized by the TLC stain, CAM, followed by heating.

Preparation of TLC stains are as follows :

Cerium-ammonium-molybdate (CAM) : 10 g of ammonium molybdate and 4 g of cerric ammonium sulfate were dissolved in 400 mL of 10% H_2SO_4 solution. After heating, blue-black spots will be observed on a light background.

Dragendorff's reagent :

Solution A: 1.7 g of bismuthyl nitrate ($BiO(NO_3)$) was dissolved in 100 mL of 20% acetic acid

Solution B: 40 g of potassium iodide (KI) was dissolved in 100 mL of water

Before use, 5 mL of solution A was mixed with 5 mL of solution B, 20 mL of acetic acid and 70 mL of water. This reagent must be freshly prepared which can be used for 1-2 weeks. Nitrogen compounds like amines, alkaloids and pyridine create brown-orange spots without heating.

Normal phase preparative TLC was performed on a silica gel (60 GF_{254}, Merck) coated glass plate (20x20 cm, 0.5 mm thickness). Fifteen milliliters of hexane in 35 mL of ethyl acetate was used as a mobile phase and the bands were viewed under UV light at 254 nm and 365 nm.

3.2.3 Mass spectrometry (MS)

Electron impact mass spectra and low resolution electrospray ionization mass spectra (LR-ESI-MS) were measured on a Waters Platform LCZ (Single Quadrupole) mass spectrometer. High resolution atmospheric solids analysis probe mass spectra (HR-ASAP-MS) were obtained with a Waters Xevo (Quadrupole Time-of-flight) mass spectrometer using leucine enkephalin as an internal standard (School of Chemistry, University of Wollongong, Australia).

3.2.4 Nuclear magnetic resonance spectroscopy (NMR)

^{1}H and ^{13}C and 2D NMR spectra were recorded relative to $CDCl_3$ (δ = 7.26 and 77.0 ppm, respectively) or MeOH-d_4 (δ = 3.30 and 49.0 ppm, respectively) with tetramethylsilane (TMS) as internal standard on a Varian Unity (VNMRS PS54 500 MHz) NMR spectrometer (School of Chemistry, University of Wollongong, Australia).

3.2.5 Infrared spectroscopy (IR)

Infrared (IR) spectra were recorded on a MIRacle 10 Shimadzu Spectrometer (School of Chemistry, University of Wollongong, Australia).

3.2.6 Optical rotations

Specific rotations ($[\alpha]_D$) were measured by using a Jasco P-2000 polarimeter (USA) at 25°C in a 10 cm path length cell (School of Chemistry, University of Wollongong, Australia).

3.2.7 Ultraviolet-visible spectroscopy

Antioxidant activity was measured by using a Beckman Coulter DTX 880 Multimode detector (Austria) at 520 nm in 96-well microtiter plates (Chiang Mai University). Ascorbic acid and trolox were used as reference standards.

3.3 Experimental

3.3.1 Bioactivity test

3.3.1.1 Preparation of crude extracts

The aerial part of *H. colorata* (2.0 kg), *P. lanceolaria* (5.0 kg), *B. strigosa* (0.5 kg) and *B. cristata* (2.4 kg) were air dried for 2 days and dried in a 50 ºC hot-air oven for 24 hours and then ground into a powder.

The dried plant powder of *H. colorata* (490.0 g) was individually extracted with 1.4 L of methanol for 7 days and then filtered. The residue was extracted again with methanol (3 x 7.5 L) for 21 days (7 days for each extraction). The filtrate was dried under vacuum to give the methanol crude extract as a dark green residue (46.73 g, 9.54%).

The dried powder of *P. lanceolaria* (1496.5 g) was individually extracted with 5 L of methanol for 7 days and then filtered. The residue was extracted again with methanol (3 x 2.5 L) for 21 days (7 days for each extraction). The filtrate was dried under vacuum to give the methanol crude extract as a dark green-red residue (184.76 g, 12.35%).

The dried powder of *B. strigosa* (135.0 g) was extracted with 500 mL methanol for 7 days at room temperature and then filtered. The residue was extracted again with

250 mL of methanol (x3). The filtrate was dried under vacuum to give a dark green residue (16.81 g, 12.45%).

The dried plant powder of *B. cristata* (630.0 g) was individually extracted with 2 L of methanol for 7 days and then filtered. The residue was extracted again with methanol (3 x 1 L) for 21 days (7 days for each extraction). The filtrate was dried under vacuum to give the methanol crude extract as a dark green residue (63.70 g, 10.11%).

3.3.1.2 Antioxidant activity

The antioxidant activities of the extracts were studied by measuring the scavenging activity on DPPH radicals. An ethanolic solution of DPPH (180 μL) was added to 20 μL sample of different concentrations of the extracts (75-175 mg/mL) in a 96-well microtiter plate. The reaction mixtures were incubated in the dark at 25°C for 30 min, then the absorbances (A_s) were measured at 520 nm. The DPPH solution was used as a negative control (A_c). The ethanol (180 μL) and the plant extract (20 μL) was used as the blank (A_b). Ascorbic acid and trolox were used as reference standards. The percentage of the DPPH scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = [(A_s - A_b)/A_c] \times 100 \quad \text{..... (3.1)}$$

The concentration providing 50% inhibition (IC_{50}) values were calculated from the linear equation of the inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging activity.

3.3.1.3 Antibacterial activity

The antimicrobial activity of the extracts were determined using the agar disc diffusion method. *Bacillus subtilis* BGE strain, *Micrococcus luteus* ATCC 9341, *Bacillus cereus* ATCC 11778, and *Escherichia coli* ATCC 11303 were used as the test organism. Working *B. subtilis* suspensions (1 x 10^7 spores/mL) were added in test agar pH 6, 7.2 and 8 (CM6, CM7.2, and CM8, respectively) with volume ratio of 0.5 mL/100 mL then working trimethoprim suspension was added to CM7.2 (0.1 mL/100 mL). Working *B. cereus* suspension (1 x 10^7 spores/mL) was added in test agar pH 6 (BC6).

Each working *M. luteus and E. coli* suspensions (1 x 10^7 cfu/mL) was added in test agar pH 8 (ML8 and EC8, respectively). About 20 mL of the test agar were poured into a sterilized petri dish and allowed to solidify at room temperature. A sterilized filter-paper disc (6 mm in diameter) was placed on the surface of the agar and filled with 10 μL of 500 mg/ml of the extract solutions. Antibacterial activities were evaluated by measuring the diameter of the inhibition zones after incubating at 30 °C (CM6, 7.2, 8 and BC6) or 37 °C (ML8 and EC8) for 24 h. Penicillin, sulfadimidine, streptomycin, erythromycin, oxytetracyclin and ciprofloxacin were used as the positive controls. The conditions for each organism are presented in Table 3.1.

Table 3.1 The pH conditions and standard controls for antibacterial activity

Bacteria	pH	Code	Standard Control	(Concentration)
B. subtilis	6	CM6	Penicillin	(0.001 IU/μL)
B. subtilis + Trimethoprim	7.2	CM7.2	Sulfadimidine	(0.05 IU/μL)
B. subtilis	8	CM8	Streptomycin	(0.05 μg/μL)
M. luteus	8	ML8	Erythromycin	(0.0025 μg/μL)
B.cereus	6	BC6	Oxytetracyclin	(0.05 μg/μL)
E.coli	8	EC8	Ciprofloxacin	(0.0003 μg/μL)

3.3.1.4 Anticancer activity

The methanol extract of *H. colorata*, *P. lanceolaria*, *B. strigosa* and *B. cristata* extracts have been tested for cytotoxicity against KB (oral cavity cancer), MCF-7 (breast cancer), and NCI-H187 (small cell lung cancer) cell lines using Resazurin Microplate Assay (REMA). Ellipticine, doxorubicin, and tamoxifen were used as positive controls. Fifty μg/mL extracts were prepared in 0.5% DMSO, thus, 0.5% DMSO was used as a negative control. Each 5 microliters of 50 extract and 45 μL of cells were put into 96 well microtiter plates, then incubated at 37 °C, 5% CO_2, for 72 h

for KB and MCF7 and 5 days for NCI-H187. After the incubation periods, each well was filled with 12.5 µL of 62.5 µL/mL Resazurin solution, and then incubated at 37 °C for 4 h. The plates were then processed for fluorescence signals using a microplate reader at excited and emission wavelengths of 530 and 590 nm. The fluorescence units of cell treated with test compound (FU_T) and untreated cell (FU_C) were used for the percentage inhibition calculation by the following equation.

$$\% \text{ Inhibition} = [1- FU_T / FU_C] \times 100 \quad \text{….. (3.2)}$$

3.3.2 Phytochemical screening

The crude methanol extracts from Section 3.3.1.1 have also been screened for phytochemical constituents such as flavonoids, alkaloids, tannins, saponins, terpenoids, and steroids.

Test for flavonoids

Ten milliliters of petroleum ether was added to 0.5 g of the crude extract and it was shaken well, then the liquid part was discarded (repeated 2 times). The remaining residue was dissolved with 10 mL of 50% methanol solution. The solution was equally divided in 2 tubes for control and test sample. Three small pieces of metal magnesium was added to the test sample tube, after that 5-6 drops of concentrated hydrochloric acid was added. A pink-red color will be observed for flavonoids and an orange color for flavones [Chhetri *et al*., 2008].

Test for alkaloids

Ten milliliters of 2% HCl was added to 0.2 g of crude methanol extract. After heating on a boiling water bath for 15 min and filtering, three drops of Kraut's reagent was added to the supernatant. Turbidity or an orange red precipitate will be observed. Kraut's reagent was prepared using the following method:

Solution A: 8 g bismuthyl nitrate ($BiO(NO_3)$) was dissolved in 12 mL 30% (w/v) nitric acid

Solution B: 27.2 g potassium iodide (KI) in 50 mL water

Before use, solution A was mixed with solution B and made up to 100 mL with water.

Test for tannins

Twenty milliliters of water was added to 0.5 g of the crude extract. After boiling and filtering, the solution was equally divided in 2 tubes for control and test sample. Three drops of 0.1% ferric chloride solution was added into the test sample tube. A blue color would be observed for hydrolysable tannins and a green black or brownish green color for condense tannins [Chhetri *et al.*, 2008].

Test for saponins

Ten milliliters of water was added to 0.4 g of the crude extract. After filtering, the solution was equally divided into 2 tubes for the froth test. The first tube was strongly shaken for 1 min and then allowed to settle for 30 min to observe the froth (appearance of creamy mist of small bubbles). The second tube was boiled with 1 M HCl, then shaken for 1 min to see a froth not in a honeycomb shape [Egwailhide and Gimba, 2007].

Test for terpenoids and steroids

Two milliliters of chloroform was added to 0.5 g of the extract then three drops of acetic anhydride was added, the mixture was shaken well and then concentrated solution of sulfuric acid was added slowly into the leaning test tube. A greenish blue color was observed for steroids and a red violet color indicated the presence of terpenoids.

Test for antraquinone

Ten milliliters of 10% HCl was added to 0.5 g of the extract then boiled for five min in a water bath. After filtration and cooling, 5 mL of the filtrate was added to 5 mL of chloroform then three drops of 10% NH_3 were added. A rose-pink color will be observed after boiling in a water bath for a few minutes [Egwailhide and Gimba, 2007].

3.3.3 Purification and structural elucidation

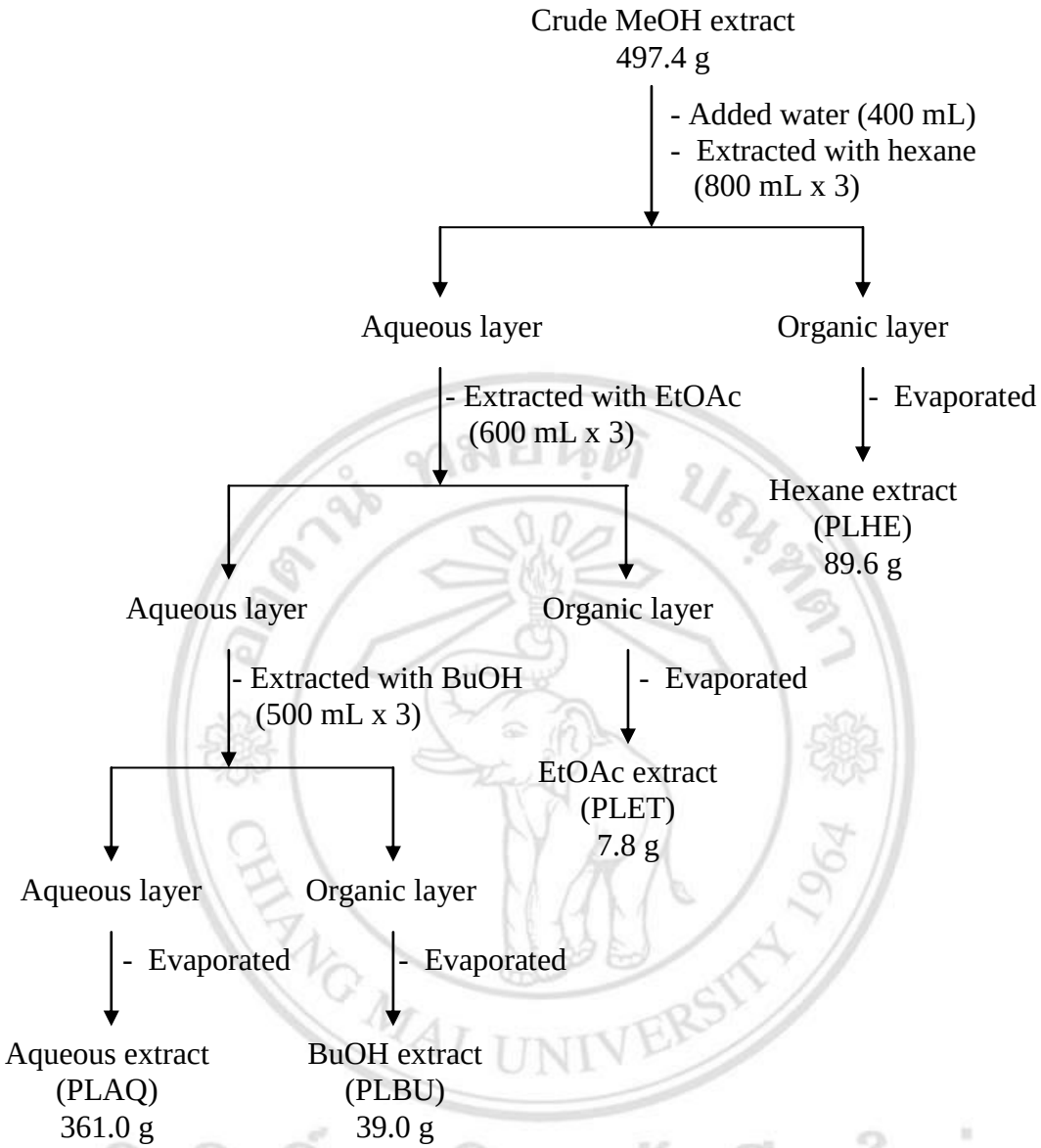
3.3.3.1 Secondary metabolites isolation from the aerial part of *P. lanceolaria*

- Preparation of crude extract

The aerial part of *P. lanceolaria* (21.0 kg) was air dried for 2 days and then dried in a 50 ºC hot-air oven for 24 h and then ground into a powder (4.074 kg). The powder was then extracted with 10 L of methanol for 7 days and then filtered. The residue was extracted again with methanol (5L x 3) for 21 days (7 days for each extraction). The filtrate was dried under vacuum to give the methanol crude extract as a dark green residue (497.4 g).

- Solvent - solvent extraction

The methanol crude extract was suspended in 400 mL of water and partitioned with hexane (800 mL x 3) to yield the crude hexane extract (dark green sticky syrup, 89.6 g). The aqueous layer was then extracted with ethyl acetate (600 mL x 3) and *n*-butanol (500 mL x 3) to yield the crude ethyl acetate extract (dark brown solid 7.8 g) and the crude *n*-butanol extract (red-brown solid 39.0 g), respectively. The extraction procedure is shown in Scheme 3.1.



Scheme 3.1 Solvent - solvent extraction scheme for *P. lanceolaria*

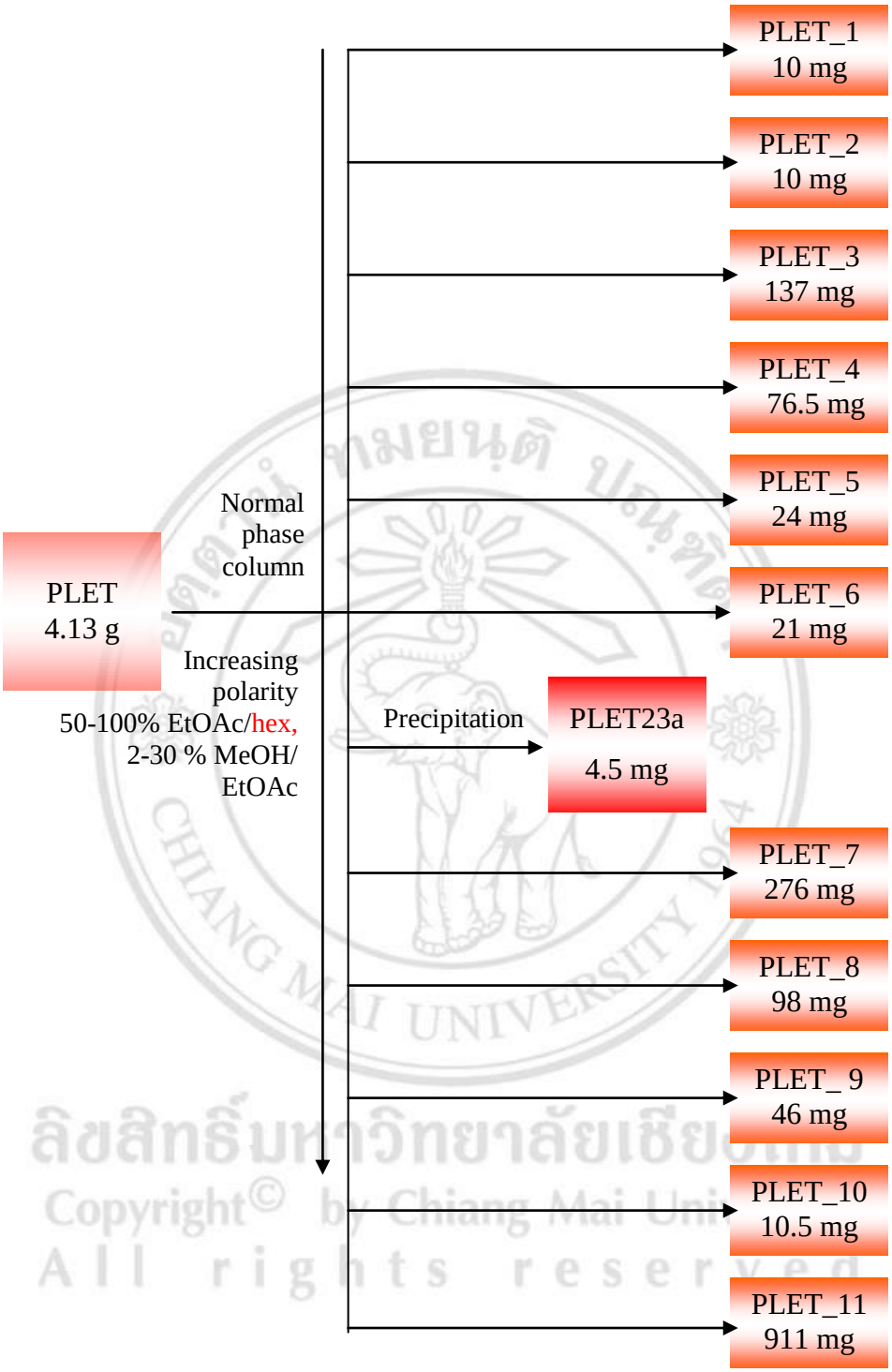
- **Isolation and Purification**

The ethyl acetate extract (PLET) and the butanol extract (PLBU) showed significant antioxidant activity (DPPH method), which were selected for isolation and purification.

Isolation of PLET23a

A portion of the ethyl acetate extract (PLET, 4.13 g) was separated by normal phase flash column chromatography (diameter 4 cm, height 38 cm) on silica gel (40-630 mesh, 250 g) using gradient elution from 1:1 to 100:0 ethyl acetate in hexane followed by 1:50 to 3:7 MeOH in ethyl acetate to give 165 fractions. The fractions were left in a fume hood over night to evaporate and an orange precipitate was obtained in fraction 23. The precipitate was then washed with petroleum ether to yield a pure compound PLET23a (4.5 mg).

All the 165 fractions were analysed by TLC with hexane and ethyl acetate (1:1), and combined together as appropriate. The solvents were dried under vacuum using a rotary evaporator to give 11 fractions, PLET_1 (10 mg), PLET_2 (10 mg), PLET_3 (137 mg), PLET_4 (77 mg), PLET_5 (24 mg), PLET_6 (21 mg), PLET_7 (276 mg), PLET_8 (98 mg), PLET_ 9 (46 mg), PLET_10 (10.5 mg) and PLET_11 (911 mg). A summary of the isolation and purification procedure to afford the pure compound PLET23a is shown in Scheme 3.2.

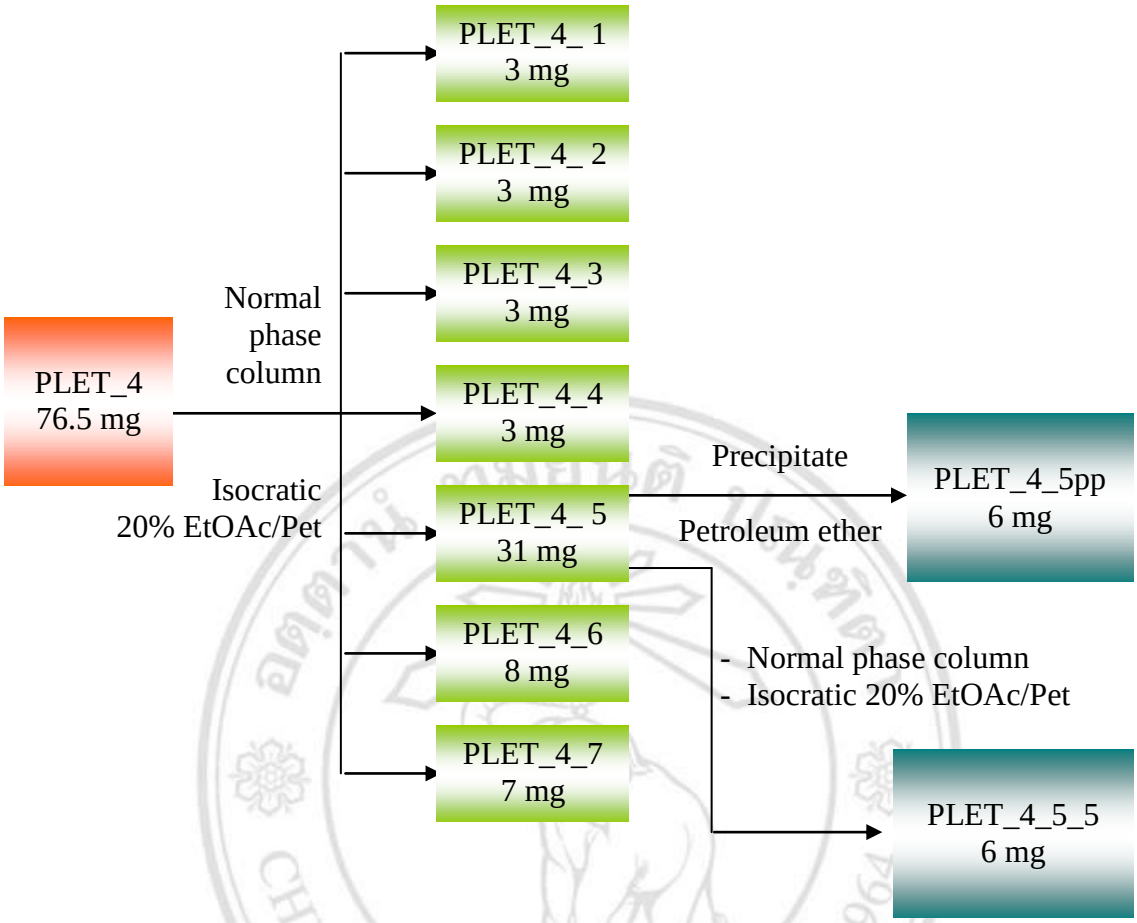


Scheme 3.2 The flash column chromatography of the ethyl acetate extract of *P. lanceolaria* (PLET) to give PLET23a

Isolation of PLET_4_5pp and PLET_4_5_5

Fractions 4 (76.5 mg) was then subjected to CC (diameter 1.4 cm, height 30 cm) over silica gel (40-630 mesh, 25 g) using isocratic elution of petroleum ether in ethyl acetate (4:1) to give 38 fractions. All fractions were analysed by TLC with petroleum ether and ethyl acetate (ratio 7:3), and combined together as appropriate to get 7 fractions, PLET_4_1 - PLET_4_7. The fractions were left in a fume hood for 12 hours an orange precipitate was obtained in fraction PLET_4_5. The precipitate was then washed with petroleum ether to yield a pure compound PLET_4_5pp (6 mg). Then the solvents in each fractions were evaporated under vacuum using a rotary evaporator to give PLET_4_1 (3 mg), PLET_4_2 (3 mg), PLET_4_3 (3 mg), PLET_4_4 (3 mg), PLET_4_5 (31 mg), PLET_4_6 (8 mg) and PLET_4_7 (7 mg).

Fractions PLET_4_5 (31 mg) was subjected to CC (diameter 0.8 cm, height 30 cm) over silica gel (40-630 mesh, 7 g) using ethyl acetate in petroleum ether (1:5) to give 20 fractions. All fractions were analysed by TLC and fractions 5-7 were combined to afford the pure compound PLET_4_5_5 (6 mg). A summary of the isolation and purification procedure for the pure compounds PLET_4_5pp and PLET_4_5_5 is presented in Scheme 3.3.



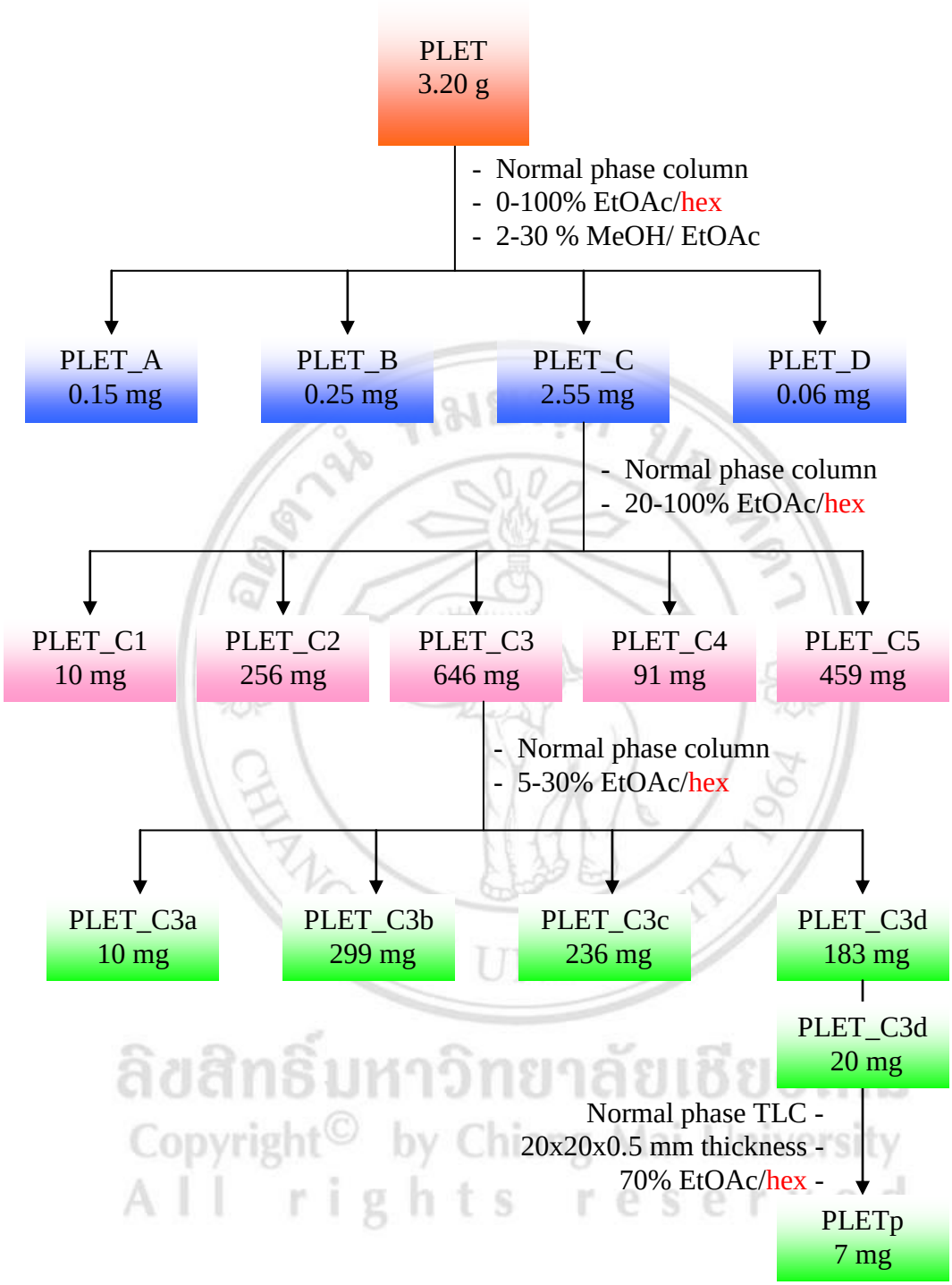
Scheme 3.3 The flash column chromatography of PLET_4 to give PLET_4_5pp and PLET_4_5_5

Isolation of PLETp

A portion of the ethyl acetate extract (PLET, 3.20 g) was separated by column chromatography (diameter 3.6 cm, height 30 cm) on silica gel (40-630 mesh, 150 g) using gradient elution from 0:100 to 100:0 ethyl acetate in hexane followed by MeOH in ethyl acetate (1:50 to 3:7) to give 140 fractions. All fractions were analysed by TLC with hexane and ethyl acetate (1:1 ratio for fractions 1-35 and 4:6 ratio for fractions 36-140), and combined together as appropriate. The solvents were evaporated under vacuum using a rotary evaporator to give 4 fractions, PLET_A (0.15 g), PLET_B (0.25 g), PLET_C (2.55 g) and PLET_D (0.06 g).

Fractions PLET_C (2.55 g) was then subjected to CC (diameter 3 cm, height 35 cm) over silica gel (40-630 mesh, 130 g) using gradient elution from 1:5 to 100:0 ethyl acetate in hexane to afford 5 fractions, PLET_C1 (10 mg), PLET_C2 (256 mg), PLET_C3 (646 mg), PLET_C4 (91 mg) and PLET_C5 (459 mg).

Fraction PLET_C3 (646 mg) was then applied to CC (diameter 2.2 cm, height 20 cm) over silica gel (40-630 mesh, 40 g) using gradient elution from 1:20 to 30:100 ethyl acetate in hexane to provide 4 fractions PLET_C3a (10 mg), PLET_C3b (299 mg), PLET_C3c (236 mg) and PLET_C3d (183 mg). A portion of fraction PLET_C3d (20 mg) was further purified by normal phase preparative TLC over a silica gel (60 GF_{254}, Merck Germany) coated glass plate (20 x 20 cm, 0.5 mm thickness). Fifteen milliliters of hexane in 35 mL of ethyl acetate was used as a mobile phase and the bands were viewed under UV light at 254 nm and 365 nm to yield pure compound PLETp (7 mg). A summary of the isolation and purification procedure of the pure compound PLETp is presented in Scheme 3.4.

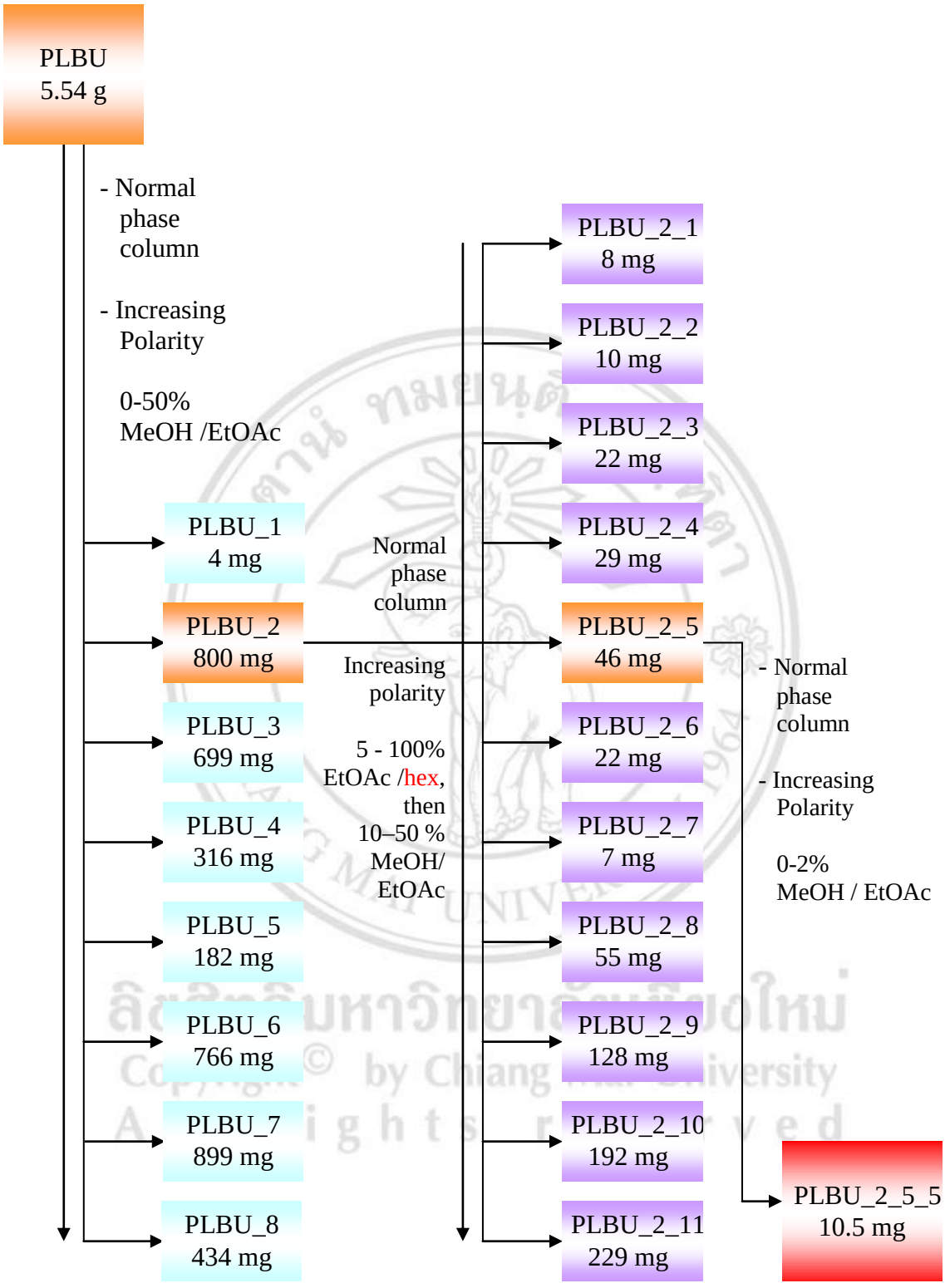


Scheme 3.4 The isolation and purification of compound PLETp

Isolation of PLBU_2_5_5

A portion of the *n*-butanol extract (PLBU, 5.54 g) was subjected to normal phase flash column chromatography (diameter 4 cm, height 38 cm) on silica gel (40-630 mesh, 250 g) using gradient elution from 0:100 to 1:1 MeOH in ethyl acetate. One hundred and fifty-four fractions were collected (50 mL each) and their composition was analysed by TLC, those fractions showing similar TLC profiles were group into eight major fractions, PLBU_1 (4 mg), PLBU_2 (800 mg), PLBU_3 (699 mg), PLBU_4 (316 mg), PLBU_5 (682 mg), PLBU_6 (766 mg), PLBU_7 (899 mg) and PLBU_8 (434 mg).

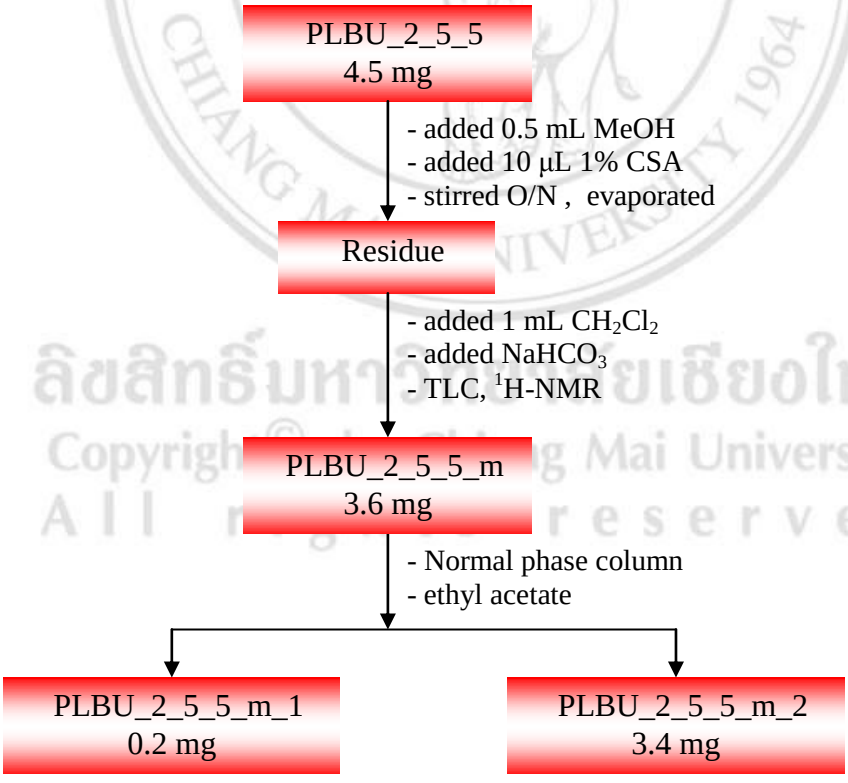
Fraction PLBU_2 (800 mg) was then subjected to normal flash CC (diameter 2 cm, height 30 cm) over silica gel (40-630 mesh, 50 g) using gradient elution from 1:20 to 100:0 ethyl acetate in hexane followed by 1:10 to 1:1 MeOH in ethyl acetate, one hundred and thirty fractions were collected (30 mL each) and analysed by TLC then grouped into eleven major fractions, PLBU_2_1 (8 mg), PLBU_2_2 (10 mg), PLBU_2_3 (22 mg), PLBU_2_4 (29 mg), PLBU_2_5 (46 mg), PLBU_2_6 (22 mg), PLBU_2_7 (7 mg), PLBU_2_8 (55 mg), PLBU_2_9 (128 mg), PLBU_2_10 (192 mg), PLBU_2_11 (229 mg). Fraction PLBU_2_5 (46 mg) was further purified by flash CC (diameter 0.8 cm, height 30 cm) over silica gel (40-630 mesh, 10 g) using gradient elution from 0:100 to 1:50 MeOH in ethyl acetate to yield pure compound PLBU_2_5_5 (10.5 mg). A summary of the isolation and purification procedure of the pure compound PLBU_2_5_5 is presented in Scheme 3.5.



Scheme 3.5 The isolation and purification of compound PLBU_2_5_5

Synthesis of compound PLBU_2_5_5_m_2

It is likely that compound PLBU_2_5_5 is an artifact from a natural product which has been reacted with the n-butanol used in the extraction process. So, this compound has converted to its methyl acetal derivative by treatment with camphor sulfonic acid (CSA) in MeOH. The reaction started with a portion of PLBU_2_5_5 (4.5 mg) dissolved in 0.5 mL of methanol in a 10 mL round bottom flask. Ten microlitters of 1% CSA was added into the flask. The mixture was stirred at room temperature for over night and then the solvent was evaporated. The residue was dissolved in 1 mL dichloromethane then washed with saturated $NaHCO_3$ solution. The progress of the reaction was monitored by TLC and ^{1}H-NMR spectrometry. The product was further purified through normal phase CC (diameter 0.5 cm, height 9 cm) over silica gel (40-630 mesh, 1 g) using ethyl acetate as a solvent to yield 3.4 mg (87%) of compound PLBU_2_5_5_m_2. A summary of the reaction and purification procedures of the pure compound PLBU_2_5_5_m_2 are presented in Scheme 3.6.



Scheme 3.6 A summary of the reaction and purification procedures of the pure compound PLBU_2_5_5_m_2

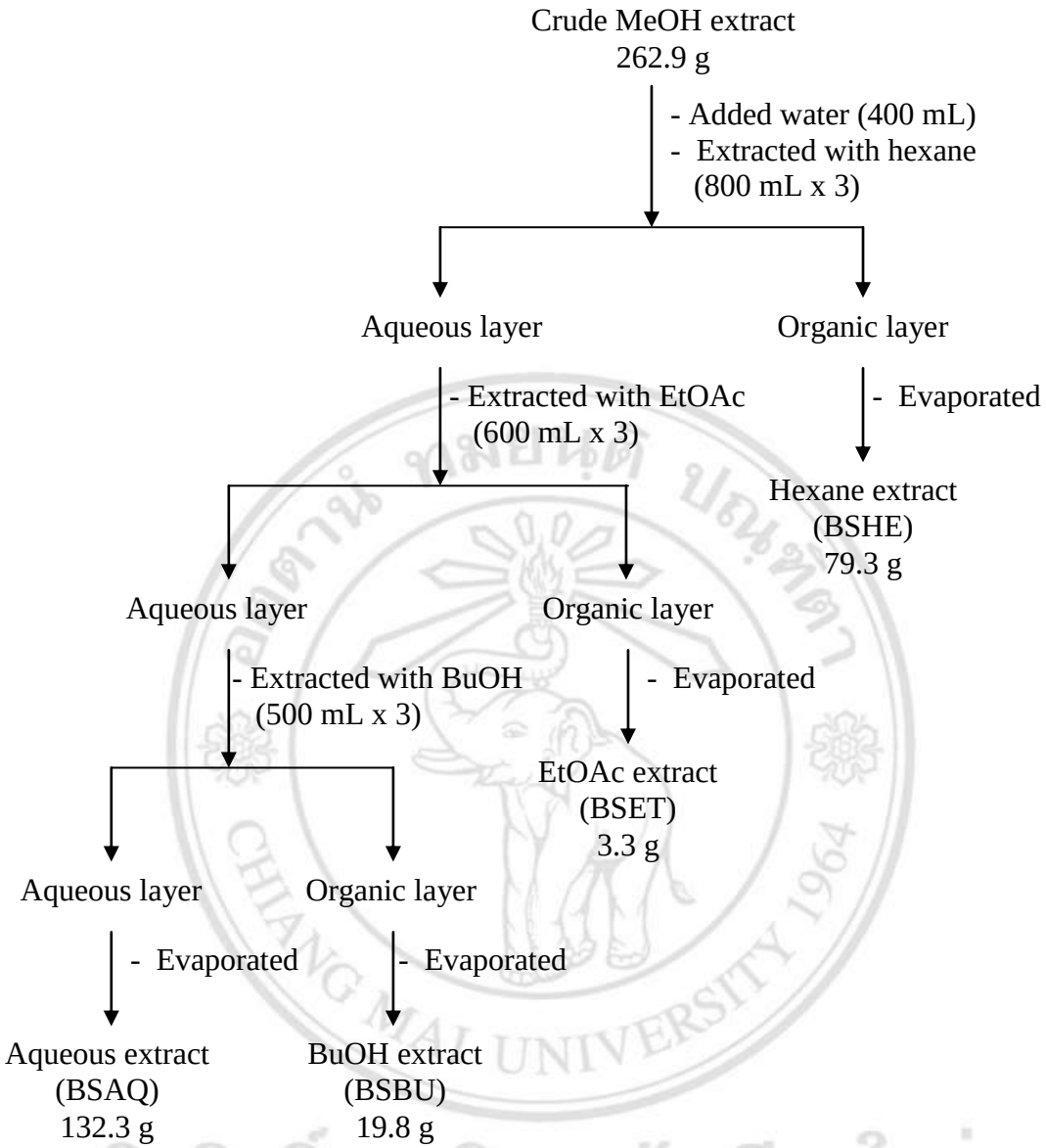
3.3.3.2 Secondary metabolites isolation from the aerial part of *B. strigosa*

- Preparation of crude extract

The aerial part of *B. strigosa* (10.5 kg) was dried under the shade for 2 days and blown in a 50 ºC hot-air blower for 2 days then ground to a fine powder. The dried plant powder (2.86 kg) was extracted with 5 L of methanol for 7 days at room temperature and then filtered. The residue was extracted again with 3 L of methanol (3 L x 3). The filtrate was dried under vacuum to give the methanol crude extract [262.9 g (9.2%)] as a dark green residue.

- Solvent - solvent extraction

The methanol crude extract was suspended in 400 mL of water and partitioned with hexane (3 x 800 mL) to yield the crude hexane extract (dark green sticky syrup, 79.3 g). The aqueous layer was then extracted with ethyl acetate (3 x 600 mL) and *n*-butanol (3 x 500 mL) to yield the crude ethyl acetate extract (dark green solid, 3.3 g), the crude *n*-butanol extract (dark green solid, 19.8 g), and the crude aqueous extract (dark brown solid, 132.3 g), respectively. The solvent - solvent extraction procedures are shown in Scheme 3.7.



Scheme 3.7 Solvent - solvent extraction scheme of *B. strigosa*

- Isolation and Purification

The ^{1}H NMR spectrum of the ethyl acetate extract (BSET) showed resonances which indicated that it may contain a mixture of aromatic compounds which was selected for isolation and purification.

Isolation of BSET_6_1_19a

The part of the ethyl acetate extract (BSET, 3.3 g) was separated by normal phase CC (diameter 4.4 cm, height 16 cm) on silica gel (40-630 mesh, 160 g) using gradient elution from 1:1 to 100:0 ethyl acetate in petroleum ether then 1:10 to 1:1 methanol in ethyl acetate to give 7 fractions, BSET_1 (10 mg), BSET_2 (207 mg), BSET_3 (530 mg), BSET_4 (227 mg), BSET_5 (94 mg), BSET_6 (1.5975 g) and BSET_7 (1.0659 g).

Fraction BSET_6 (1.5975 g) was then subjected to normal phase CC (diameter 4.0 cm, height 23 cm) on silica gel (150 g) using isocratic elution of 1:20 MeOH in ethyl acetate to afford 5 fractions, BSET_6_1 (396 mg), BSET_6_2 (802 mg), BSET_6_3 (90 mg), BSET_6_4 (5 mg) and BSET_6_5 (92 mg).

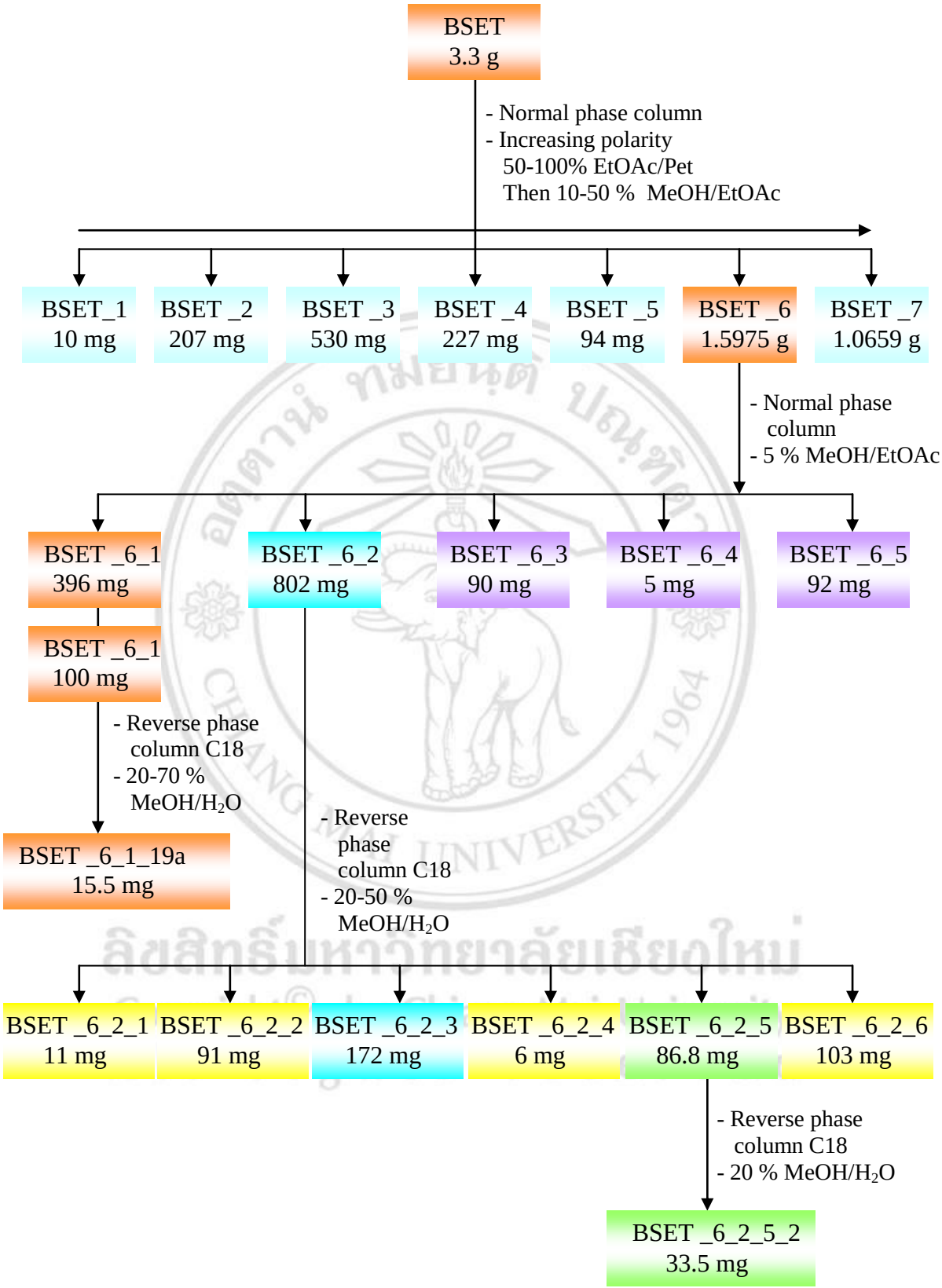
A portion of fraction BSET_6_1 (100 mg) was separated by CC (diameter 1.4 cm, height 23 cm) on RP-C18 bonded silica gel (35 g) using gradient elution from 1:5 to 7:3 MeOH in water to yield compound BSET_6_1_19a (15.5 mg) (Scheme 3.8).

Isolation of BSET_6_2_3

Fraction BSET_6_2 (802 mg) was separated by CC on RP-C18 bonded silica gel (35 g) using gradient elution from 1:5 to 1:1 MeOH in water to afford 6 fractions, BSET_6_2_1 (11 mg), BSET_6_2_2 (91 mg), pure compound BSET_6_2_3 (172 mg), BSET_6_2_4 (6 mg), BSET_6_2_5 (87 mg) and BSET_6_2_6 (103 mg). A summary of the isolation and purification procedures of the pure compounds BSET_6_1_19a and BSET_6_2_3 are presented in Scheme 3.8.

Isolation of BSET_6_2_5_2

Fraction BSET_6_2_5 (86.8 mg) was separated by RP-C18 bonded silica gel (35 g) CC using isocratic elution of MeOH in water (1:5) to yield pure compound BSET_6_2_5_2 (33.5 mg). A summary of the isolation and purification procedures of the pure compound BSET_6_2_5_2 are presented in Scheme 3.8.



Scheme 3.8 A summary of the isolation and purification procedure of the pure compound BSET_6_1_19a, BSET_6_2_3 and BSET_6_2_5_2

CHAPTER 4

Results and discussion

4.1 Bioactivity test

4.1.1 Preparation of crude extracts

The crude methanol extracts of *H. colorata*, *P. lanceolaria*, *B. strigosa* and *B. cristata* were prepared for bioactivity and chemical tests. Each plant was air dried for 2 days and dried in a 50 ºC hot-air oven for 24 hour, then ground into a powder. Each plant powder was individually extracted with methanol that provided the methanol crude extracts of *H. colorata*, *P. lanceolaria*, *B. strigosa* and *B. cristata* in yields of 9.5, 12.4, 12.4 and 10.1% dry weight, respectively. The preparation results are shown in Table 4.1.

Table 4.1 Percentage yields of methanol crude extracts prepared from *H. colorata*, *P. lanceolaria*, *B. strigosa* and *B. cristata*

Plants	Weight of dry material (g)	Weight of crude extract (g)	% Yield[a]	Appearance
H. colorata	490.0	46.73	9.5	dark green solid
P. lanceolaria	1496.5	184.76	12.4	dark green-red solid
B. strigosa	135.0	16.81	12.4	dark green solid
B. cristata	630.0	63.70	10.1	dark green solid

[a] The percentage yield was calculated on the crude methanol extract weight basis

4.1.2 Antioxidant activity

The antioxidant activity of the crude methanol extracts of *H. colorata*, *P. lanceolaria*, *B. strigosa* and *B. cristata* were evaluated spectrophotometrically using the

DPPH method. Ascorbic acid and trolox were used as reference standards. The results are shown in Table 4.2. All extracts showed strong antioxidant activity against the DPPH radical. The IC_{50} values of these extracts (18 - 176 μg/mL) were quite similar to each other and also close to those of the standards ascorbic acid and trolox (4 and 6 μg/mL, respectively). *B. cristata* crude methanol extract exhibited the highest antioxidant activity with an IC_{50} value of 18 μg/mL. *P. lanceolaria* extract showed the lowest antioxidant activity with an IC_{50} value of 176 μg/mL. Moreover, the crude methanol extracts of *H. colorata* and *B. strigosa* possessed good activities with the IC_{50} values of 53 and 73 μg/mL, respectively. However, these values were slightly lower than those of the standards ascorbic acid and trolox.

Table 4.2 Antioxidant activity of the crude methanol extracts *H. colorata*, *P. lanceolaria*, *B. strigosa*, *B. cristata* and the standards ascorbic acid and trolox

sample	**IC_{50} (μg/mL)**
H. colorata	53
P. lanceolaria	176
B. strigosa	73
B. cristata	18
Ascorbic acid	4
Trolox	6

4.1.3 Antibacterial activity

The agar disc diffusion method was used to evaluate the antimicrobial activity of the extracts of *H. colorata*, *P. lanceolaria*, *B. strigosa* and *B. cristata*. The studies were carried out *in vitro* against Gram-positive (*Bacillus subtilis*, *Micrococcus luteus*, and *Bacillus cereus*) and Gram-negative (*Escherichia coli*) organisms. Penicillin, sulfadimidine, streptomycin, erythromycin, oxytetracyclin and ciprofloxacin were used as the positive controls. The methanol extracts of *P. lanceolaria* and *B. cristata* showed weak antibacterial activities. The extract of *P. lanceolaria* exhibited antibacterial activity against *B. subtilis* at pH 6 and pH 7.2 with the inhibition zones of 8.0 and 10.0

mm, respectively. The *B. cristata* extract showed antibacterial activity against *B. subtilis* at pH 7.2, *B. subtilis* at pH 8 and *B. cereus* at pH 6 with the inhibition zones of 7.0, 9.0 and 7.0 mm, respectively. However, *H. colorata* and *B. strigosa* extracts were inactive to all the test organisms. The results are presented in Table 4.3.

Table 4.3 The inhibition zone of plant extracts using the agar disk diffusion method

Bacteria strains	**Inhibition zone (mm)**[a]					
	Standard Controls	**Blank Control**	***H. colorata***	***P. lanceolaria***	***B. strigosa***	***B. cristata***
B. subtilis (pH 6)	28 Penicillin	≤6.0	≤6.0	8.0	≤6.0	≤6.0
B. subtilis (pH 7.2)	29 Sulfadimidine	≤6.0	≤6.0	10.0	≤6.0	7.0
B. subtilis (pH 8)	25 Streptomycin	≤6.0	≤6.0	≤6.0	≤6.0	9.0
M. luteus (pH 8)	29 Erythromycin	≤6.0	≤6.0	≤6.0	≤6.0	≤6.0
B. cereus (pH 6)	17 Oxytetracyclin	≤6.0	≤6.0	≤6.0	≤6.0	7.0
E. coli (pH 8)	28 Ciprofloxacin	≤6.0	≤6.0	≤6.0	≤6.0	≤6.0

[a] Diameter of inhibition zones are the mean diameter on 6.0 mm paper disc

4.1.4 Anticancer activity

The *H. colorata*, *P. lanceolaria*, *B. strigosa* and *B. cristata* extracts have been tested at 50 μg/mL concentration for anticancer activity against KB (oral cavity cancer), MCF-7 (breast cancer), and NCI-H187 (small cell lung cancer) cell lines. Ellipticine, doxorubicin, and tamoxifen were used as the positive controls. It was found that all of the extracts were inactive due to their percentage inhibitions being less than 50%. The results of the anticancer activities are shown in Table 4.4.

Table 4.4 Anticancer activities of *H. colorata*, *P. lanceolaria*, *B. strigosa* and *B. cristata* crude methanol extracts on human cancer cell lines

Samples	% Inhibitions[b]			activity
	KB	MCF-7	NCI-H187	
H. colorata	16.02	29.17	14.28	Inactive
P. lanceolaria[a]	36.35	43.06	25.45	Inactive
B. strigosa[a]	40.84	26.81	22.87	Inactive
B. cristata[a]	29.13	43.97	16.33	Inactive
	IC_{50} (µg/mL)[c]			
Ellipticine[d]	0.84	-	1.20	Active
Doxorubicin[d]	0.48	8.63	0.08	Active
Tamoxifen[d]	-	9.87	-	Active

[a] Partially soluble in 100% DMSO

[b] Percentage inhibition at 50 µg/mL

[c] Concentration that killed 50% of cell lines

[d] Positive control

4.2 Phytochemical screening

The phytochemical screening of the crude extracts was investigated. The crude extracts were tested for flavonoids, alkaloids, tannins, saponins, terpenoids, and steroids. The extracts of *B. cristata*, *B. strigosa*, *H. colorata* were found to contain tannins, saponins and steroids. Whereas the extract of *P. lanceolaria* contained saponins, steroids and flavonoids. However, all extracts showed a negative test for alkaloids and antraquinones. The results are summarized in Table 4.5.

Table 4.5 Phytochemical constituents of the plant extracts

Plants	***H. colorata***	***P. lanceolaria***	***B. strigosa***	***B. cristata***
Flavonoids	Negative	Pink solution	Negative	Negative
Alkaloids	Negative	Negative	Negative	Negative
Tannins	Brownish green solution with green precipitate	Negative	Brownish green solution with green precipitate	Brownish green solution with green precipitate
Saponins (Tube1/Tube2)	Honeycomb shape froth/ No froth	Honeycomb shape froth/ No froth	Honeycomb shape froth/ No froth	Honeycomb shape froth/ No froth
Steroids	Greenish blue	Greenish blue	Greenish blue	Greenish blue
Antraquinones	Negative	Negative	Negative	Negative

4.3 Purification and structural elucidation

4.3.1 Secondary metabolites isolation from the aerial part of *P. lanceolaria*

4.3.1.1 Preparation of crude extract

The dried aerial part of *P. lanceolaria* was ground into a powder (4.074 kg) then extracted with methanol at room temperature to yield the methanol crude extract as a dark green residue, with the percentage yield of 12.2% w/w (497.4 g). It was further extracted with different polarity solvents using solvent-solvent extraction procedures.

4.3.1.2 Solvent-solvent Extraction

The methanol crude extract (497.4 g) was suspended in water and then successively partitioned with *n*-hexane, ethyl acetate, and *n*-butanol to provide hexane extract, ethyl acetate extract, butanol extract and aqueous extract, respectively. The percentage yields of all extracts were calculated as shown in Table 4.6. The aqueous

extract provided the largest percentage yield at 72.6% w/w. The hexane and butanol extracts were lower yielding (18.0 and 7.8%, respectively), whereas the ethyl acetate extract gave the lowest yield at 1.6%.

Table 4.6 The percentage yields of *P. lanceolaria* extracts by solvent-solvent extraction

extracts	Weight (g)	% yield[a]	Appearance
Hexane extract	89.6	18.0	dark green sticky syrup
Ethyl acetate extract	7.8	1.6	dark brown solid
Butanol extract	39.0	7.8	red-brown solid
Aqueous extract	361.0	72.6	dark-red solid

[a] The percentage yield was calculated on the crude methanol extract weight basis

All extracts were tested for their antioxidant activities using the DPPH method. The extracts possessed high activities with IC_{50} values of 50-227 μg/mL. Therefore, the ethyl acetate extract (PLET) and the butanol extract (PLBU) were selected for isolation and purification due to their significantly higher antioxidant activities, with the IC_{50} values of 57 μg/mL and 50 μg/mL, respectively. The results are presented in Table 4.7.

Table 4.7 Antioxidant activity of *P. lanceolaria* extracts

sample	Sample code	IC_{50}[a] (μg/mL)
Hexane extract	PLHE	227
Ethyl acetate extract	PLET	57
n-Butanol extract	PLBU	50
Aqueous extract	PLAQ	148
Ascorbic acid[b]	-	4
Trolox[b]	-	6

[a] The concentration that scavenged 50 % of DPPH radicals

[b] The standard controls

4.3.1.3 Isolation and structural elucidation of compound PLET23a

The ethyl acetate extract of *P. lanceolaria* (PLET) was separated by normal phase flash column chromatography using gradient elution. Ethyl acetate, hexane, and methanol were used as eluents and 165 fractions were collected. After leaving the fractions in a fume hood over night to evaporate, compound **PLET23a** was obtained as an orange-red amorphous powder. It was an optically active compound ($[\alpha]_D^{25}$ +149.7°, c 0.00225 g/mL, $CHCl_3$). The EI mass spectrum showed the molecular-ion peak at *m/z* 568 (M^+, $C_{40}H_{56}O_2$) which corresponding to the molecular formula of $C_{40}H_{56}O_2$ (Figure 4.1).

The ^1H and ^{13}C NMR spectrum of compound **PLET23a** (Table 4.8, Figure 4.2 and 4.3) showed the characteristics of an asymmetric carotenoid which consisted of one optically active β-type moiety and an optically active ε-type moiety. Due to it asymmetrical nature, there were several signals for methyl groups in the aliphatic part of the ^1H NMR spectrum of compound **PLET23a**. Singlet signals corresponding to the methyl groups H-16′, H-17′, H-18′, H-18, and H-19′ appeared at δ 0.85, 1.00, 1.62, 1.74, and 1.91 ppm, respectively. Two singlet signals with integrations for six and nine protons were evident at δ 1.07 (H-16, and H-17) and 1.97 (H-19, H-20, and H-20′). The aliphatic proton signals for the methylene protons at H-2 and H-4 in the β-ring (conjugated with the polyene chain) showed as ABX systems at δ 1.77 (*br. dt*, ^2J=11.9, H-2*α*) and δ 1.47 (*t*-like, ^2J=11.9, *J*(H-2*β*,3)=11.9, H-2*β*) and at δ 2.36 (*dd*, ^2J=16.9, *J*(H-4*α*,3)=5.5, H-4*α*) and δ 2.05 (*dd*, ^2J=16.9, *J*(H-4*β*,3)=9.8, H-4*β*), respectively. The multiplet resonance at δ 4.00 was assigned to H-3 due to its coupling with H-2 and H-4 including the geminal coupling to the hydroxyl group in the β-ring. The olefinic proton (H-4′) in the ε-ring showed a broad singlet signal at δ 5.54. The aliphatic proton signals from the β-ring also presented as an ABX pattern at δ 1.84 (*dd*, ^2J=13.1, *J*(H-2′*α*,3′)=5.9, H-2′*α*), δ 1.36 (*dd*, ^2J=13.1, *J*(H-2′*β*,3′)=6.8, H-2′*β*), and δ 4.25 (*br. s*, H-3′).

The conjugated olefinic protons of compound **PLET23a** showed several overlapped signals; H-8 and H-8′ presents a broad singlet at δ 6.12; H-11, H-15, H-15′ appeared as multiplets at δ 6.65, δ 6.64, and δ 6.62, respectively; H-12, H-12′, H-14 and H-14′ showed doublets at δ 6.36 (*d*, *J*(11,12)=14.8), δ 6.35 (*d*, *J*(11′,12′)=14.9), δ 6.27 (*d*, *J*(14,15)=11.8), and δ 6.23 ((*d*, *J*(14′,15′)=12.2), respectively. Because it is adjacent

to the β-ring system, the signal of H-7 at δ 6.10 (*d*, *J*(7,8)=16.4) was down field shifted more than that of H-7′ δ 5.43 (*dd*, *J*(7′,8′)=15.4, *J*(6′,7′)=9.9).

The assignment of ^{1}H and ^{13}C NMR signals of compound **PLET23a** were supported by two-dimensional correlated spectroscopy, correlation spectroscopy (COSY), heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC), and nuclear Overhauser effect spectroscopy (NOESY), experiments. Moreover, the specific rotation of compound **PLET23a** ($[\alpha]_D^{25}$ +149.7° (c 0.00225 g/mL, $CHCl_3$) was comparable to that the previously reported ($[\alpha]_D^{20}$ +160.5° (c 1.0 g/mL, $CHCl_3$) compound lutein [Gigoshvili and Alaniya, 2003]. From this data and by comparing the previously reported ^{1}H and ^{13}C NMR signal assignments for lutein, which were published earlier [Molnar *et al.*, 2004], it was suggested that compound **PLET23a** was lutein.

The complete ^{1}H and ^{13}C NMR assignments for both lutein and **PLET23a** isolated from the aerial part of *P. lanceolaria* are given in Table 4.8.

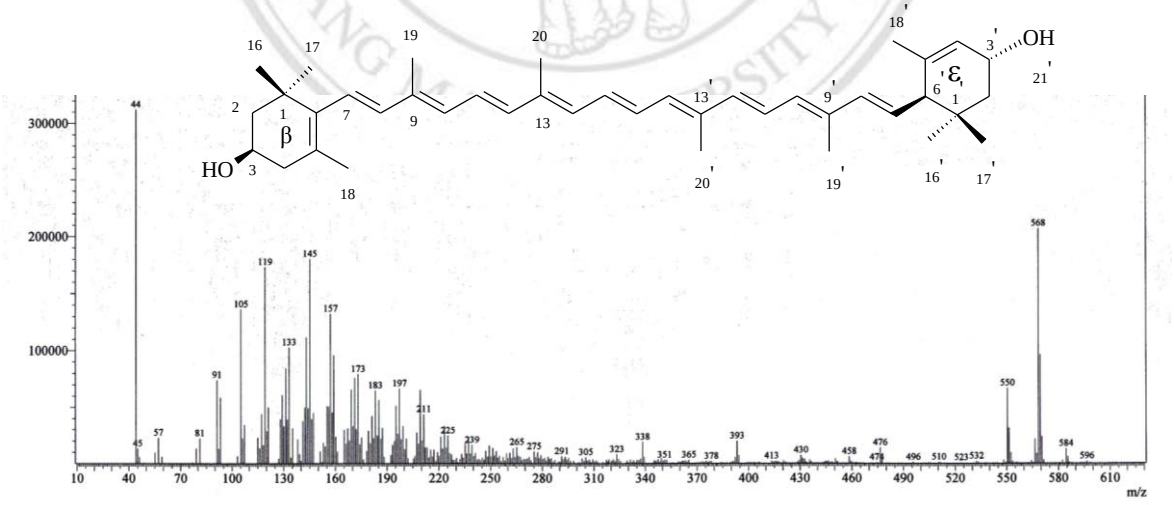


Figure 4.1 Chemical structure and mass spectrum of compound **PLET23a**

Table 4.8 ^{1}H- and ^{13}C-NMR data of lutein and compound **PLET23a** isolated from the aerial part of *P. lanceolaria*

Position	Lutein[a]		PLET23a[b]	
	^{1}H δ (H) (*J*)	^{13}C δ (C)	^{1}H δ (H) (*J*)	^{13}C δ (C)
1	-	37.1	-	37.1
2	1.77 (*ddd*, *J*(2*α*,3)=3.4, *J*(2α,4α)=2.1), 1.47 (*t*-like, ^{2}J=11.9, *J*(2*β*,3)=11.9)	48.4	1.77 (*br. dt*, ^{2}J=11.9, *J* ca 2), 1.47 (*t*-like, ^{2}J=11.9, *J*(2*β*,3)=11.9)	48.4
3	3.99 (*m*)	65.9	4.00 (*m*)	65.1
4	2.38 (*dd*, *J*(4*α*,3)=5.7) 2.04 (*dd*, ^{2}J=16.8, *J*(4*β*,3)=9.5)	42.5	2.36 (*dd*, ^{2}J=16.9, *J*(4*α*,3)=5.5) 2.05 (*dd*, ^{2}J=16.9, *J*(4*β*,3)=9.8)	42.5
5	-	126.2	-	126.2
6	-	138.0	-	137.8
7	6.09 (*m*, *J*(7,8)=16.3)	124.9	6.10 (*d*, *J*(7,8)=16.4)	125.6
8	6.12 (*m*)	138.5	6.12 (*m*)	138.5
9	-	135.7	-	135.7
10	6.15 (*m*)	131.3	6.16 (*d*, *J*(10,11)=10.4)	131.3
11	6.64 (*m*)	124.8	6.65 (*m*)	124.9
12	6.35 (*d*, *J*(11,12)=14.8)	137.5	6.36 (*d*, *J*(11,12)=14.8)	137.6
13	-	136.5	-	136.5
14	6.26 (*m*)	132.6	6.27 (*d*, *J*(14,15)=11.8)	132.6
15	6.62 (*m*)	130.1	6.64 (*m*)	130.1
16	1.07 (*s*)	28.7	1.07 (*s*)	28.7

Table 4.8 (Continued)

Position	Lutein[a]		PLET23a[b]	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)	δ (H) (*J*)	δ (C)
17	1.07 (*s*)	30.2	1.07 (*s*)	30.2
18	1.73 (*s*)	21.6	1.74 (*s*)	21.6
19	1.97 (*s*)	12.7	1.97 (*s*)	12.7
20	1.96 (*s*)	12.8	1.97 (*s*)	12.8
1′	-	34.0	-	34.0
2′	1.84 (*dd*), 1.37 (*dd*, ^{2}J=12.9, *J*(2′,3′)=6.7)	44.6	1.84 (*dd*, ^{2}J=13.1, *J*(2′*α*,3′)=5.9), 1.36 (*dd*, ^{2}J=13.1, *J*(2′*β*,3′)=6.8)	44.6
3′	4.24 (*m*)	65.1	4.25 (*br. s*)	65.9
4′	5.54 (*br. s*)	125.6	5.54 (*br. s*)	124.5
5′	-	137.7	-	138.0
6′	2.40 (*d*, *J*(6′,7′)=10.1)	54.9	2.40 (*d*, *J*(6′,7′)=9.9)	55.0
7′	5.42 (*dd*, *J*(7′,8′)=15.5)	128.7	5.43 (*dd*, *J*(7′,8′)=15.4, *J*(6′,7′)=9.9)	128.7
8′	6.13 (*m*)	137.7	6.12 (*m*)	138.5
9′	-	135.1	-	135.1
10′	6.14 (*m*)	130.8	6.14 (*d*, *J*(10′,11′)=10.4)	130.8
11′	6.60 (*dd*, *J*(10′,11′)=11.4)	124.5	6.59 (*dd*, *J*(11′,12′)=14.9) *J*(10′,11′)=10.4)	124.8
12′	6.35 (*d*, *J*(11′,12′)=14.8)	137.5	6.35 (*d*, *J*(11′,12′)=14.9)	137.5
13′	-	136.4	-	136.4

Table 4.8 (Continued)

Position	Lutein[a]		PLET23a[b]	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)	δ (H) (*J*)	δ (C)
14′	6.24 (*m*)	132.6	6.23 ((*d*, *J*(14′,15′)=12.2)	132.6
15′	6.62 (*m*)	130.0	6.62 (*m*)	130.0
16′	0.84 (*s*)	29.5	0.85 (*s*)	24.3
17′	0.99 (*s*)	24.3	1.00 (*s*)	29.5
18′	1.61 (*s*)	22.9	1.62 (*s*)	22.8
19′	1.90 (*s*)	13.1	1.91 (*s*)	13.1
20′	1.96 (*s*)	12.8	1.97 (*s*)	12.8

[a] H- and ^{13}C-NMR data of compound **PLET23a** isolated from the aerial part of *P. lanceolaria*, Conditions: at 500 and 125 MHz, respectively, in $CDCl_3$ solution (*T*=25°) ; *δ* in ppm, *J* in Hz.

[b] H- and ^{13}C-NMR data of **Lutein**, Conditions: at 400 and 100 MHz, respectively, in $CDCl_3$ solution (*T*=25°) ; *δ* in ppm, *J* in Hz [Molnar *et al.*, 2004]

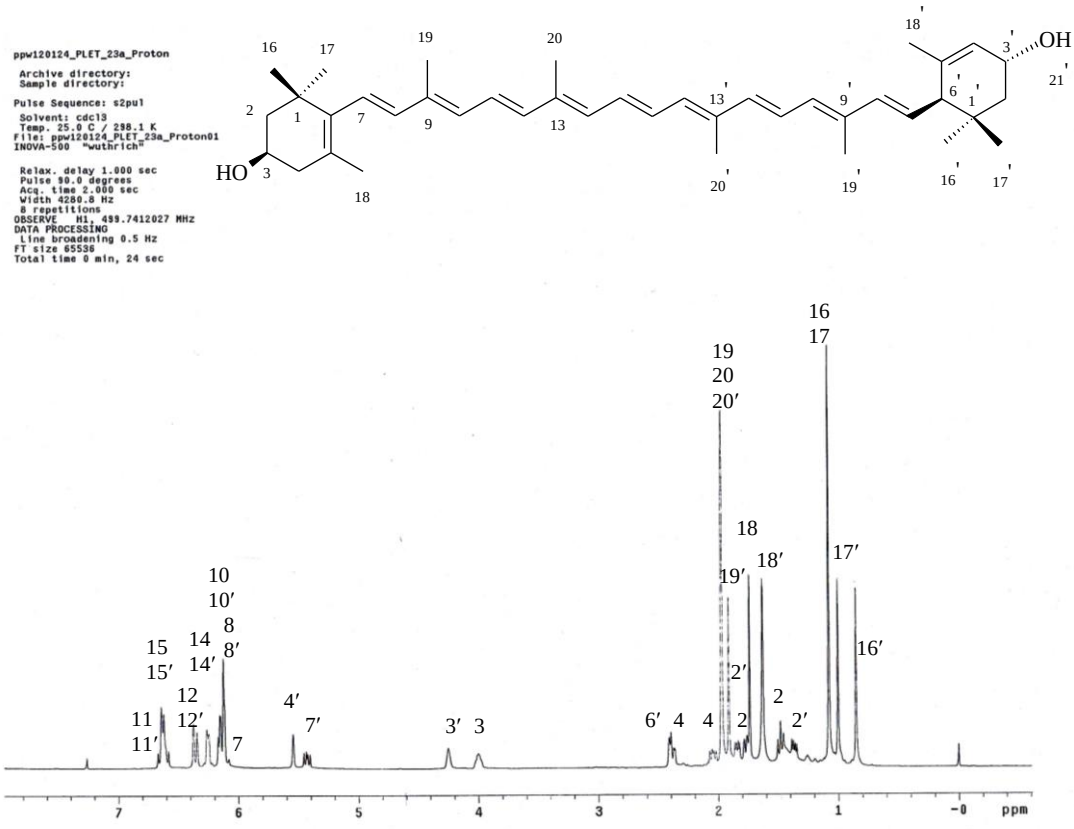


Figure 4.2 ^{1}H NMR spectrum of compound **PLET23a** (500 MHz, $CDCl_3$)

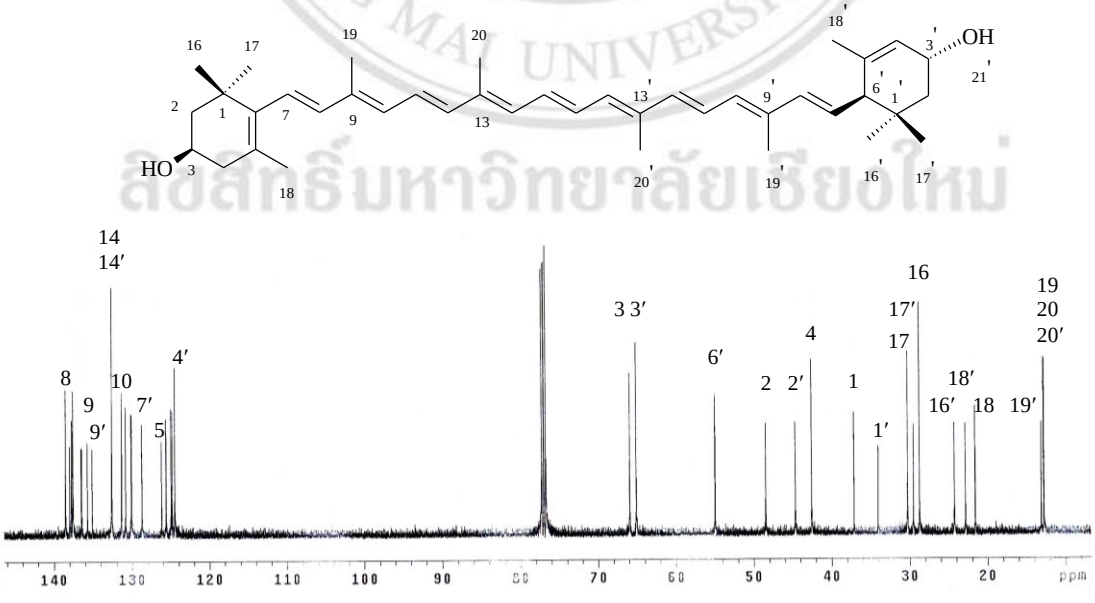


Figure 4.3 ^{13}C NMR spectrum of compound **PLET23a** (125 MHz, $CDCl_3$)

This is the first report of the isolation of lutein from the aerial part of *P. lanceolaria*. Lutein (all-*E*,3*R*,3′*R*,6′*R*)-4′,5′-didehydro-5′,6′-dihydro-*β*,*β*-carotene-3,3′-diol) is one of the well known caroteoniods. It is the main xanthophyll found in the higher plant pigment. Its function is to transfer energy in the electron transport chain mechanisms during photosynthesis [Molnar *et al.*, 2004]. It is known as a potential antioxidant found in high concentrations in egg yolks, yellow-orange fruits and vegetables including dark green leaf vegetables. Lutein is one of two major carotenoids found in the retina of the human eye. It also plays an important role in the prevention of age-related macular degeneration which is the leading cause of legal blindness in over 65-year-old people [Ma *et al*., 2012]. However, humans are not able to synthesize carotenoids, consequently, man needs to take it from the diet [Granado *et al*., 2003].

4.3.1.4 Isolation and structural elucidation of compound PLET_4_5pp

Fraction PLET_4, which was described earlier, was further purified by flash column chromatography using gradient elution with petroleum ether and ethyl acetate to yield 7 fractions, PLET_4_1 - PLET_4_7. An orange precipitate of compound **PLET_4_5pp** was obtained from fraction PLET_4_5 after it was left in a fume hood for 12 hours.

Compound **PLET_4_5pp** was an orange-red amorphous powder; $[\alpha]_D^{25}$ +41.4° (c 0.0025 g/mL, $CHCl_3$) The EI mass spectrum showed the molecular-ion peak at *m/z* 582 (M^+, $C_{41}H_{58}O_2$), and the high resolution atmospheric solids analysis probe mass spectrum (HR-ASAP-MS) was obtained with positive HR-ASAP-MS at *m/z* 583.4564 $[M+H]^+$ (calculated for 583.4515), which corresponding to the molecular formula of $C_{41}H_{58}O_2$ (Figure 4.4). The assignment of ^1H and ^{13}C NMR signals of compound **PLET_4_5pp** were also supported by two-dimensional correlated spectroscopy, COSY, HSQC, and HMBC experiments. The ^1H and ^{13}C NMR spectra of **PLET_4_5pp** presented the signal pattern quite similar to those of compound **PLET23a** except for the appearance of a methoxy group signal. The ^1H and ^{13}C NMR signals of the methoxy group were at δ 3.36 (singlet, 3H, H-21′) and δ 55.8 (C-21′), respectively (Figure 4.5

and 4.6). The signal of proton on the ε-ring geminal to the methoxy group shifted upfield to δ 3.78 (*br. s*, H-3′). The methylene protons at C-2′ shifted to δ 1.77 (*dd*, ^{2}J=13.0, $J(2'\alpha,3')$=5.1, H-2′α) and δ 1.40 (*dd*, ^{2}J=13.0, $J(2'\beta,3')$=7.0, H-2′β). The olefinic proton signal for H-4′ slightly shifted to δ 5.60 (*br. s*, H-4′). The complete ^{1}H- and ^{13}C-NMR assignments for both **PLET23a** and **PLET_4_5pp** isolated from the aerial part of *P. lanceolaria* are given in Table 4.9. From these data, it can be concluded that compound **PLET_4_5pp** was a known compound, lutein 3′-methyl ether. This is the first report of the isolation of lutein and lutein 3′-methyl ether (all-*E*,3*R*,3′*R*,6′*R*)-4′,5′-didehydro-5′,6′-dihydro-,3′-methoxy-*β*,*β*-carotene-3-ol) from the aerial part of *P. lanceolaria* and the first report of lutein 3′-methyl ether as a natural product.

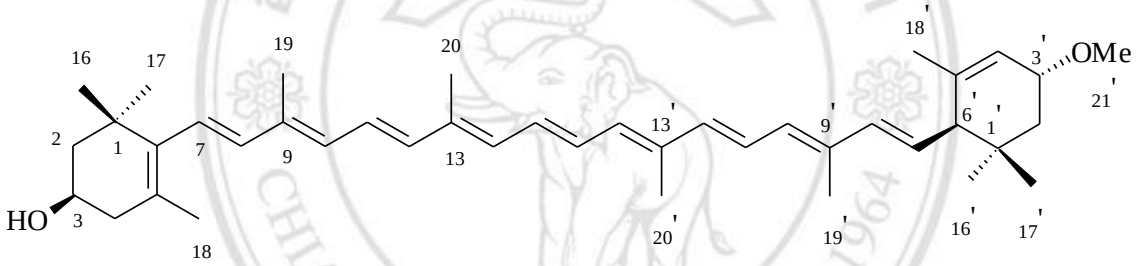


Figure 4.4 The chemical structure of **PLET_4_5pp** (lutein 3′-methyl ether)

Table 4.9 ^{1}H- and ^{13}C-NMR data of **PLET23a** (lutein) , and **PLET_4_5pp** (lutein 3′-methyl ether) isolated from the aerial part of *P. lanceolaria*

	PLET23a		**PLET_4_5pp**	
Position	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
	δ (H) (*J*)	**δ (C)**	**δ (H) (*J*)**	**δ (C)**
1	-	37.1	-	37.1
2	1.77 (*br. dt*, ^{2}J=11.9, *J* ca 2), 1.47 (*t*-like, ^{2}J=11.9, $J(2\beta,3)$=11.9)	48.4	1.76 (*br. dt*, ^{2}J=12.0, *J* ca 2), 1.48 (*t*-like, ^{2}J=12.0, $J(2\beta,3)$=11.7)	48.4

Table 4.9 (Continued)

Position	PLET23a		PLET_4_5pp	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)	δ (H) (*J*)	δ (C)
3	4.00 (*m*)	65.1	4.00 (*m*)	65.1
4	2.36 (*dd*, ^{2}J=16.9, *J*(4*α*,3)=5.5) 2.05 (*dd*, ^{2}J=16.9, *J*(4*β*,3)=9.8)	42.5	2.39 (*dd*, ^{2}J=16.6, *J*(4*α*,3)=5.4) 2.04 (*dd*, ^{2}J=16.6, *J*(4*β*,3)=9.8)	42.5
5	-	126.2	-	126.2
6	-	137.8	-	137.7
7	6.10 (*d*, *J*(7,8)=16.4)	125.6	6.10 (*d*, *J*(7,8)=16.6)	125.6
8	6.12 (*m*)	138.5	6.12 (*m*)	138.5
9	-	135.7	-	135.7
10	6.16 (*d*, *J*(10,11)=10.4)	131.3	6.16 (*d*, *J*(10,11)=11.0)	131.3
11	6.65 (*m*)	124.9	6.65 (*dd*, *J*(10,11)=11.0, *J*(11,12)=14.9)	124.9
12	6.36 (*d*, *J*(11,12)=14.8)	137.6	6.36 (*d*, *J*(11,12)=14.9)	137.5
13	-	136.5	-	136.5
14	6.27 (*d*, *J*(14,15)=11.8)	132.6	6.27 (*d*, *J*(14,15)=12.0)	132.6
15	6.64 (*m*)	130.1	6.64 (*m*)	130.0
16	1.07 (*s*)	28.7	1.07 (*s*)	28.7
17	1.07 (*s*)	30.2	1.07 (*s*)	30.2
18	1.74 (*s*)	21.6	1.74 (*s*)	21.6
19	1.97 (*s*)	12.7	1.96 (*s*)	12.8
20	1.97 (*s*)	12.8	1.96 (*s*)	12.8

Table 4.9 (Continued)

Position	PLET23a		PLET_4_5pp	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)	δ (H) (*J*)	δ (C)
1′	-	34.0	-	33.9
2′	1.84 (*dd*, ^{2}J=13.1, *J*(2′*α*,3′)=5.9), 1.36 (*dd*, ^{2}J=13.1, *J*(2′*β*,3′)=6.8)	44.6	1.77 (*dd*, ^{2}J=13.0, *J*(2′*α*,3′)=5.1), 1.40 (*dd*, ^{2}J=13.0, *J*(2′*β*,3′)=7.0)	40.4
3′	4.25 (*br. s*)	65.9	3.78 (*m*)	74.6
4′	5.54 (*br. s*)	124.5	5.60 (*br. s*)	121.8
5′	-	138.0	-	138.2
6′	2.40 (*d*, *J*(6′,7′)=9.9)	55.0	2.42 (*d*, *J*(6′,7′)=9.8)	55.1
7′	5.43 (*dd*, *J*(7′,8′)=15.4, *J*(6′,7′)=9.9)	128.7	5.44 (*dd*, *J*(7′,8′)=15.4, *J*(6′,7′)=9.8)	129.0
8′	6.12 (*m*)	138.5	6.12 (*m*)	137.6
9′	-	135.1	-	135.1
10′	6.14 (*d*, *J*(10′,11′)=10.4)	130.8	6.14 (*d*, *J*(10′,11′)=11.5)	130.7
11′	6.59 (*dd*, *J*(11′,12′)=14.9) *J*(10′,11′)=10.4)	124.8	6.60 (*dd*, *J*(11′,12′)=14.9) *J*(10′,11′)=11.5)	124.8
12′	6.35 (*d*, *J*(11′,12′)=14.9)	137.5	6.35 (*d*, *J*(11′,12′)=14.9)	137.5
13′	-	136.4	-	136.4
14′	6.23 ((*d*, *J*(14′,15′)=12.2)	132.6	6.23 ((*d*, *J*(14′,15′)=12.0)	132.5
15′	6.62 (*m*)	130.0	6.62 (*m*)	130.0
16′	0.85 (*s*)	24.3	0.84 (*s*)	24.2

Table 4.9 (Continued)

Position	PLET23a		PLET_4_5pp	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)	δ (H) (*J*)	δ (C)
17′	1.00 (*s*)	29.5	0.97 (*s*)	29.4
18′	1.62 (*s*)	22.8	1.62 (*s*)	23.0
19′	1.91 (*s*)	13.1	1.91 (*s*)	13.1
20′	1.97 (*s*)	12.8	1.96 (*s*)	12.8
21′	-	-	3.36 (*s*)	55.8

Conditions: at 125 and 500 MHz, resp., in $CDCl_3$ solution (*T*=25°) ; *δ* in ppm, *J* in Hz.

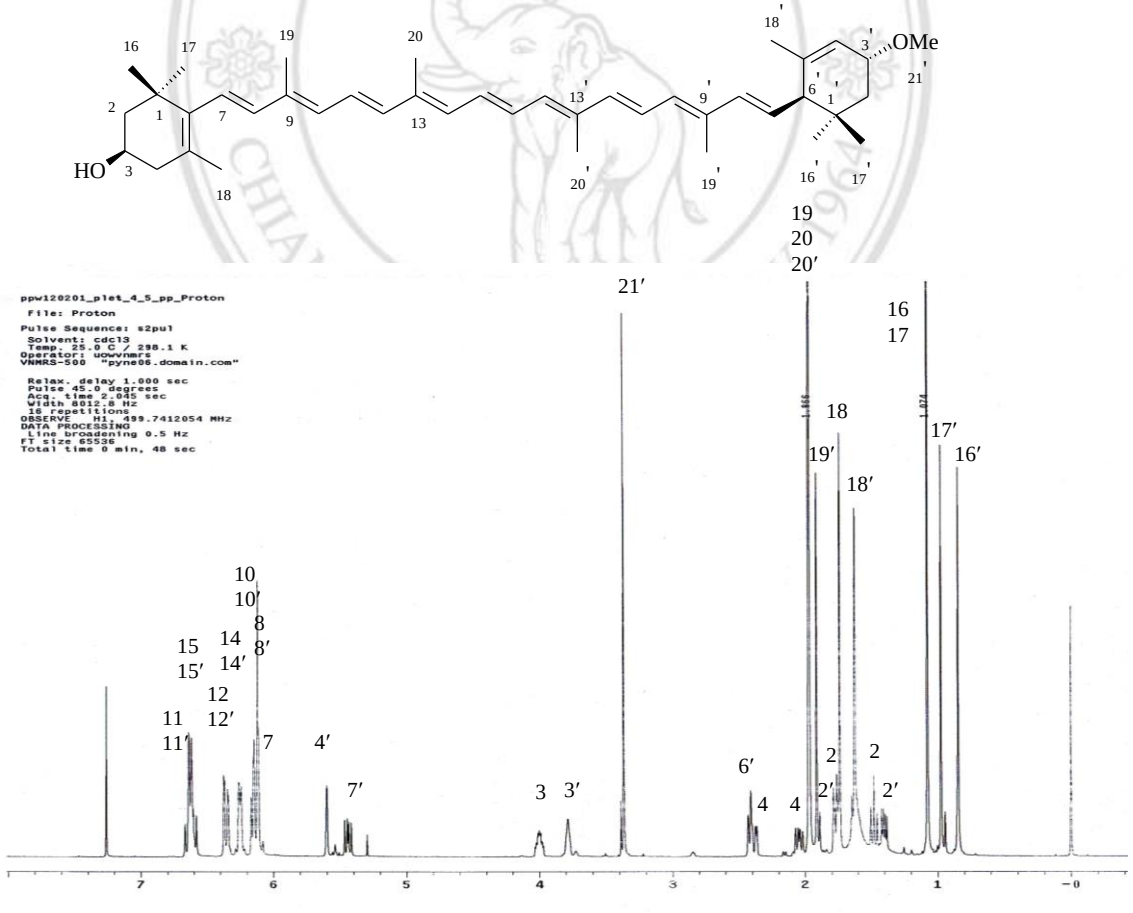


Figure 4.5 ^{1}H NMR spectrum of compound **PLET_4_5pp** (500 MHz, $CDCl_3$)

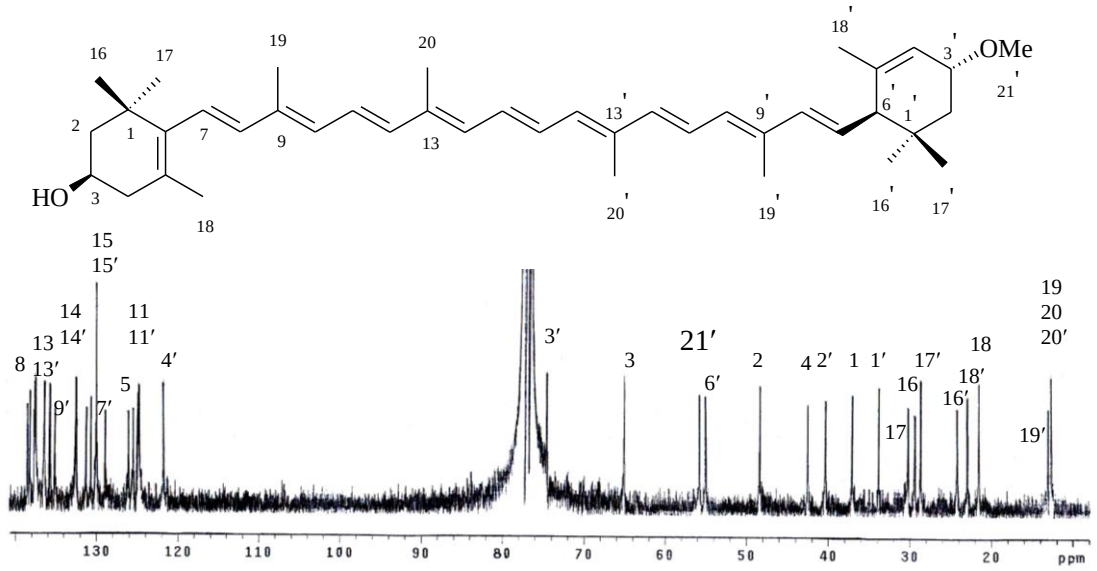


Figure 4.6 ^{13}C NMR spectrum of compound **PLET_4_5pp** (125 MHz, $CDCl_3$)

4.3.1.5 Isolation and structural elucidation of compound PLETp

The ethyl acetate extract of *P. lanceolaria* (PLET) was separated by normal phase flash column chromatography and preparative TLC to yield pure compound **PLETp.** Compound **PLETp** was a brick-red amorphous powder. The mass spectrum showed the molecular-ion peak at *m/z* 272 (M^+) and HR-ASAP-MS *m/z* at 273.0869 $[M+H]^+$ (calculated for $C_{14}H_{13}N_2O_4$ 273.0875), that corresponding to the molecular formula of $C_{14}H_{12}N_2O_4$ (Figure 4.7). ^1H NMR spectrum showed 4 singlet aromatic proton signals at δ 7.21 (1H, H-9), δ 6.92 (1H, H-6), δ 6.50 (1H, H-1), and δ 6.44 (1H, H-4). The methoxy protons appeared as two singlet signals at δ 3.99 (3H, OCH_3-12) and δ 3.97 (3H, OCH_3-13). The broad singlet signal at δ 5.06 (2H, NH_2-11) was assigned to two protons on a nitrogen atom (Figure 4.8). The assignment of the ^1H and ^{13}C NMR signals of compound **PLETp** were also supported by two-dimensional correlated spectroscopy, HSQC and HMBC, experiments (Figure 4.9). The NMR spectroscopic data of compound **PLETp** agreed well with those reported in the literature for peristrophine [Qin *et al.*, 1999]. It was found however, some differences in the ^{13}C NMR chemical shifts, these are shown in Table 4.9. Because they are adjacent to methoxy groups (H-12 and H-13), two signals at δ 151.5 and δ 147.6 were assigned to C-7 and C-8, respectively. These assignments were supported by the HMBC correlation between the C-7 and H-12 (δ 3.99) and the C-8 and H-13 (δ 3.96), respectively. Due to their

aromatic system and two methoxy groups on the adjacent carbon atoms, the signals of H-6 at δ 6.92 and H-9 at δ 7.21 were more down field shifted than those of H-1 (δ 6.50) and H-4 (δ 6.43). The proton resonances at δ 6.50 (H-1), δ 6.43 (H-4), δ 6.92 (H-6), and δ 7.21 (H-9) were assigned to δ 101.1 (C-1), δ 103.7 (C-4), δ 98.7 (C-6), and δ 109.1 (C-9), respectively via HSQC correlations. A resonance for a quaternary aromatic carbon at δ 138.4 in ^{13}C NMR spectrum was assigned to C-5′ which was confirmed by the HMBC experiment, where correlations were seen between the C-5′ and H-6 (δ 6.92) including the C-5′ and H-9 (δ 7.21). In the same way, a resonance at δ 103.7 was suggested to C-4′ which was also supported by the HMBC experiment, where correlations were seen between the C-4′ and H-1 (δ 6.50) together with the C-4′ and H-4 (δ 6.43).

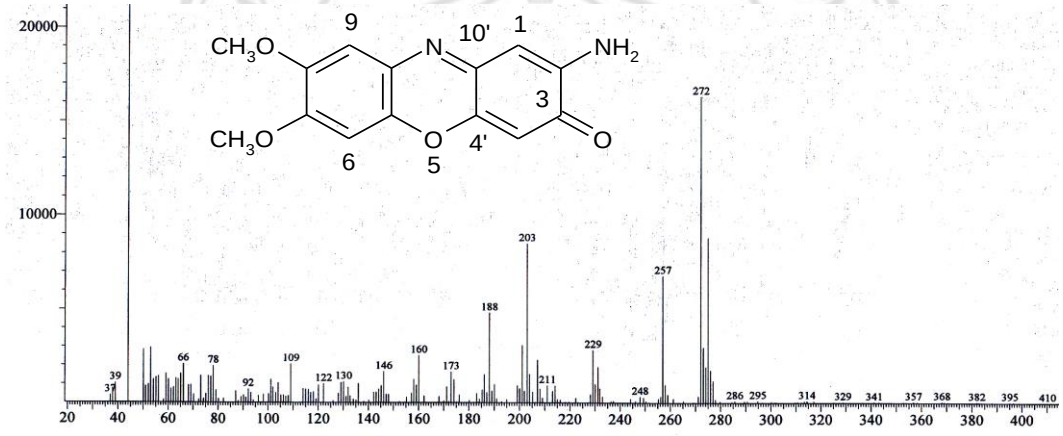


Figure 4.7 Chemical structure and mass spectrum of compound **PLETp** (peristrophine)

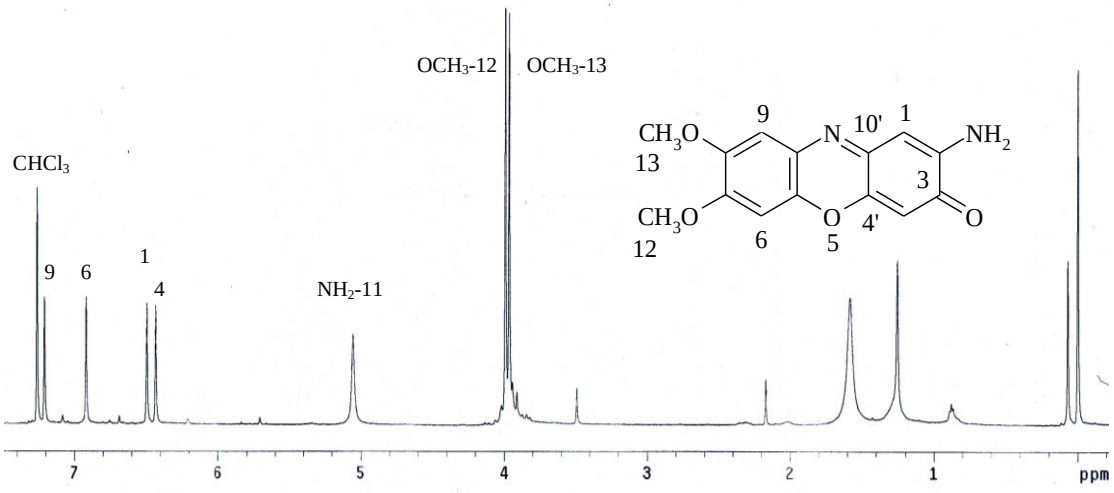


Figure 4.8 ^{1}H NMR spectrum of compound **PLETp** (500 MHz, $CDCl_3$)

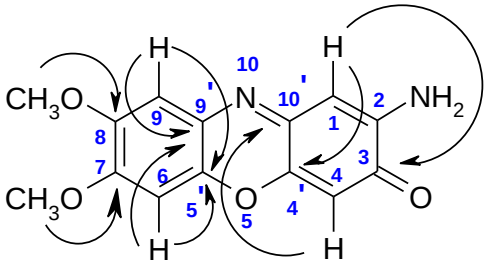


Figure 4.9 HMBC correlations of compound **PLETp** (peristrophine)

Table 4.10 ^{1}H- and ^{13}C-NMR data of compound **PLETp** isolated from the aerial part of *P. lanceolaria* compared with peristrophine

Position	Peristrophine		PLETp	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**
	δ (H) (*J*)	**δ (C)**	**δ (H) (*J*)**	**δ (C)**
1	6.50 (*s*)	108.4	6.50 (*s*)	101.1
2	-	145.3	-	145.7
3	-	179.1	-	179.6
4	6.44 (*s*)	102.4	6.43 (*s*)	103.7
4′	-	137.1	-	146.3
5	-	O	-	O
5′	-	147.0	-	138.4
6	6.93 (*s*)	98.3	6.92 (*s*)	98.7
7	-	146.8	-	151.5
8	-	146.8	-	147.6
9	7.22 (*s*)	98.9	7.21 (*s*)	109.15
9′	-	127.6	-	128.6
10	-	N	-	N
10′	-	148.8	-	149.6
11	5.06 (*br. s*)	NH_2	5.06 (*br. s*)	NH_2
12	4.00 (*s*)	56.0	3.99 (*s*)	56.7
13	3.97 (*s*)	55.7	3.97 (*s*)	56.5

Conditions: at 300 MHz (Peristrophine) and 500 MHz (compound PLETp), respectively, in $CDCl_3$ solution (T=25°); δ in ppm, J in Hz.

4.3.1.6 Isolation and structural elucidation of compound PLBU_2_5_5

Compound **PLBU_2_5_5** was isolated from the *n*-butanol extract by normal phase flash CC over silica gel using gradient elution of ethyl acetate in hexane and MeOH in ethyl acetate. It was isolated as a brick-red amorphous powder. The positive HR-ASAP-MS mass spectrum showed the molecular ion peak at *m/z* 296.1498 $[M+H]^+$, calculated for $C_{15}H_{22}NO_5$ 296.1498, which corresponded to the molecular formula $C_{15}H_{21}NO_5$ (M = 295).

The ^1H and ^{13}C NMR spectra of compound **PLBU_2_5_5** showed characteristic signals for the aromatic protons at δ 6.66 (1H, *s*, H-10) and δ 6.60 (1H, *s*, H-7), which were assigned to δ 105.1 (C-10) and δ 109.6 (C-7), respectively via HSQC correlations. The singlet resonance for an aromatic methoxy proton was also observed at δ 3.80 (3H, *s*, H-11). The chain of acyclic aliphatic proton resonances at δ 3.47 (2H, *m*, H-2′), δ 1.57 (2H, *m*, H-3′), δ 1.35 (2H, *m*, H-4′), δ 0.90 (3H, *t*, *J*=7.5 Hz, H-5′) were assigned to 67.6 (C-2′), 31.5 (C-3′), 19.1 (C-4′) and 13.7 (C-5′), respectively. Two pairs of diastereotopic protons represented signals at δ 2.19 (1H, *dd*, ^2J=13, *J*(3,4)=9 Hz, H-3) and δ 2.33 (1H, *m*, H-3) and at δ 2.49 (1H, *dd*, ^2J=13, *J*(4,3)=9 Hz, H-4) and δ 2.80 (1H, *dd*, ^2J=17.5, *J*(4,3)=9 Hz, H-4), were correlated to ^{13}NMR signals at 25.8 (C-3) and 28.7 (C-4), respectively. The lone methine group, with a resonance at δ 5.13 (1H, *d*, *J*= 5.5 Hz, H-2), was suggested to be attached to two oxygen atoms based on its downfield shift. This methine proton also showed a through space interaction with the protons signals at δ 3.47 (2H, *m*, H-2′) and δ 6.66 (1H, *s*, H-10) via a NOESY experiment. The NOESY interaction of H-11 and H-10, H-10 and H-2, H-2 and H-2′ are shown in Figure 4.12. The carbonyl carbon at 175.9 (C-5), adjacent to C-4 (28.7), was established by the HMBC experiment, as shows in Figure 4.12.

The optical rotation of $[\alpha]_D^{25}$ +29.2 (c 0.00525 g/mL, $CHCl_3$) confirmed the presence of a chiral carbon. However, NMR methods are not suited to establish the absolute configuration at the chiral carbon (C-2) of this compound.

There has been no previously reported on the discovery of this compound. This compound would be named as **1,6-benzoxazocine-5-one**.

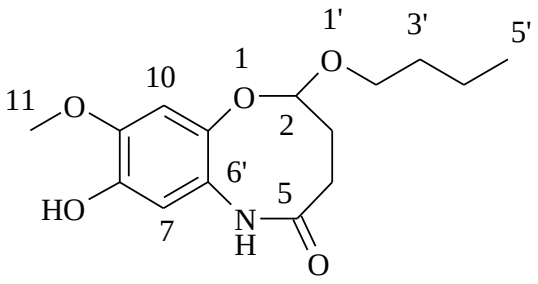


Figure 4.10 Chemical structure of compound **PLBU_2_5_5**

Table 4.11 ^{1}H- and ^{13}C-NMR Data of **PLBU_2_5_5** isolated from the aerial part of *P. lanceolaria*

Position	PLBU_2_5_5	
	^{1}H	**^{13}C**
	δ (H) (*J*)	**δ (C)**
1	-	-
2	5.13 (*d*, *J*= 5.5)	93.0
3	2.33 (*m*) 2.19 (*dd*, ^{2}J=13) *J*(3,4)=9)	25.8
4	2.80 (*dd*, ^{2}J=17.5) *J*(4,3)=9) 2.49 (*dd*, ^{2}J=13) *J*(4,3)=9)	28.7
5	-	175.9
6	-	NH
6′	-	115.9
7	6.60 (*s*)	105.1
8	-	146.5
9	-	140.6
10	6.66 (*s*)	109.6
10′	-	147.0

Table 4.11 (Continued)

Position	PLBU_2_5_5	
	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)
11	3.80 (*s*)	56.5
1′	-	O
2′	3.47 (*m*)	67.6
3′	1.57 (*m*)	31.5
4′	1.35 (*m*)	19.1
5′	0.90 (*t*, *J*=7.5)	13.7

Conditions: at 500 MHz, in $CDCl_3$ solution (*T*=25°) ; *δ* in ppm, *J* in Hz.

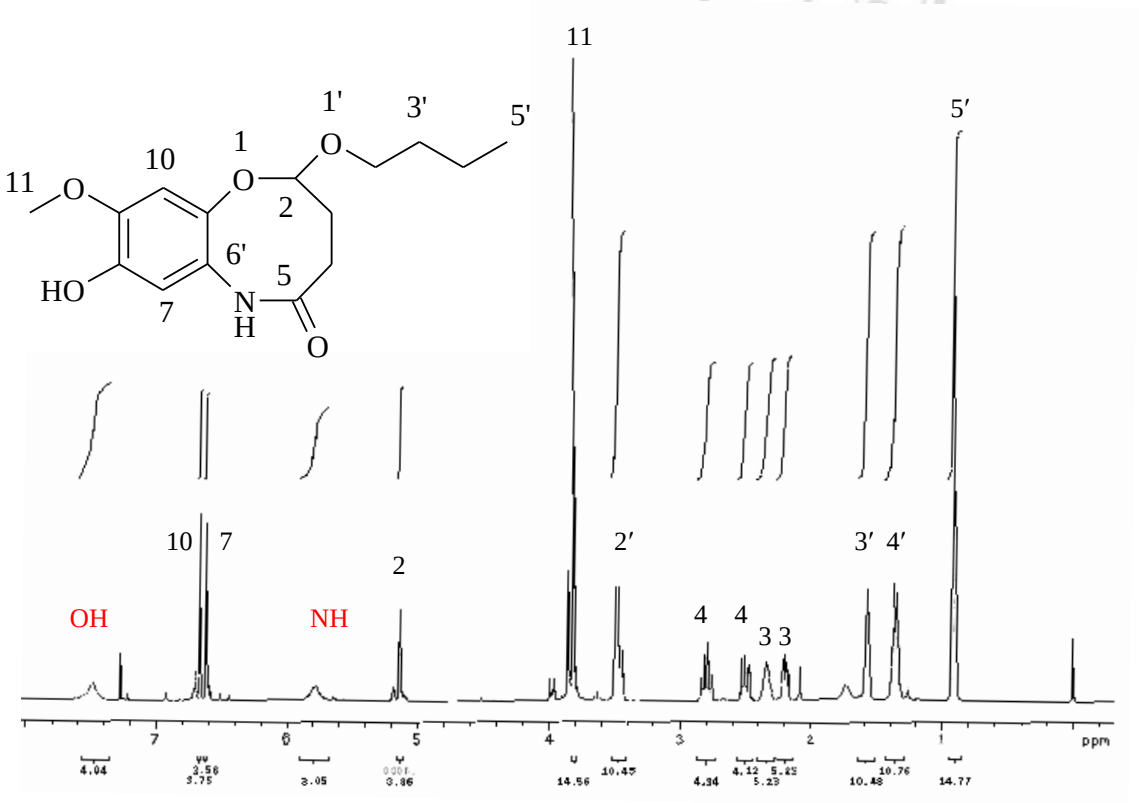
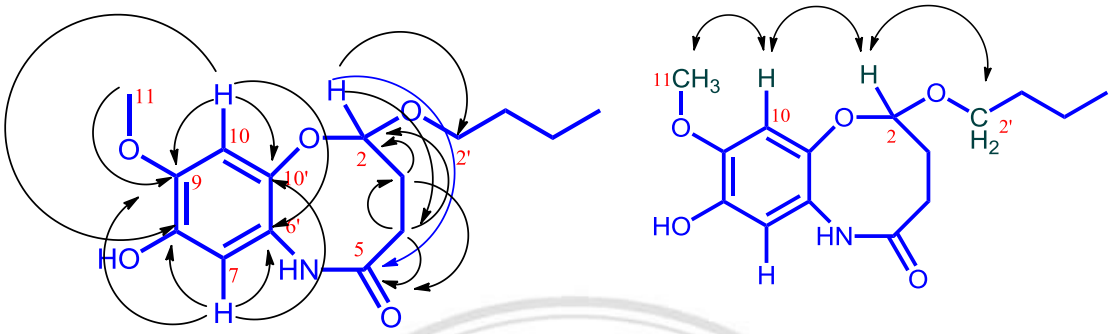


Figure 4.11 ^{1}H NMR spectrum of compound **PLBU_2_5_5** (500 MHz, $CDCl_3$)



HMBC correlation NOESY 1D

Figure 4.12 HMBC and NOESY 1D correlations of compound **PLBU_2_5_5** (1,6-benzoxazocine-5-one)

4.3.1.7 Synthesis, purification and structural elucidation of compound PLBU_2_5_5_m_2

Compound **PLBU_2_5_5** have been converted to its methyl acetal derivative, compound **PLBU_2_5_5_m_2**, by treatment with camphor sulfonic acid (CSA) in MeOH.

Compound **PLBU_2_5_5_m_2** was a brick-red amorphous powder. The positive HR-ASAP-MS mass spectrum showed the molecular ion peak at *m/z* 254.1040 $[M+H]^+$, calculated for $C_{12}H_{16}NO_5$ 254.1028, which corresponding to the molecular formula $C_{12}H_{16}NO_5$ (M = 253). The molecular structure of compound **PLBU_2_5_5_m_2** is shown in Figure 4.13.

The ^1H and ^{13}C NMR spectra of compound **PLBU_2_5_5_m_2** (Figure 4.14) was quite similar to compound **PLBU_2_5_5** (Table 4.11), the only difference being the presence of the methoxy group, instead of the butoxy group, which observed as a sharp singlet at δ 3.40 (3H, *s*, H-2′).

It is likely that compound **PLBU_2_5_5** is an artifact and the natural product is the hemi-acetal, where the O-butyl is an OH group. This compound can react with the

n-butanol used in the extraction process to give compound **PLBU_2_5_5**, as was demonstrated in the synthesis of compound **PLBU_2_5_5_m_2** from compound **PLBU_2_5_5**. The ^{1}NMR spectrum of **PLBU_2_5_5_m_2** indicated a small amount of an impurity with aromatic ^{1}H NMR signals at δ 6.70 and δ 6.61, a doublet signal at δ 5.17 and an aromatic methoxy signal at δ 3.86 (Figure 4.14). The identity of this compound could not be determined.

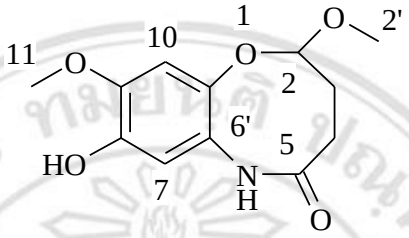
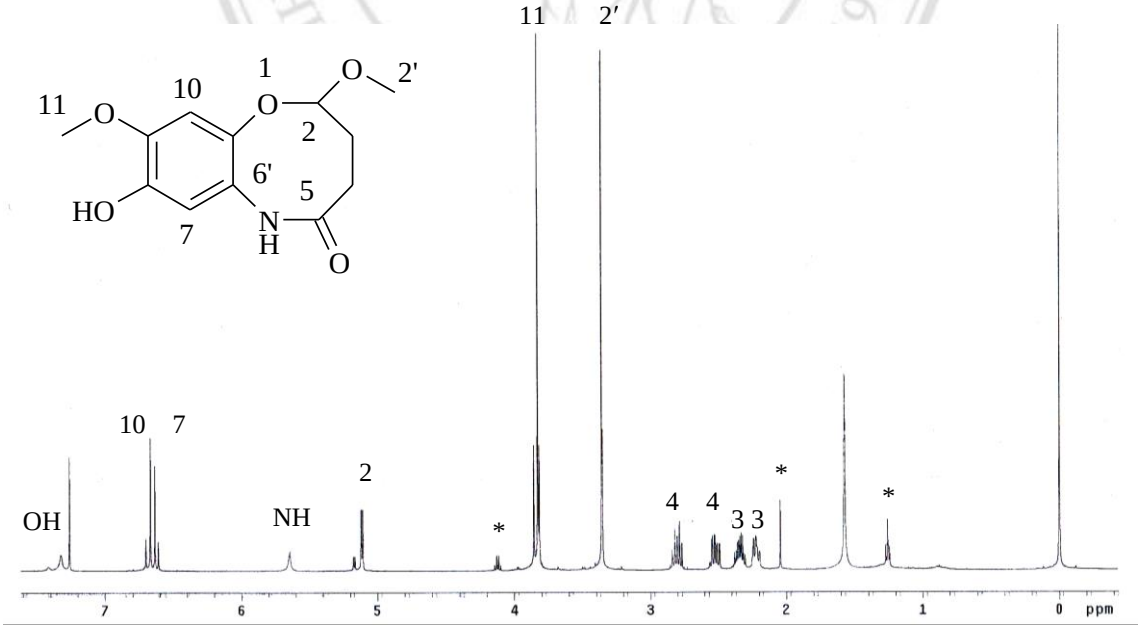


Figure 4.13 Chemical structure of compound **PLBU_2_5_5_m_2**



*EtOAc

Figure 4.14 ^{1}H NMR spectrum of compound **PLBU_2_5_5_m_2** (500 MHz, $CDCl_3$)

Table 4.11. ^{1}H- and ^{13}C-NMR Data of **PLBU_2_5_5** (isolated from the aerial part of *P. lanceolaria*) and **PLBU_2_5_5_m_2** (synthesized from **PLBU_2_5_5**)

Position	PLBU_2_5_5		PLBU_2_5_5_m_2	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**
	δ (H) (*J*)	**δ (C)**	**δ (C)**	**δ (C)**
1	-	O	-	O
2	5.13 (*d*, *J*= 5.5)	93.0	5.12 (*d*, *J*= 5.9)	94.1
3	2.33 (*m*) 2.19 (*dd*, ^{2}J=13) *J*(3,4)=9)	25.8	2.33 (*m*) 2.22 (*dd*, ^{2}J=12.2) *J*(3,4)=9.3)	25.3
4	2.80 (*dd*, ^{2}J=17.5) *J*(4,3)=9) 2.49 (*dd*, ^{2}J=13) *J*(4,3)=9)	28.7	2.80 (*dd*, ^{2}J=17.6) *J*(4,3)=9.3) 2.52 (*dd*, ^{2}J=17.6) *J*(4,3)=9.3)	28.8
5	-	175.9	-	175.9
6	-	NH	-	NH
6′	-	115.9	-	115.9
7	6.60 (*s*)	105.1	6.64 (*s*)	105.4
8	-	146.5	-	146.5
9	-	140.6	-	140.6
10	6.66 (*s*)	109.6	6.67 (*s*)	108.9
10′	-	147.0	-	146.8
11	3.80 (*s*)	56.5	3.83 (*s*)	56.5
1′	-	O	-	O
2′	3.47 (*m*)	67.6	3.40 (*s*)	54.6
3′	1.57 (*m*)	31.5	-	-
4′	1.35 (*m*)	19.1	-	-

Table 4.11 (Continued)

Position	PLBU_2_5_5		PLBU_2_5_5_m_2	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)	δ (H) (*J*)	δ (C)
5′	0.90 (*t*, *J*=7.5)	13.7	-	-

Conditions: at 500 MHz, in $CDCl_3$ solution (*T*=25°) ; *δ* in ppm, *J* in Hz.

4.3.2 Secondary metabolites isolation from the aerial part of *B. strigosa*

4.3.2.1 Preparation of crude extract

The dried aerial part of *B. strigosa* was extracted with methanol at room temperature give the methanol crude extract as a dark green residue. The percentage yield was 9.21% (262.9 g). It was further extracted with different polarity solvents using solvent-solvent extraction procedures.

4.3.2.2 Extraction

The methanol crude extract (262.9 g) was suspended in water and partitioned with hexane to yield crude hexane extract. The aqueous layer was then extracted with ethyl acetate and *n*-butanol to yield crude ethyl acetate extract, crude *n*-butanol extract, and crude aqueous extract, respectively. The percentage yields of all extracts were calculated which were shown in Table 4.12. The aqueous extract provided the largest percentage yield at 50.3%. The hexane and butanol extracts were obtained with lower yields (30.2 and 7.5%, respectively). Whereas the ethyl acetate extract gave the lowest yield at 1.2%.

Table 4.12 The percentage yields of *B. strigosa* extracts by solvent-solvent extraction

extracts	Weight (g)	%yield[a]	Description properties
Hexane extract	79.3	30.2	dark green sticky syrup
Ethyl acetate extract	3.3	1.2	dark green solid
n-Butanol extract	19.8	7.5	dark green solid
Aqueous extract	132.3	50.3	dark brown solid

[a] The percentage yield was calculated on the crude methanol extract weight basis

All extracts were analysed by TLC and ^{1}H NMR. The ^{1}H NMR spectrum of the ethyl acetate extract (BSET) showed some interesting resonances, which indicated that it may contain a mixture of aromatic compounds. This extract was selected for isolation and purification.

4.3.2.3 Isolation and structural elucidation of compound BSET_6_1_19a

The ethyl acetate extract of *B. strigosa* was separated by normal phase CC using gradient elution (ethyl acetate and petroleum ether) to give fraction BSET_6, which was then subjected to NP-CC using isocratic elution of 5% MeOH in ethyl acetate. Fraction BSET_6_1 was separated on RP-C18 CC using gradient elution of MeOH and water to yield compound **BSET_6_1_19a.**

Compound **BSET_6_1_19a** was a pale yellow amorphous powder which was optically active. The specific rotation was $[\alpha]_D^{25}$ -94.4° (c 0.007 g/mL, CH_3OH). The IR spectrum showed bands at ν_{max} 3355, 1689, 1602, 1520, 1447, 1369, 1267, 1158, and 1023 cm^{-1}. The negative EI-MS spectrum exhibited a molecular ion peak at *m/z* 623[$(M-H)^-$ and the positive ion EI-MS showed an ion at *m/z* 647 $[M+Na]^+$. This information together with NMR data allowed its molecular formula to be assigned as $C_{29}H_{36}O_{15}$ (Figure 4.15).

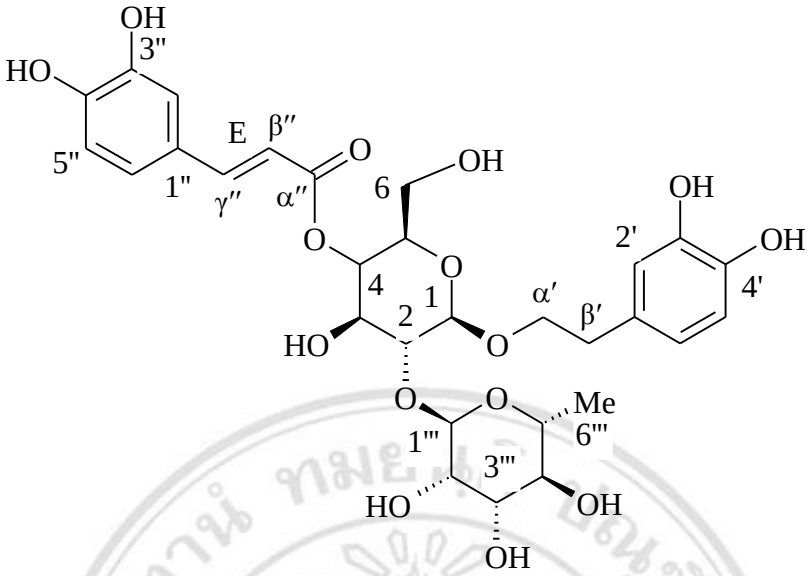


Figure 4.15 Chemical structure of compound **BSET_6_1_19a**

The ^{1}H NMR spectrum of **BSET_6_1_19a** exhibited the characteristic signals of a phenolic glycoside contained caffeic acid and phenylethanol moieties. Two sets of ABX-type aromatic proton signals appeared at δ 6.52 (1H, *d*, *J*(6′,5′) = 7.8 Hz, H-6′), δ 6.63 (1H, *d*, *J*(5′,6′) = 7.8 Hz, H-5′) and δ 6.65 (1H, *s*, H-2′) and at δ 6.91 (2H, *d*, *J* *J*(6″,5″) = 7.8 Hz, H-6″), δ 6.73 (1H, *d*, *J*(5″,6″) = 8.3 Hz, H-5″) and δ 7.01 (1H, *s*, H-2″). Two doublet signals at δ 6.23 (1H, *d*, *J*(β″,γ″) = 15.6 Hz, H-β″) and δ 7.55 (1H, *d*, *J*(γ″,β″) = 15.6 Hz, H-γ″) were assigned as a pair of *trans*-olefinic protons in the caffeoyl part. A multiplet benzylic methylene proton signal at δ 2.79 (2H, m, H-β′) and two diastereotopic proton signals at δ 4.00 (1H, *dd*, ^{2}J = 16.6, *J*(α′,β′) = 7.8 Hz, H- α′) and δ 3.80 (1H, *dd*, ^{2}J = 16.6, *J*(α′,β′) = 7.8 Hz, H-α′) were assigned as a side chain of the aglycone part.

Two sugar anomeric proton resonances were observed as a singlet and a doublet signals at δ 5.14 (1H, *s*, H-1‴) and δ 4.33 (1H, *d*, *J* = 7.8 Hz, H-1) which were assigned to δ 103.0 (C-1‴) and δ 104.2 (C-1) in the ^{13}C NMR spectrum, respectively. The ^{1}H NMR spectrum also indicated a secondary methyl group at δ 1.09 (3H, *d*, *J*=6.0 Hz, H-6‴) which indicated the presence of a rhamnose sugar unit in the compound. In addition, all connectivity within compound **BSET_6_1_19a** were supported by the HMBC

experiment, where correlations were seen between H-1 (δ 4.33) of the glucose unit and the C-α′ (δ 72.2) of the aglycone moiety, H-2′ (δ 3.77) of the glucose unit and the C-1‴ (δ 103.0) of the rhamnose unit, and H-4 (δ 4.87) of the glucose unit and the C-α (δ 168.3) of the caffeoyl moiety (Figure 4.16). Moreover, the correlations between H-2′ (δ 3.77) of the glucose unit and H-1‴ (δ 5.14) of the rhamnose unit in NOSEY experiment confirmed the attachment of the sugar units (Figure 4.16).

Comparisons of all this data with the literature (Table 4.13) suggest that the structure of compound **BSET_6_1_19a** as parvifloroside A (2-(3,4-dihydroxyphenyl)-ethyl-*O*-*α*-L-rhamnopyranosyl-(1→2)-4-*O*-*E*-caffeoyl-*β*-D-glucopyranoside.

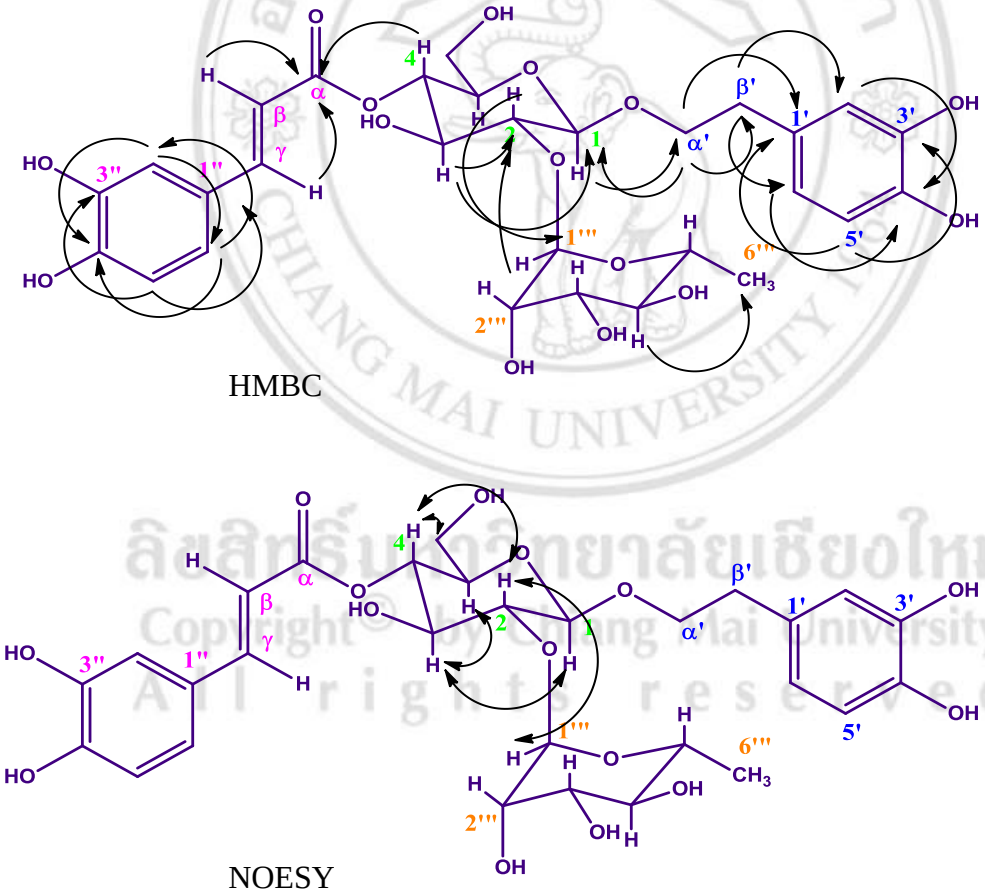


Figure 4.16 Selected HMBC and NOESY correlations of compound **BSET_6_1_19a**

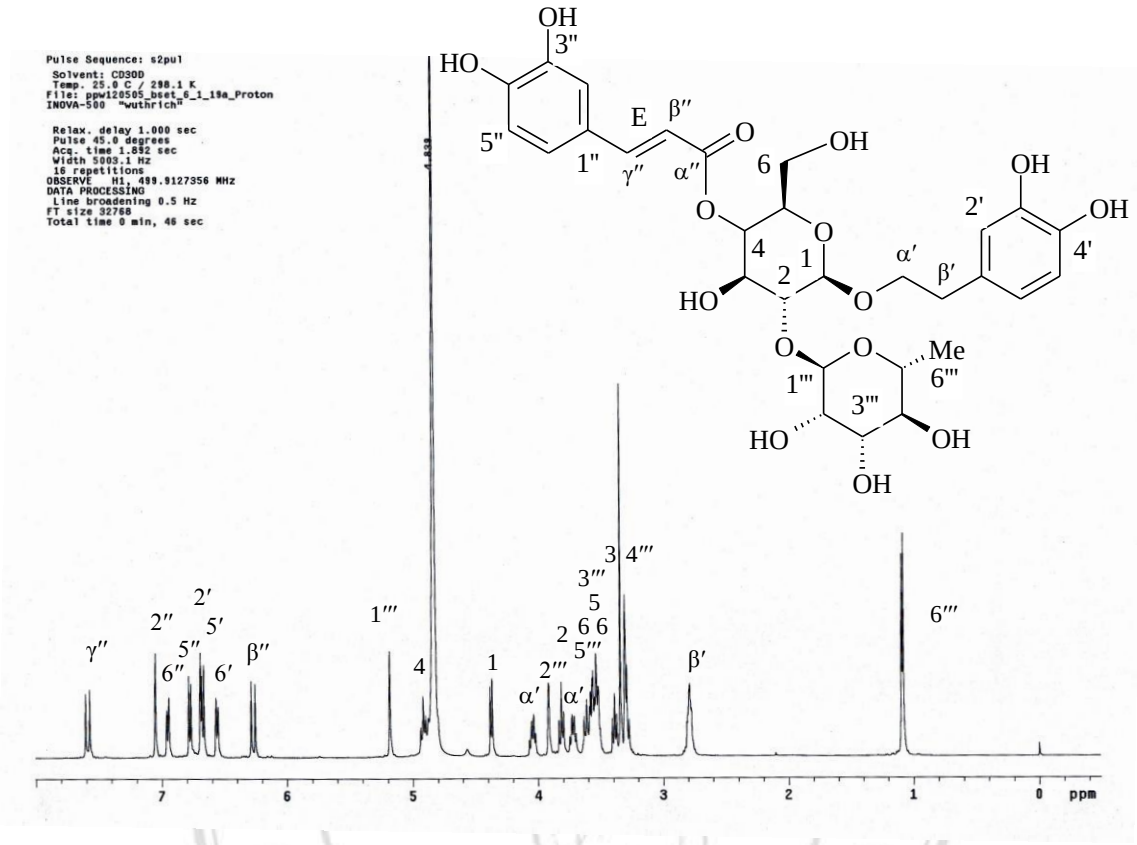


Figure 4.17 ^{1}H NMR spectrum of compound **BSET_6_1_19a** (500 MHz, CD_3OD-d_4)

Table 4.13. ^{1}H- and ^{13}C-NMR data of parvifloroside A and **BSET_6_1_19a** (isolated from the aerial part of *B. strigosa*)

Position	Parvifloroside A[a]		BSET_6_1_19a[b]	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)	δ (H) (*J*)	δ (C)
Aglycone				
1′		131.5		131.5
2′	6.68 (*d*, *J*=1.7)	116.3	6.65 (*s*)	117.1
3′		144.7		146.1
4′		146.1		144.7

Table 4.13 (Continued)

Position	Parvifloroside A[a]		BSET_6_1_19a[b]	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)	δ (H) (*J*)	δ (C)
5′	6.65 (*d*, *J*=8.0)	117.1	6.63 (*d*, *J*=7.8)	116.3
6′	6.56 (*dd*, ^{2}*J*=7.9, 1.7)	121.3	6.52 (*d*, *J*=7.8)	121.2
α′	3.82 (*dt*, *J*=11.4, 7.9)	72.3	4.00 (*dd*, ^{2}*J*=16.6, 7.8) 3.82 (*dd*, ^{2}*J*=16.6, 7.8)	72.2
β′	2.80 (*t*, *J*=7.9)	36.6	2.79 (*m*)	36.6
Caffeic acid				
1″		127.7		127.7
2″	7.04 (*d*, *J*=1.7)	115.2	7.01 (*s*)	115.2
3″		146.8		146.8
4″		149.8		149.8
5″	6.78 (*d*, *J*=8.1)	116.5	6.73 (*d*, *J*=8.3)	116.1
6″	6.93 (*dd*, ^{2}*J*=8.2, 1.8)	123.2	6.91 (*d*, *J*=7.8)	123.2
γ″	7.60 (*d*, *J*=15.8)	148.0	7.59 (*d*, *J*=15.6)	148.0
β″	6.28 (*d*, *J*=15.8)	114.7	6.27 (*d*, *J*=15.6)	114.7
α″		168.3		168.3
Glucose				
1	4.37 (*d*, *J*=7.8)	104.2	4.33 (*d*, *J*=7.8)	104.2
2	3.38 (*t*, *J*=8.4)	81.6	3.77 (*t*, *J*=9.3)	81.6
3	3.60 (*m*)	76.2	3.34 (*t*, *J*=8.3)	76.0
4	4.90 (*t*, *J*=9.3)	72.0	4.87 (*t*, *J*=9.3)	70.6

Table 4.13 (Continued)

Position	Parvifloroside A[a]		BSET_6_1_19a[b]	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)	δ (H) (*J*)	δ (C)
5	3.89 (*m*)	76.1	3.49 (*m*)	76.0
6	3.75 (*m*)	62.4	3.58 (*m*)	62.4
	3.90 (*m*)		3.47 (*m*)	
Rhamnose				
1″′	5.10 (*s*)	103.3	5.14 (*s*)	103.0
2″′	3.49 (*m*)	70.4	3.87 (*br. s*)	72.3
3″′	3.58 (*m*)	72.4	3.54 (*m*)	72.0
4″′	3.44 (*m*)	73.8	3.26 (*m*)	73.8
5″′	4.03 (*m*)	70.6	3.58 (*m*)	70.4
6″′	1.09 (*d*, *J*=6.6)	18.4	1.09 (*d*, *J*=6.0)	18.4

[a] Assignment based on HMQC experiments, conditions: at 500 and 125 MHz, resp., in CD_3OD-d_4 solution; δ in ppm, *J* in Hz. [Ahmad *et al*., 2006]

[b] Assignment based on COSY, HSQC, HMBC, AND NOESY experiments, conditions: at 500 and 125 MHz, resp., in CD_3OD-d_4 solution; δ in ppm, *J* in Hz.

4.3.2.4 Isolation and structural elucidation of compound BSET_6_2_5_2

Fraction BSET_6_2 was separated by RP-C18 CC using a MeOH-water gradient for elution to afford fraction BSET_6_2_3 which was later identified as compound **BSET_6_1_19a**. Fraction BSET_6_2_5 was also separated by RP-C18 CC using isocratic by elution with MeOH in water (1:5) to yield pure compound **BSET_6_2_5_2**.

Compound **BSET_6_2_5_2** was isolated as a pale-yellow amorphous powder. The specific rotation was $[\alpha]_D^{25}$ -50.5 (c 0.01075, MeOH); The IR spectrum showed bands at ν_{max} 3354, 1684, 1603, 1520, 1450, 1370, 1264, 1162, 1115, and 1033 cm^{-1}. The chemical structure of compound **BSET_6_2_5_2** is shown in Figure 4.18.

The ^{1}H NMR spectrum of compound **BSET_6_2_5_2** showed similar features to that of compound **BSET_6_1_19a** except for the downfield shift of the H-6 protons at δ 4.50 (1H, *dd*, ^2J = 11.8, J(6,5) = 1.8 Hz, H-6), 4.36 (1H, *dd*, ^2J = 12.0, J(6,5) = 5.8 Hz, H-6). These signals showed HSQC correlations to C-6 at δ 64.6 (Table 4.14). The data from the HMBC experiment exhibited the correlation of the H-6 protons at δ 4.50 and δ 4.36 with C-α″ at δ 169.1, which suggested the attachment of caffeoyl moiety at C-6 of glucose unit rather than C-4. The HMBC and NOESY correlations are shown in Figure 4.19. Furthermore, the NOESY correlations also supported that compound **BSET_6_2_5_2** was parvifloroside B (2-(3,4-dihydroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl-(1→2)-6-*O*-*E*-caffeoyl-β-D-glucopyranoside.

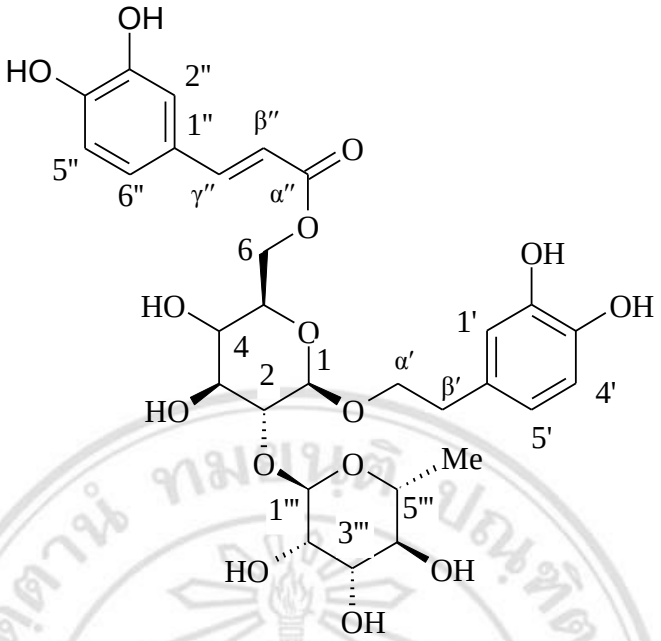


Figure 4.18 Chemical structure of compound **BSET_6_2_5_2**

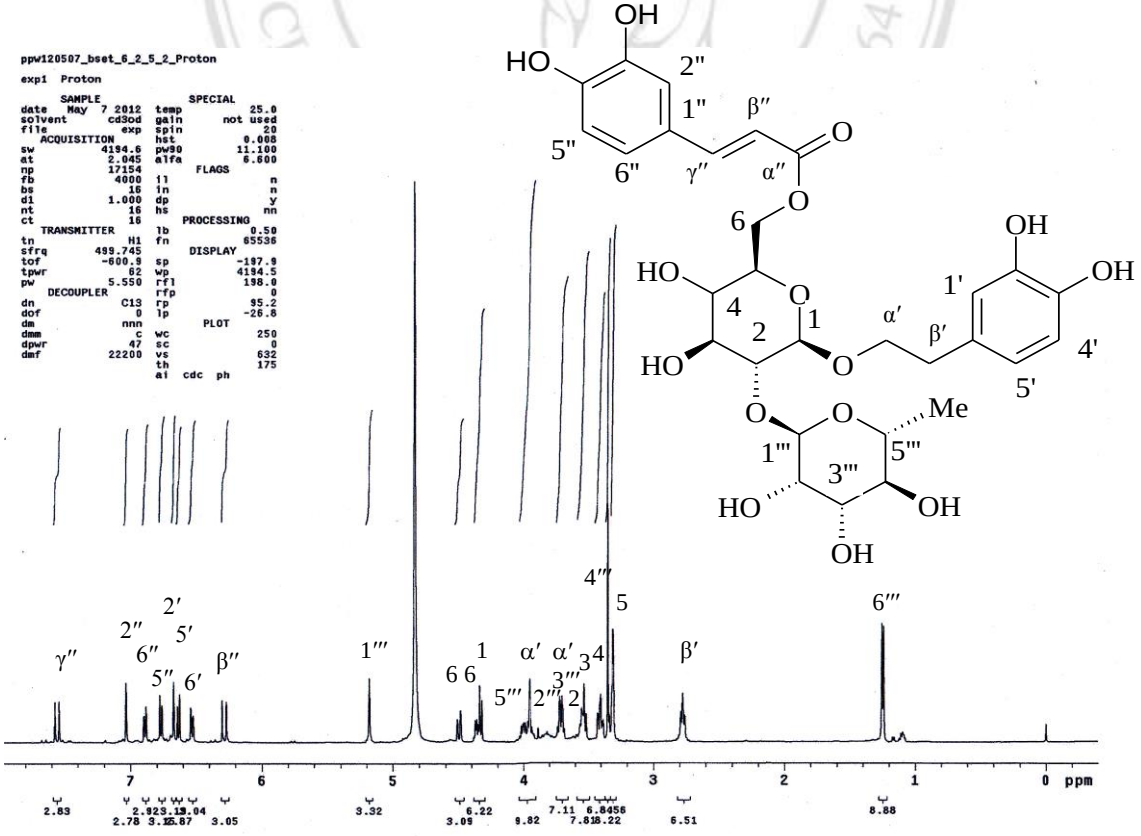
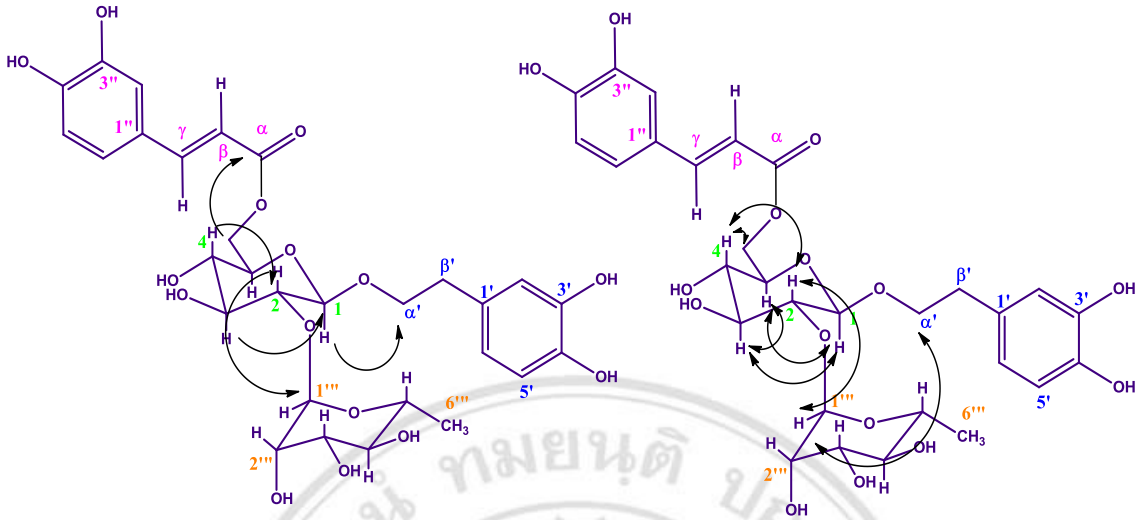


Figure 4.19 ^{1}H NMR spectrum of compound **BSET_6_2_5_2** (500 MHz, CD_3OD-d_4)



HMBC NOESY

Figure 4.20 Selected HMBC and NOESY correlations of compound **BSET_6_2_5_2** (parvifloroside B)

Table 4.14 ^{1}H- and ^{13}C-NMR data of **BSET_6_1_19a** and **BSET_6_2_5_2** isolated from the aerial part of *B. strigosa*

Position	**BSET_6_1_19a**		**BSET_6_2_5_2**	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**
	δ (H) (*J*)	**δ (C)**	**δ (H) (*J*)**	**δ (C)**
Aglycone				
1′		131.5		131.4
2′	6.65 (*s*)	117.1	6.67 (*d*, *J*=1.8)	117.1
3′		146.1		146.1
4′		144.7		144.7
5′	6.63 (*d*, *J*=7.8)	116.3	6.64 (*d*, *J*=8.0)	116.4
6′	6.52 (*d*, *J*=7.8)	121.2	6.53 (*dd*, ^{2}*J*=8.0, 1.8)	121.3

Table 4.13 (Continued)

Position	BSET_6_1_19a		BSET_6_2_5_2	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	**δ (C)**	**δ (H) (*J*)**	**δ (C)**
α′	4.00 (*dd*, ^{2}J=16.6, 7.8) 3.82 (*dd*, ^{2}J=16.6, 7.8)	72.2	3.72 (*m*) 3.95 (*m*)	72.4
β′	2.79 (*m*)	36.6	2.78 (*t*, *J*=7.1)	36.7
Caffeic acid				
1″		127.7		127.7
2″	7.01 (*s*)	115.2	7.04 (*d*, *J*=1.9)	115.1
3″		146.8		146.8
4″		149.8		149.6
5″	6.73 (*d*, *J*=8.3)	116.1	6.77 (*d*, *J*=8.1)	116.5
6″	6.91 (*d*, *J*=7.8)	123.2	6.89 (*dd*, ^{2}J=8.1, 1.9)	123.1
γ″	7.59 (*d*, *J*=15.6)	148.0	7.56 (*d*, *J*=16.0)	147.2
β″	6.27 (*d*, *J*=15.6)	114.7	6.29 (*d*, *J*=16.0)	114.8
α″		168.3		169.1
Glucose				
1	4.33 (*d*, *J*=7.8)	104.2	4.33 (*d*, *J*=7.8)	104.4
2	3.77 (*t*, *J*=9.3)	81.6	3.52 (*t*, *J*=8.9)	83.9
3	3.34 (*t*, *J*=8.3)	76.0	3.55 (*m*)	75.4
4	4.87 (*t*, *J*=9.3)	70.6	3.42 (*t*, *J*=9.2)	70.4
5	3.49 (*m*)	76.0	3.31 (*m*)	75.7

Table 4.13 (Continued)

Position	BSET_6_1_19a		BSET_6_2_5_2	
	^{1}H	^{13}C	^{1}H	^{13}C
	δ (H) (*J*)	δ (C)	δ (H) (*J*)	δ (C)
α′	4.00 (*dd*, ^{2}J=16.6, 7.8) 3.82 (*dd*, ^{2}J=16.6, 7.8)	72.2	3.72 (*m*) 3.95 (*m*)	72.4
6	3.58 (*m*) 3.47 (*m*)	62.4	4.50 (*dd*, ^{2}J=11.8, 1.8) 4.36 (*dd*, ^{2}J=12.0, 5.8)	64.6
Rhamnose				
1‴	5.14 (*s*)	103.0	5.18 (*s*)	102.7
2‴	3.87 (*br. s*)	72.3	3.96 (*m*)	72.2
3‴	3.54 (*m*)	72.0	3.70 (*m*)	72.3
4‴	3.26 (*m*)	73.8	3.39 (*t*, *J*=9.2)	74.0
5‴	3.58 (*m*)	70.4	4.00 (*m*)	70.4
6‴	1.09 (*d*, *J*=6.0)	18.4	1.25 (*d*, *J*=6.2)	17.9

CHAPTER 5

Conclusions

Four Thai Acanthaceous plants, *B. cristata* Linn., *B. strigosa* Willd., *H. colorata* Blume, and *P. lanceolaria* (Roxb.) Nees, were studied in this research. Previously, there are very few literature reports on the chemical constituents and biological activities of these selected plants. The purpose of this study was to search for biological activities of these selected plants and with the expectation to isolate their secondary metabolites which would lead to novel compounds and new drug discovery.

The methanol crude extracts of these plants were screened for antimicrobial, antioxidant, and anticancer activities. Each extract possessed high antioxidant activity against the DPPH radical. The methanol extract of *P. lanceolaria* exhibited antibacterial activity against *B. subtilis*, while the *B. cristata* extract showed antibacterial activity against *B. subtilis* and *B. cereus*. However, *H. colorata* and *B. strigosa* extracts were in active to all the test organisms. Unfortunately, none of the extracts exhibited cytotoxic activity against KB oral cavity cancer, MCF-7 breast cancer, and NCI-H187 small cell lung cancer cell lines.

The phytochemical screening showed that the extracts of *B. cristata, B. strigosa, H. colorata* consisted of tannins, soponins and steroids, whereas the extract of *P. lanceolaria* contained saponins, steroids and flavonoids.

The methanol extract of *P. lanceolaria* was partitioned with hexane, ethyl acetate, and *n*-butanol to afford the hexane, ethyl acetate butanol and aqueous extracts. The ethyl acetate and butanol extracts exhibited the greatest antioxidant activity. Therefore these two extracts were selected for purification by column chromatography.

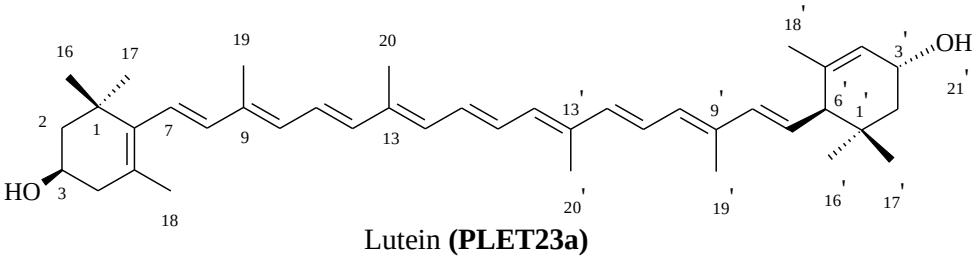
Two isolated carotenoids, lutein and lutein 3′ methyl ether, and an isolated alkaloid, peristrophine, were obtained from the ethyl acetate extract of *P. lanceolaria*.

This is the first report on the isolation of these compounds from the aerial part of *P. lanceolaria*. Lutein is one of the well known caroteoniods found in the higher plant pigment. It is known as a high potential antioxidant found with a high concentration in fruits and vegetables. In the retina of human eye is also found lutein which plays important role in the prevention of ultraviolet damage. However, human is not able to synthesize carotenoids, that's why we need to take it from dietary.

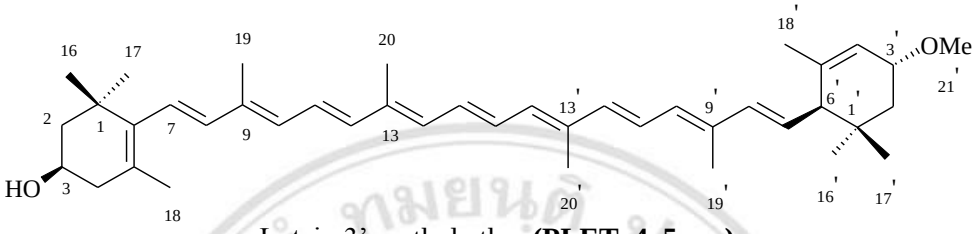
A novel compound**,** 1,6-benzoxazocine-5-one, was obtained from the butanol extract of *P. lanceolaria*. There has been no previously reported on the discovery of this compound and it is likely that this compound is an artifact. The hemi acetal, where O-butyl is OH group, is suggested to be the natural product. This compound can react with the n-butanol used in the extraction process as was demonstrated in the synthesis section.

The methanol crude extract of *B. strigosa* was also subjected to isolation and purification, where parvifloroside A and parvifloroside B were obtained. Because of they are diastereoisomers, they were not able to separate using normal phase column chromatography on silica gel. However, the purified in success for C18 reverse phase column chromatography.

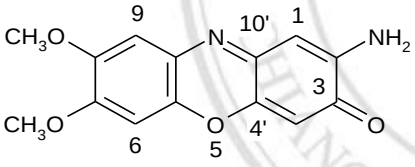
The molecular structure of all the isolated compounds obtained from this selected Acanthaceous plants are shown in Figure 5.1. All of them have never been previously reported from the isolation of *P. lanceolaria* and *B. strigosa*.



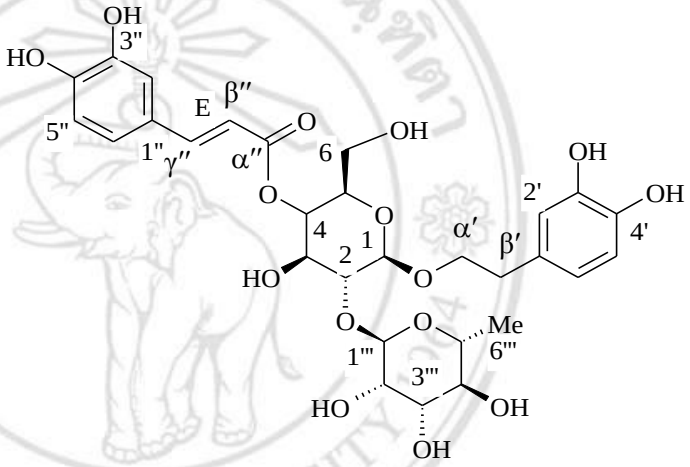
Lutein **(PLET23a)**



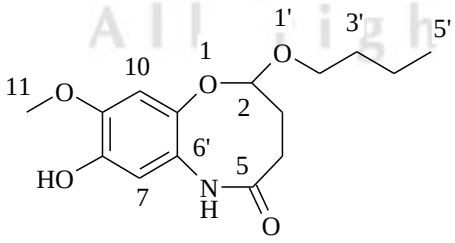
Lutein 3’-methyl ether **(PLET_4_5_pp)**



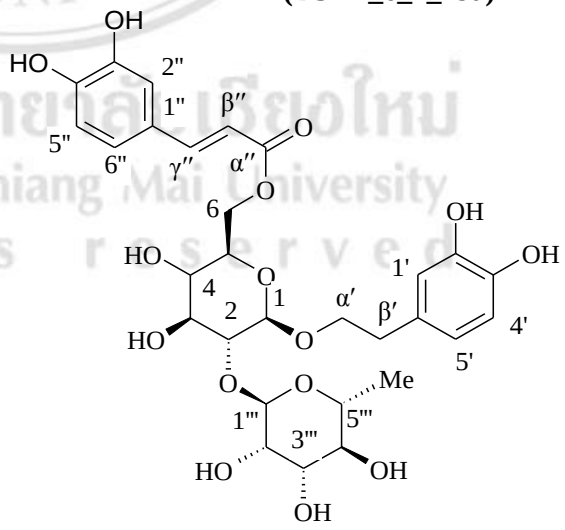
Peristrophine
(PLETp)



Parvifloroside A
(BSET_6_1_19a)



1,6-Benzoxazocine-5-one
butyl acetal derivative
(PLBU_2_5_5)



Parvifloroside B
(BSET_6_2_5_2)

Figure 5.1 The isolated compounds from the selected Acanthaceous plants

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APPENDIX

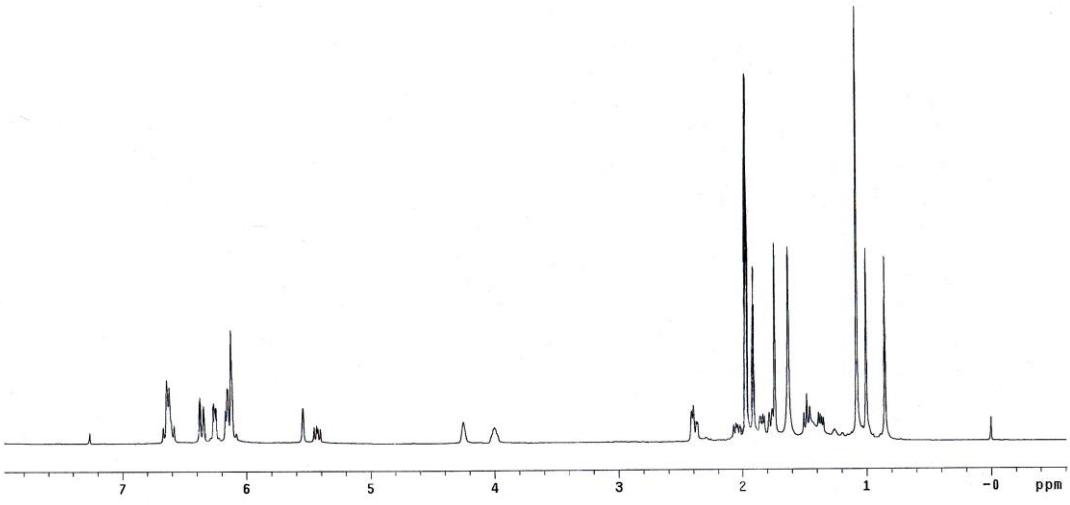

Figure A.1 ^{1}H NMR spectrum of compound **PLET23a** (500 MHz, $CDCl_3$)

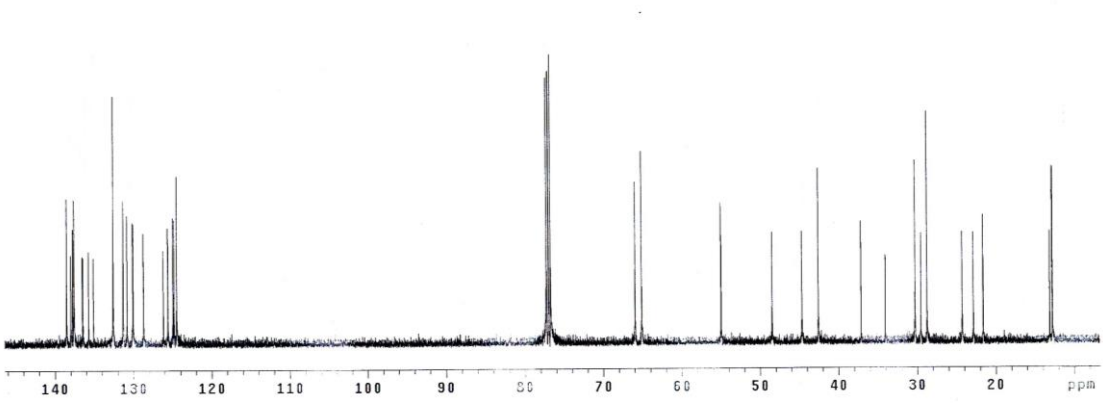

Figure A.2 ^{13}C NMR spectrum of compound **PLET23a** (125 MHz, $CDCl_3$)

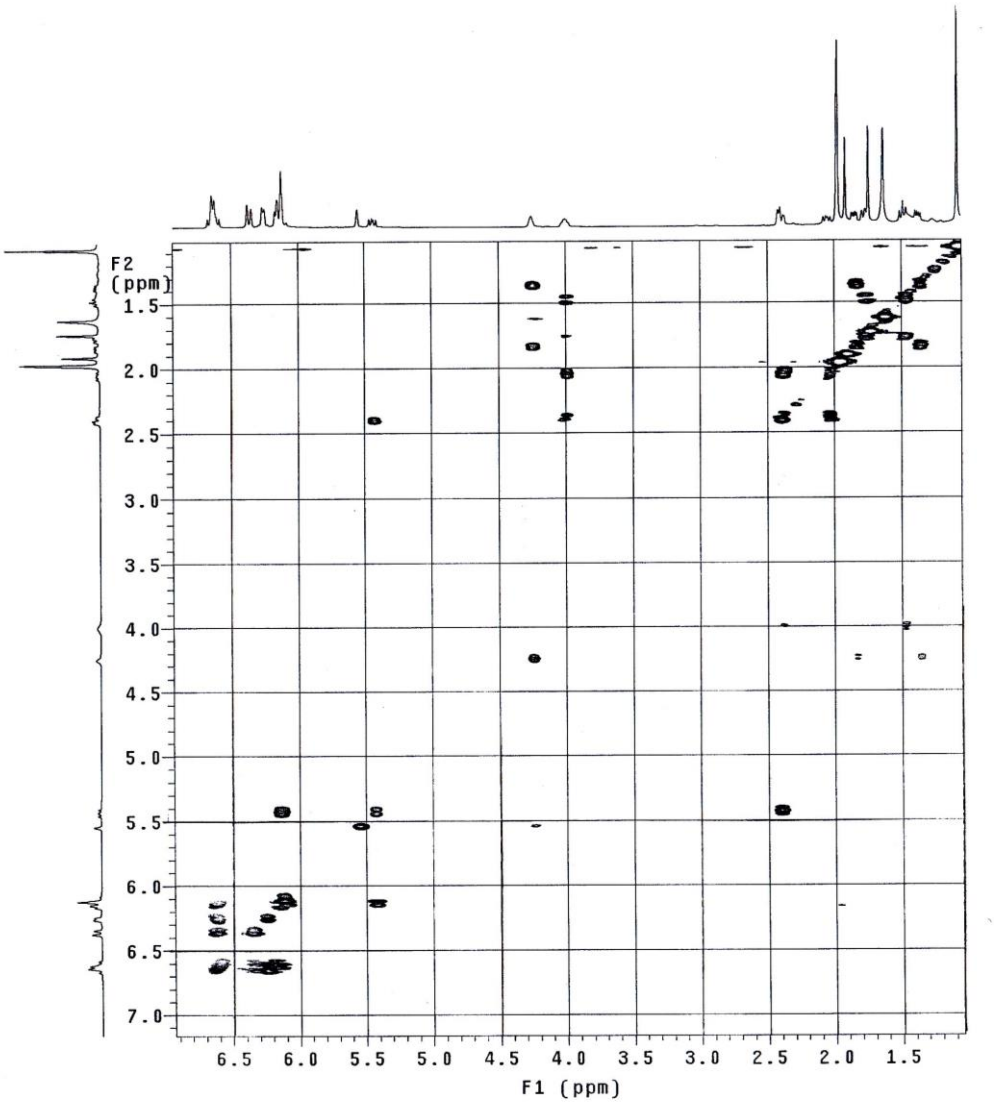

Figure A.3 COSY spectrum of compound **PLET23a** (500 MHz, $CDCl_3$)

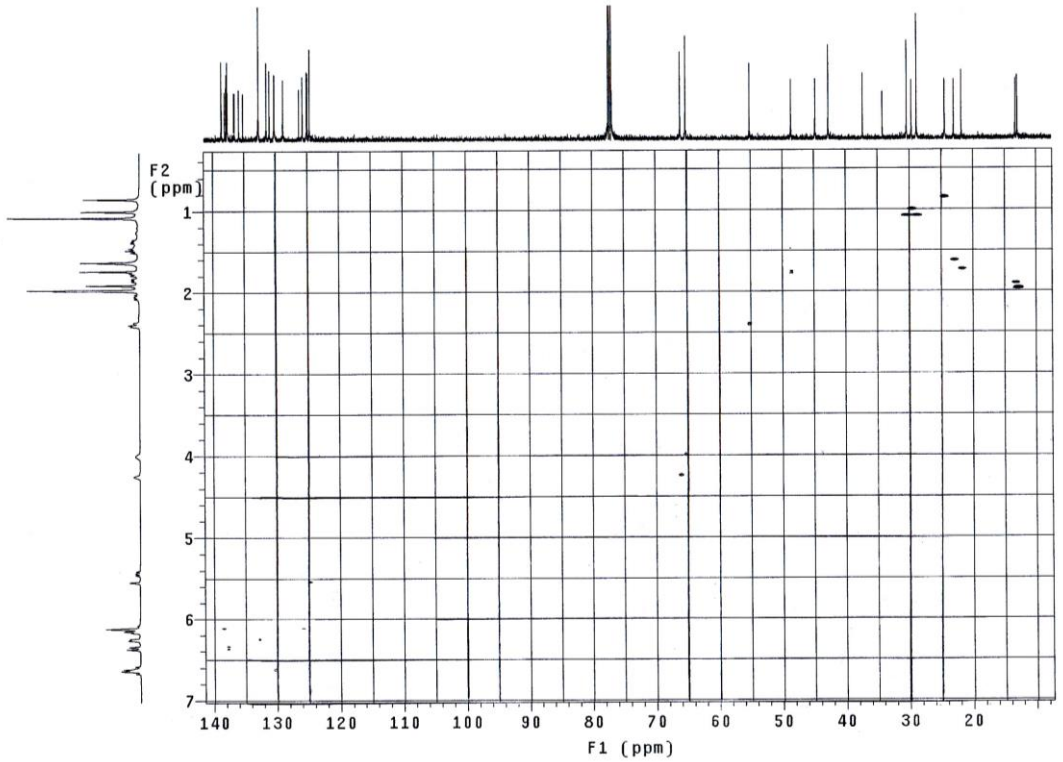

Figure A.4 HSQC spectrum of compound **PLET23a** (500 MHz, $CDCl_3$)

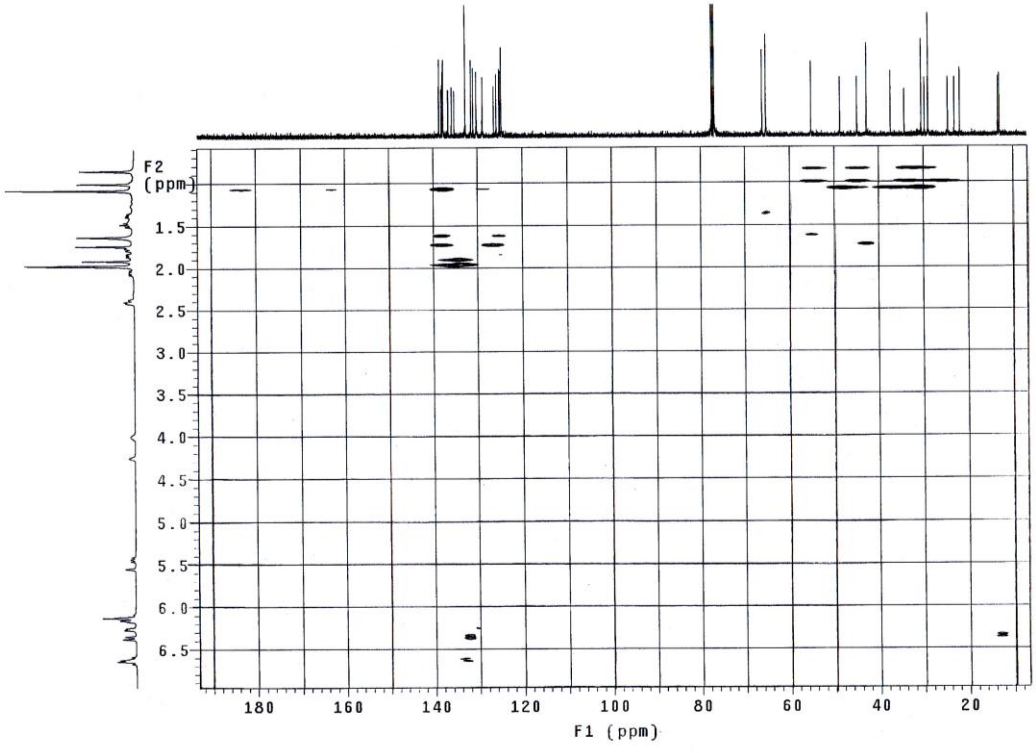

Figure A.5 HMBC spectrum of compound **PLET23a** (500 MHz, $CDCl_3$)

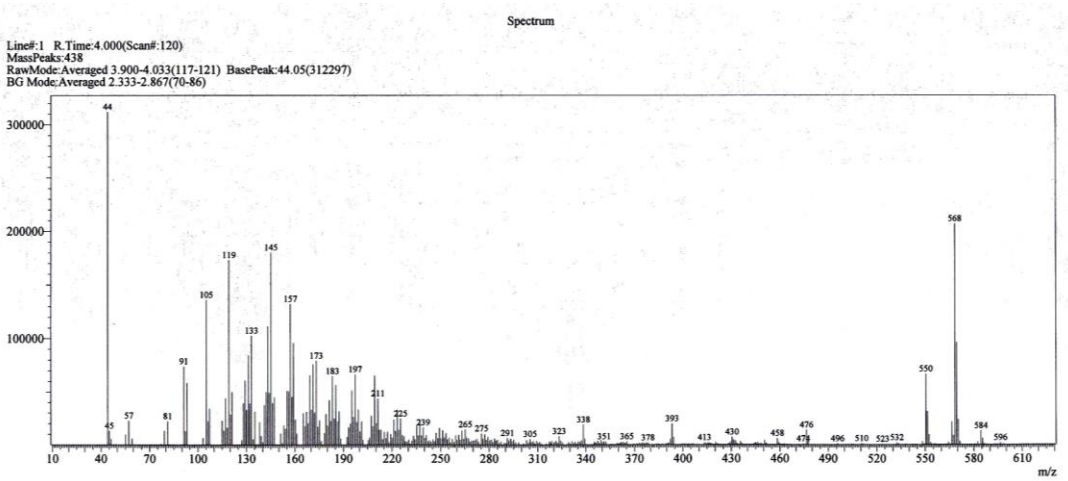

Figure A.6 Mass spectrum of compound **PLET23a**

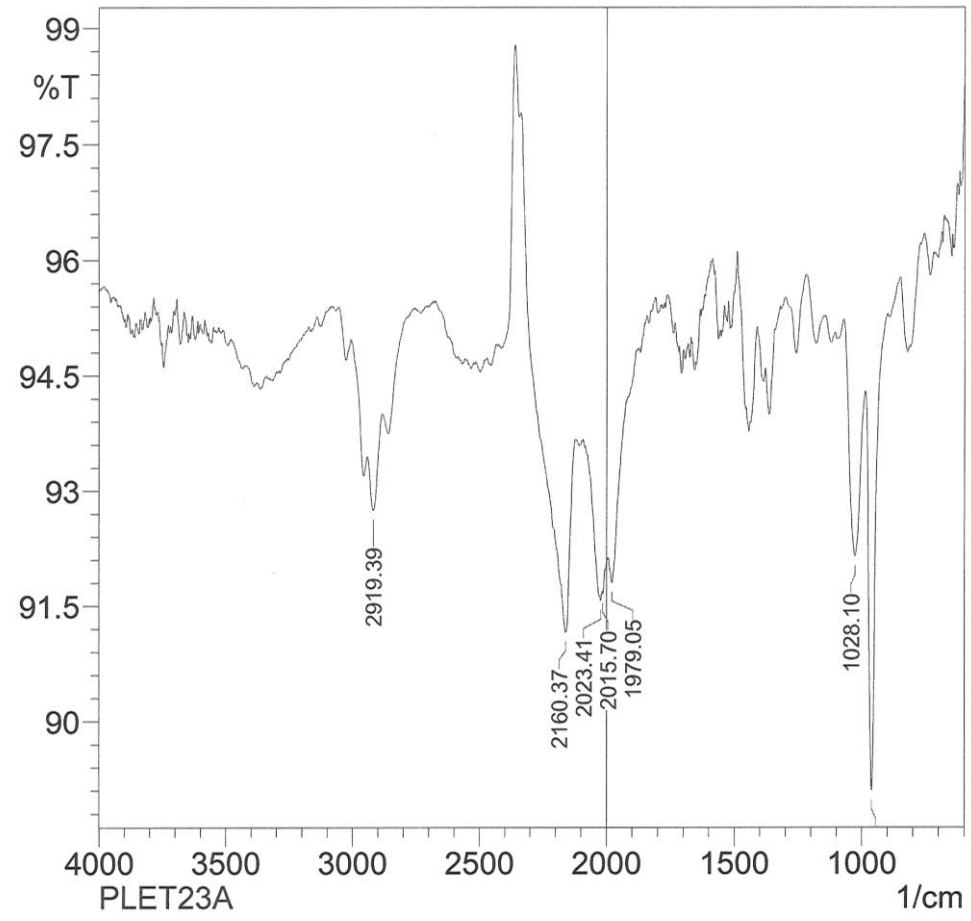

Figure A.7 IR spectrum of compound **PLET23a**

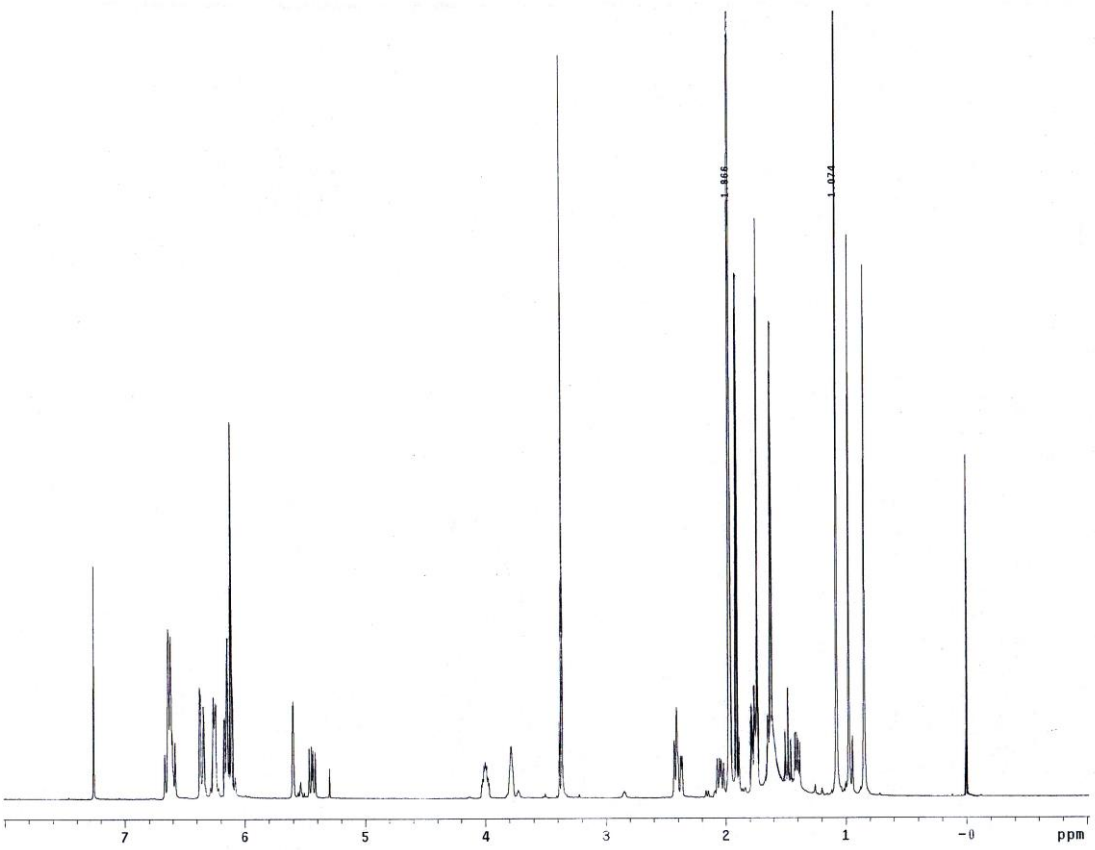

Figure A.8 ^{1}H NMR spectrum of compound **PLET_4_5pp** (500 MHz, $CDCl_3$)

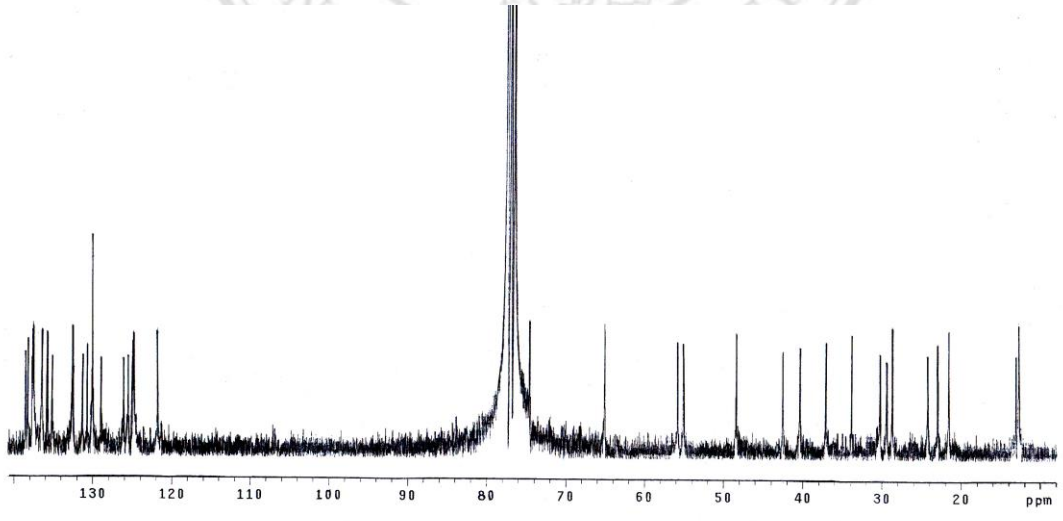

Figure A.9 ^{13}C NMR spectrum of compound **PLET_4_5pp** (125 MHz, $CDCl_3$)

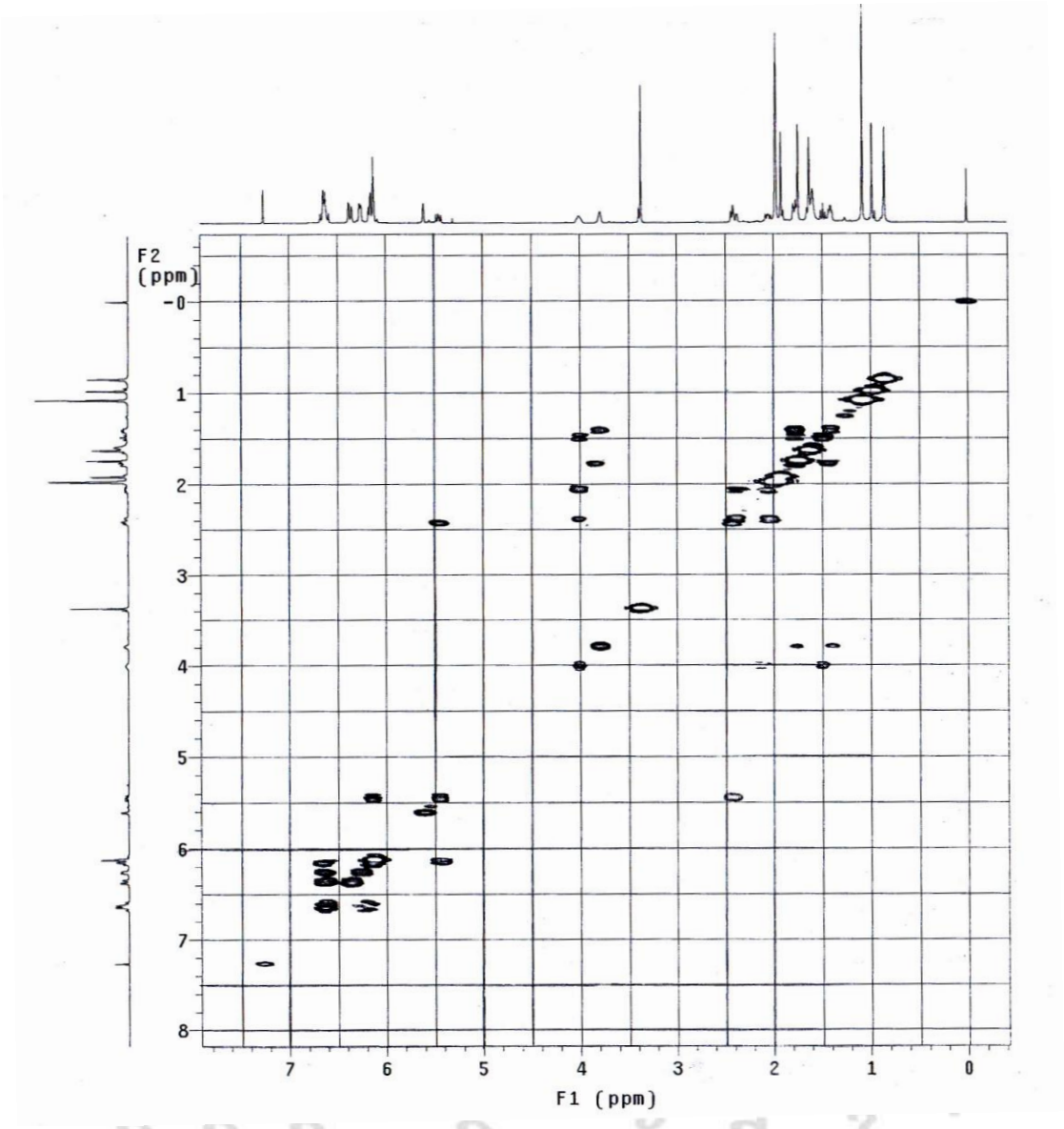

Figure A.10 COSY spectrum of compound **PLET_4_5pp** (500 MHz, $CDCl_3$)

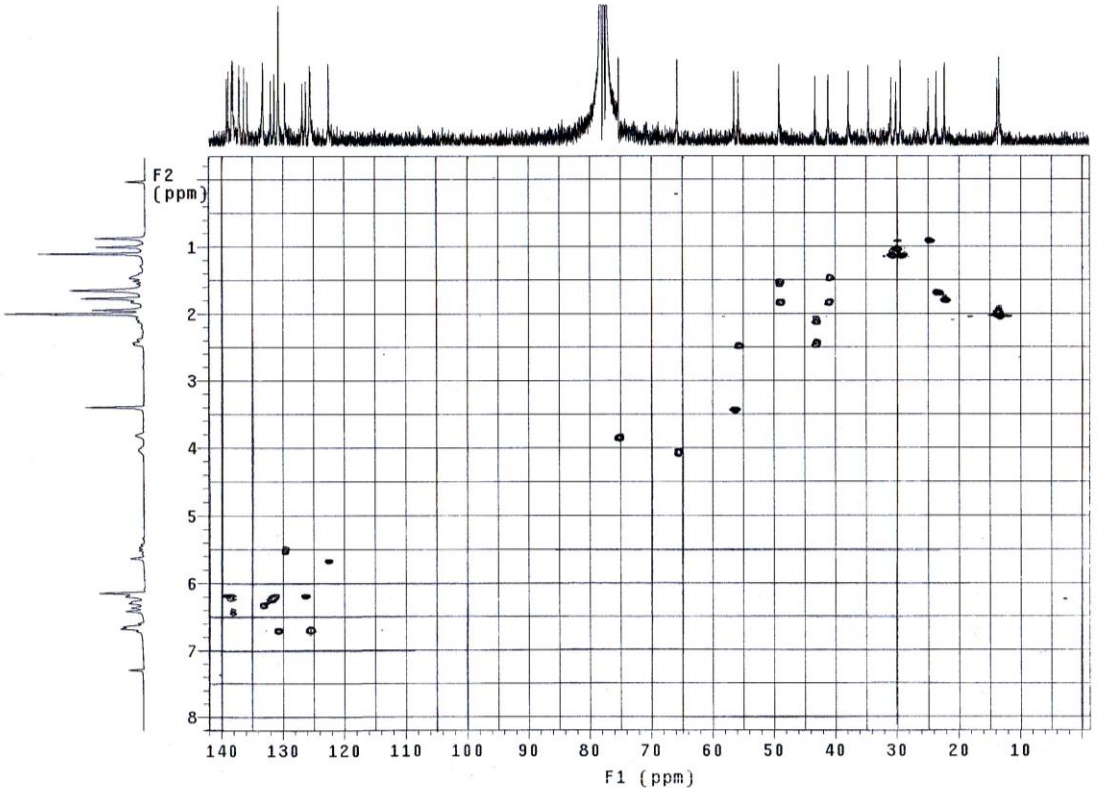

Figure A.11 HSQC spectrum of compound **PLET_4_5pp** (500 MHz, $CDCl_3$)

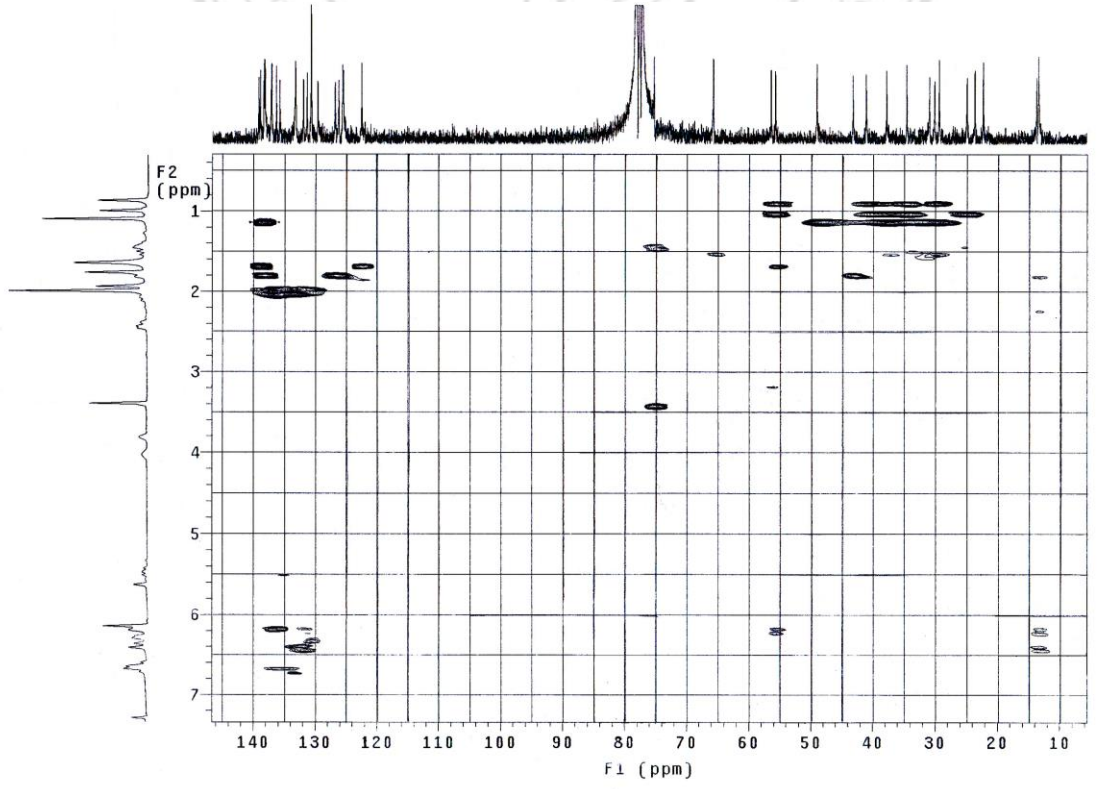

Figure A.12 HMBC spectrum of compound **PLET_4_5pp** (500 MHz, $CDCl_3$)

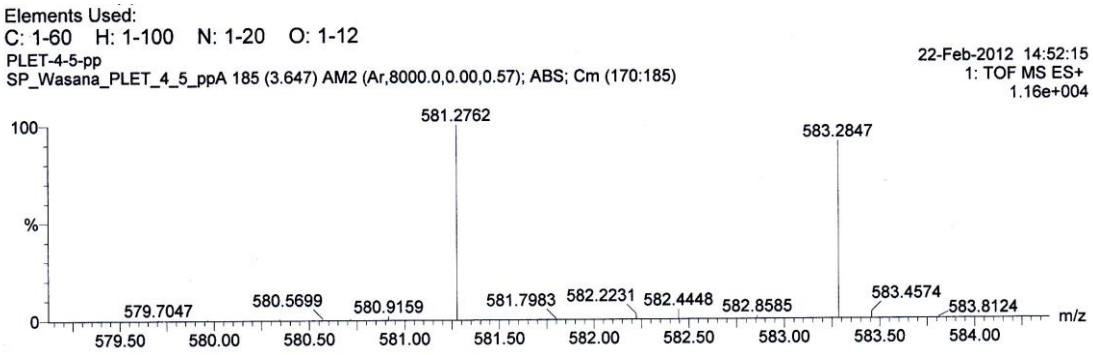

Figure A.13 HR-MS spectrum of compound **PLET_4_5pp**

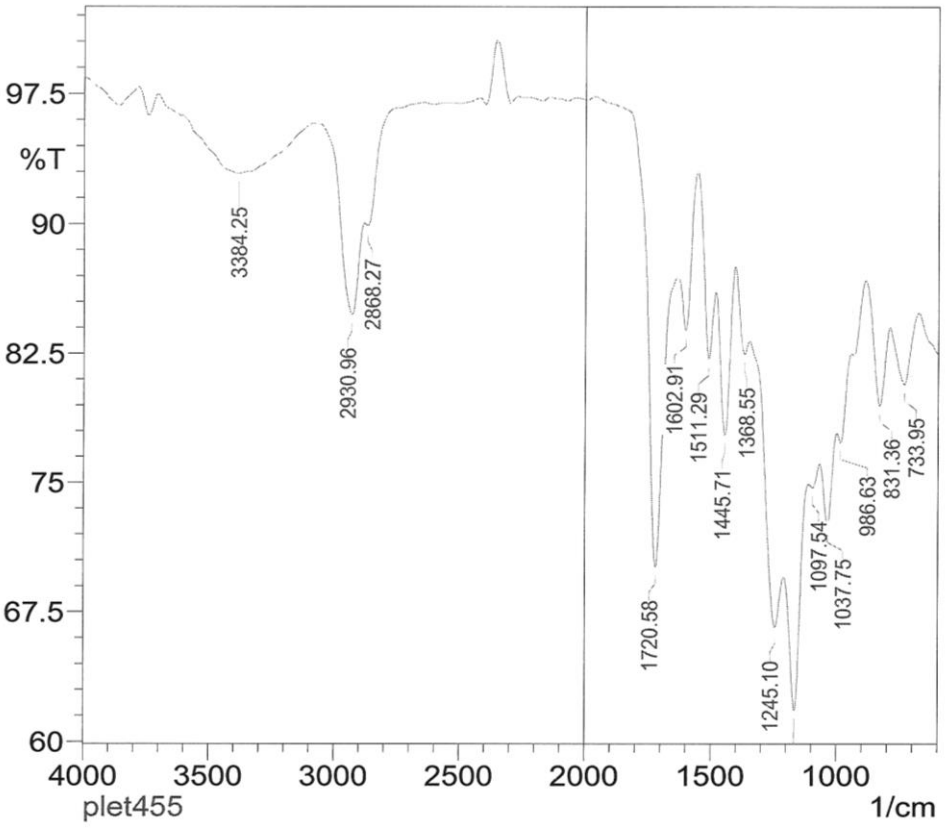

Figure A.14 IR spectrum of compound **PLET_4_5pp**

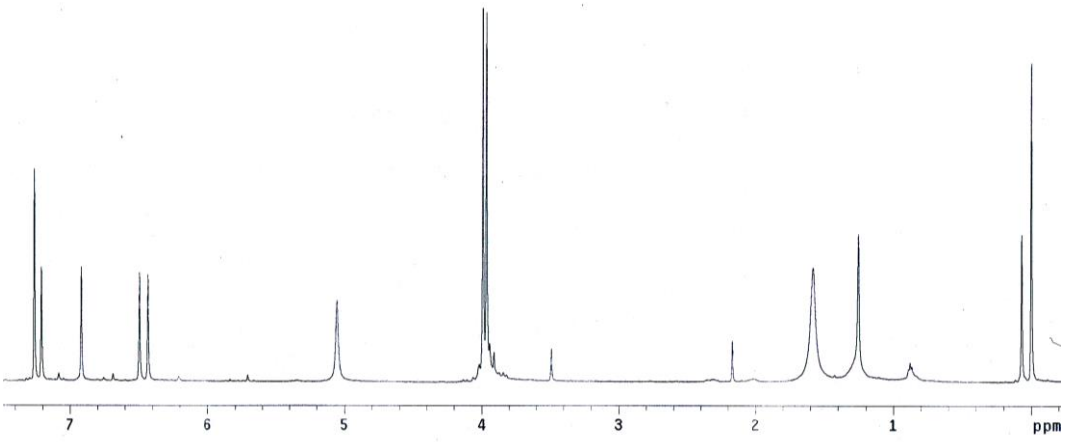

Figure A.15 ^{1}H NMR spectrum of compound **PLETp** (500 MHz, $CDCl_3$)

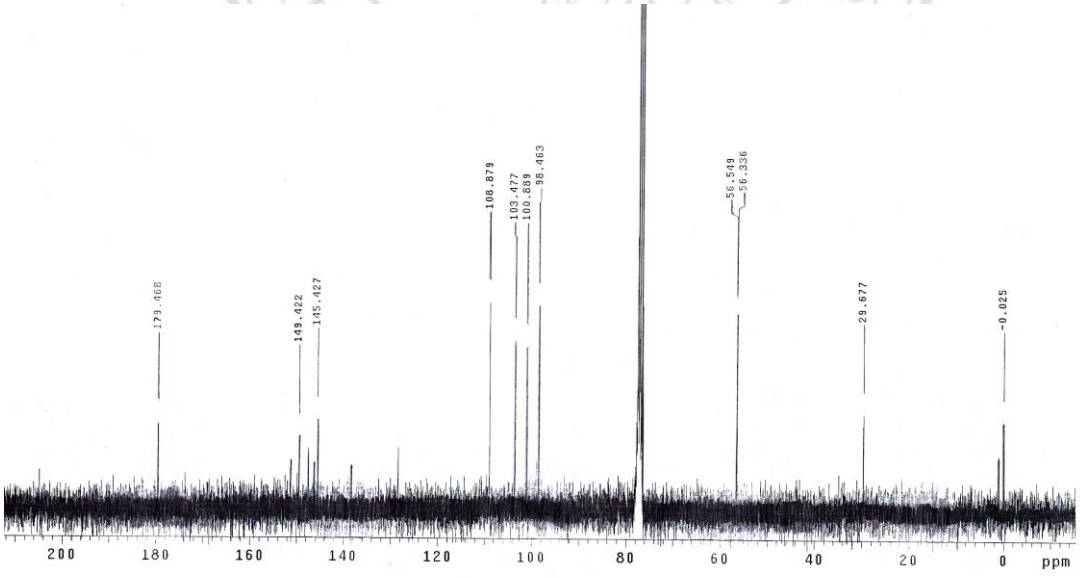

Figure A.16 ^{13}C NMR spectrum of compound **PLETp** (125 MHz, $CDCl_3$)

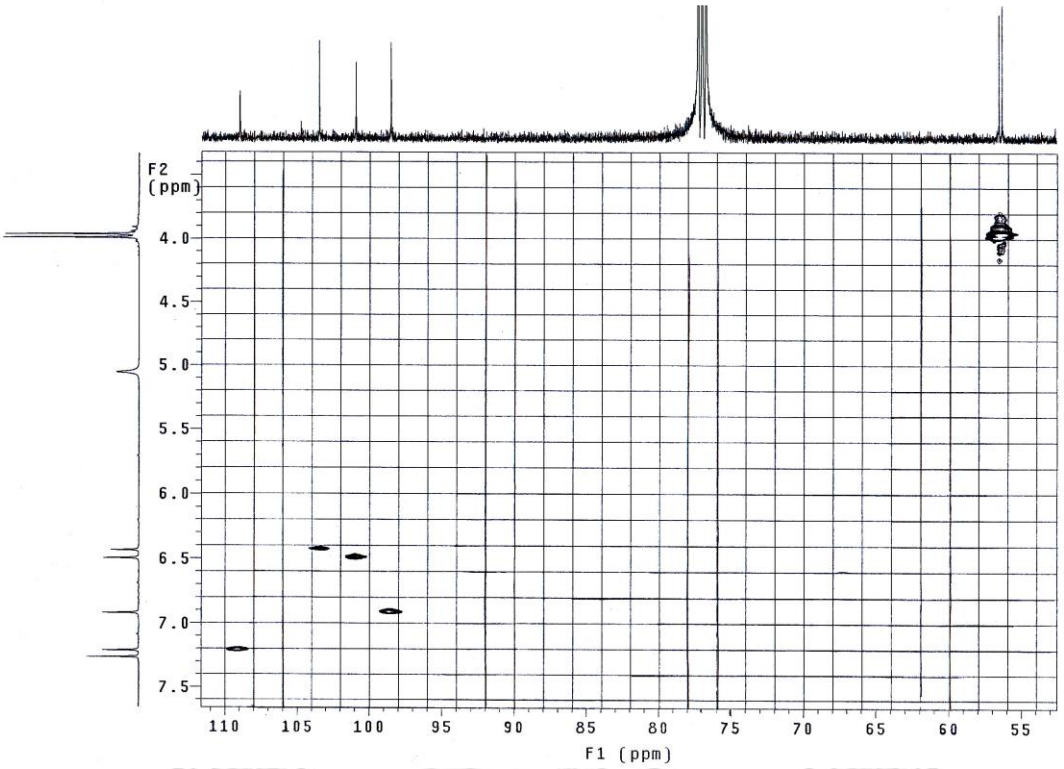

Figure A.17 HSQC spectrum of compound **PLETp** (500 MHz, $CDCl_3$)

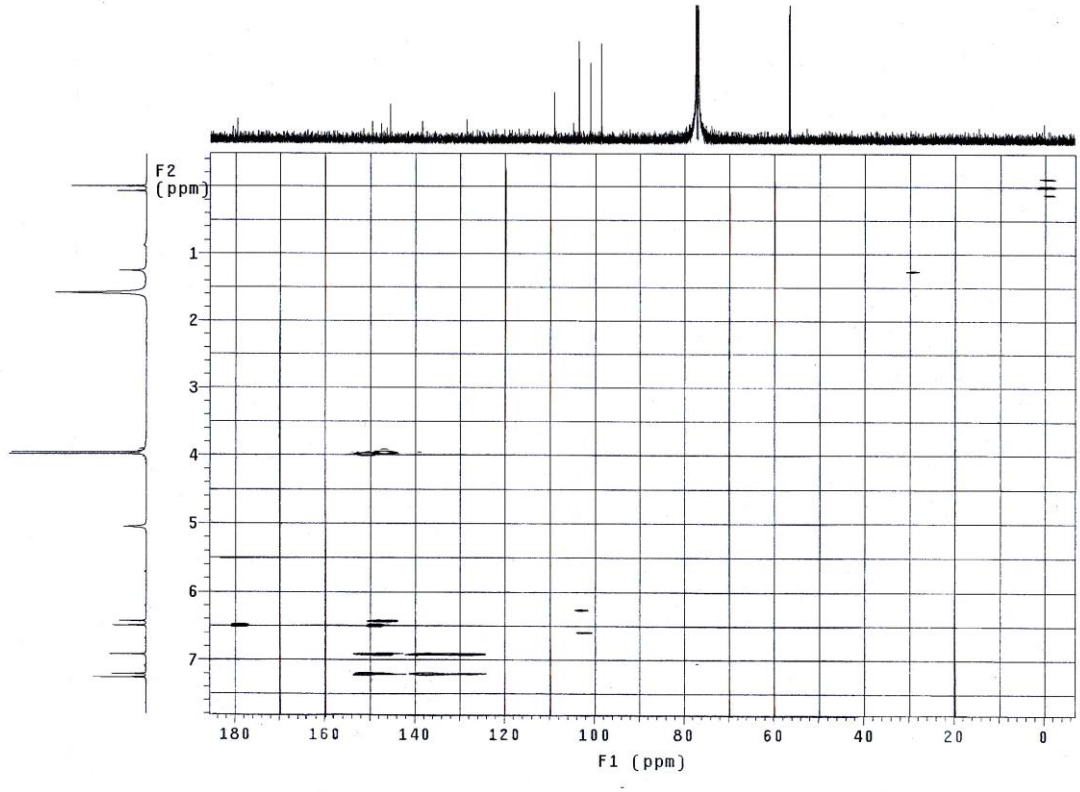

Figure A.18 HMBC spectrum of compound **PLETp** (500 MHz, $CDCl_3$)

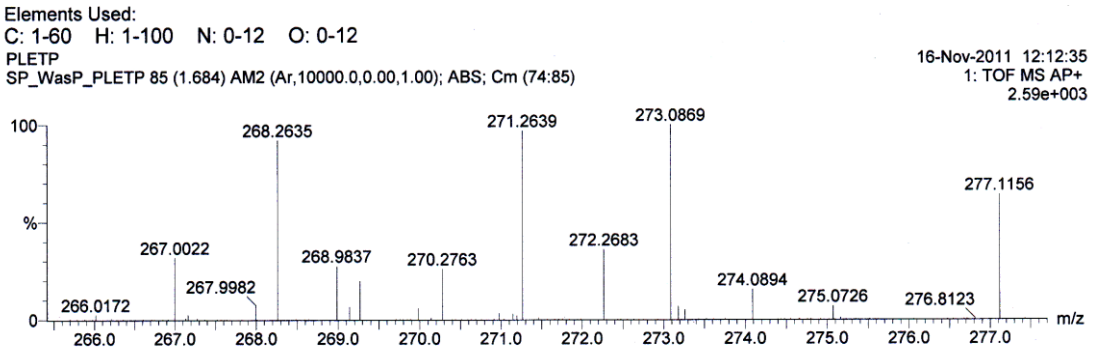

Figure A.19 HR-MS spectrum of compound **PLETp**

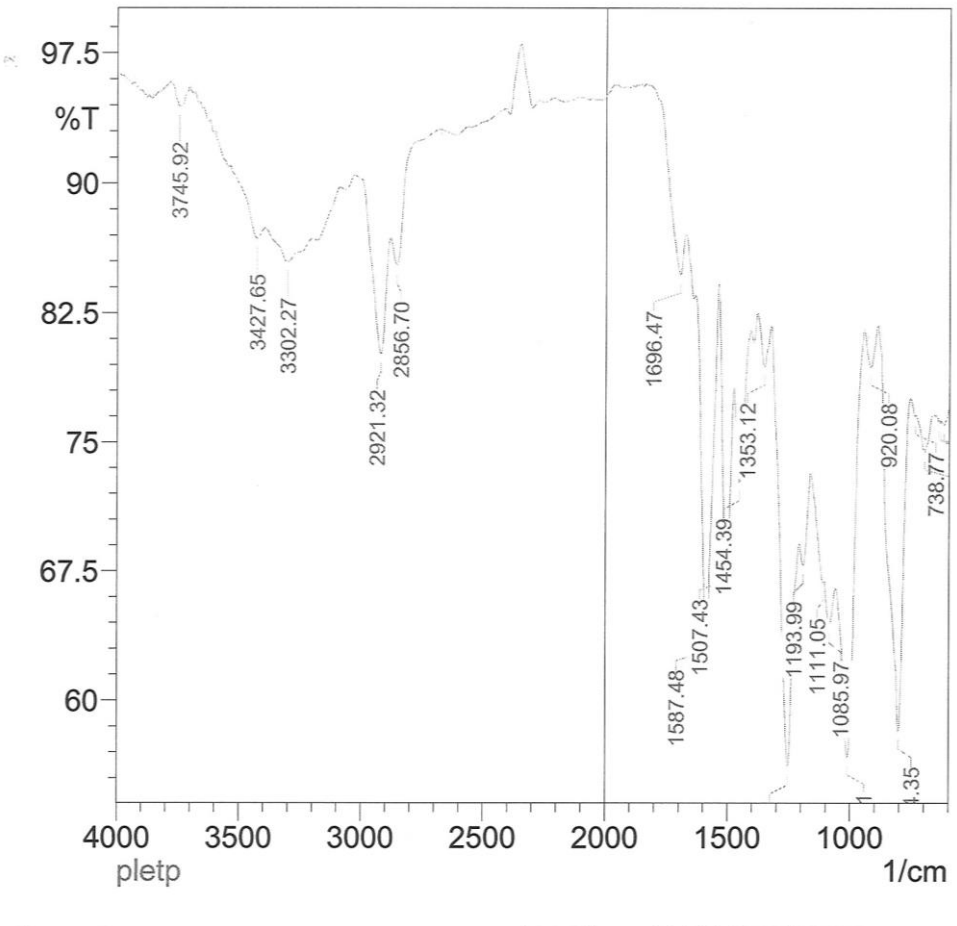

Figure A.20 IR spectrum of compound **PLETp**

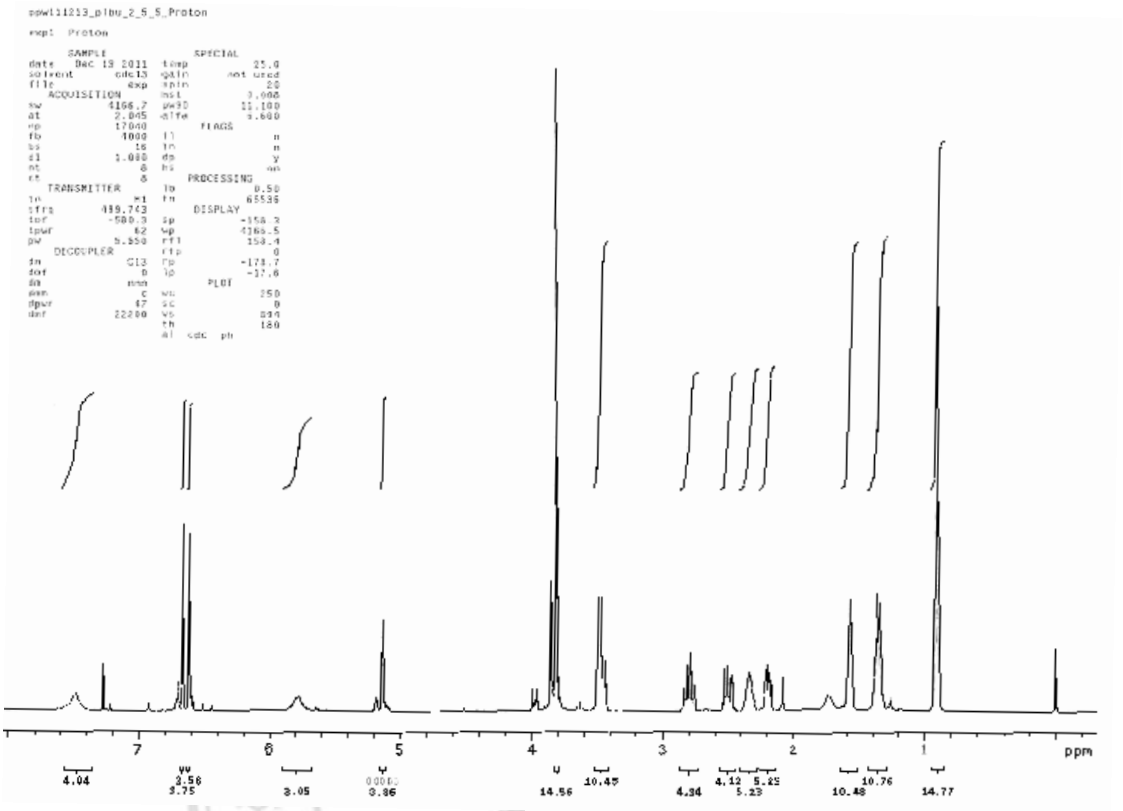

Figure A.21 ^{1}H NMR spectrum of compound **PLBU_2_5_5** (500 MHz, $CDCl_3$)

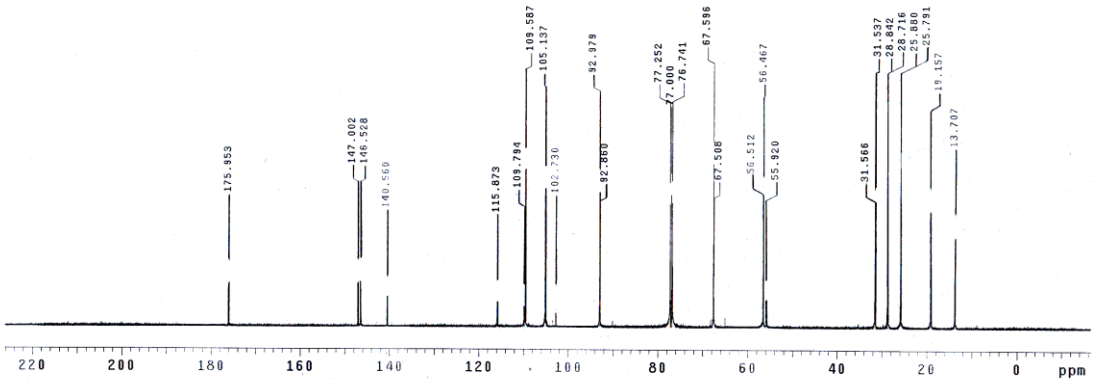

Figure A.22 ^{13}C NMR spectrum of compound **PLBU_2_5_5** (125 MHz, $CDCl_3$)

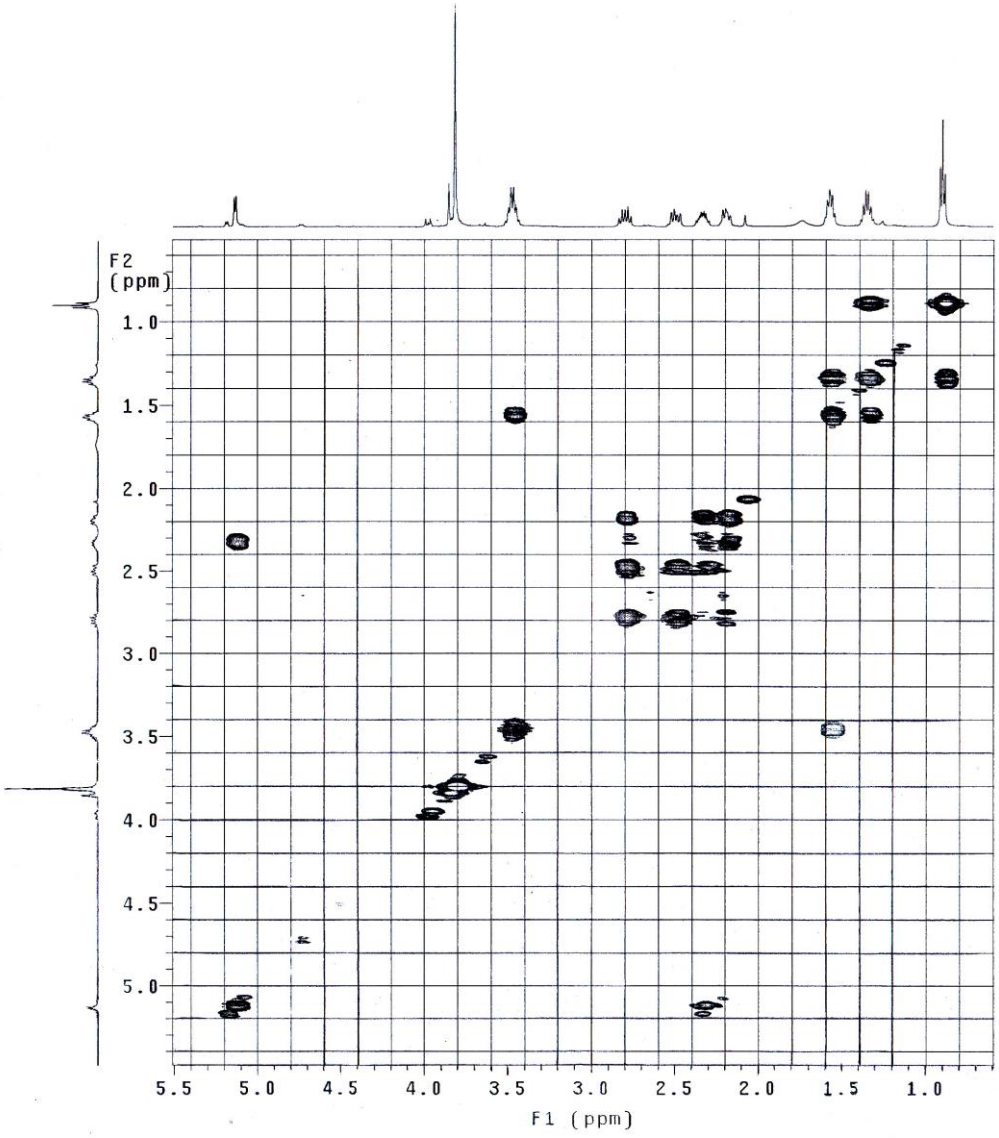

Figure A.23 COSY spectrum of compound **PLBU_2_5_5** (500 MHz, $CDCl_3$)

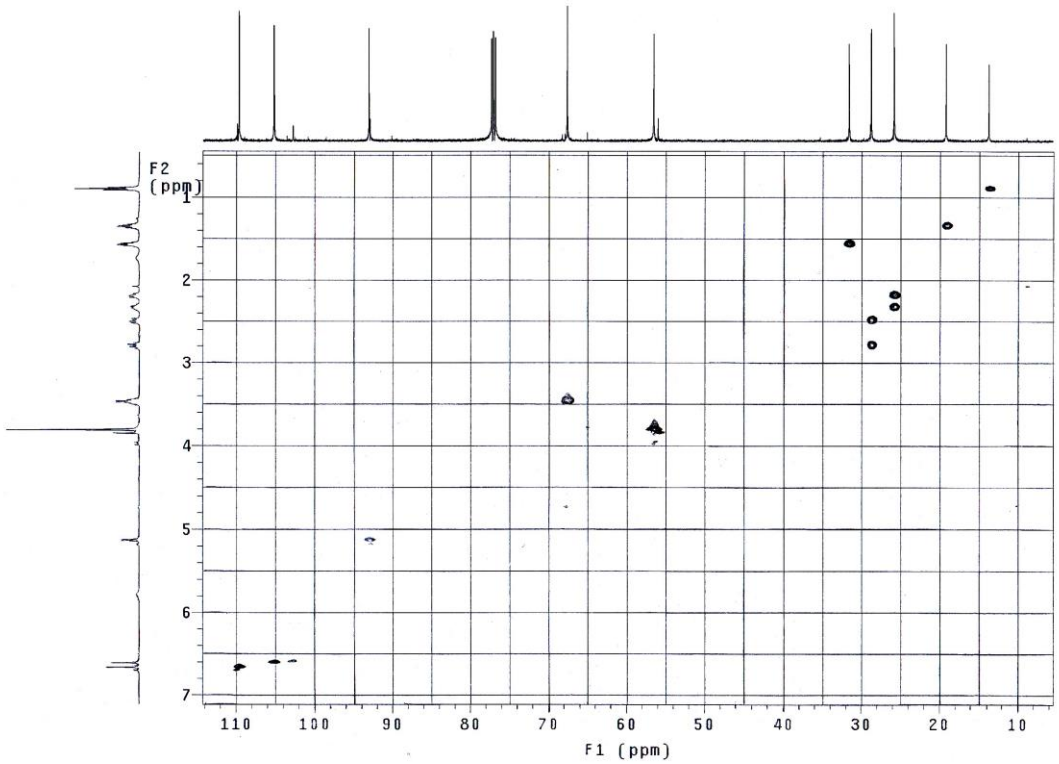

Figure A.24 HSQC spectrum of compound **PLBU_2_5_5** (500 MHz, $CDCl_3$)

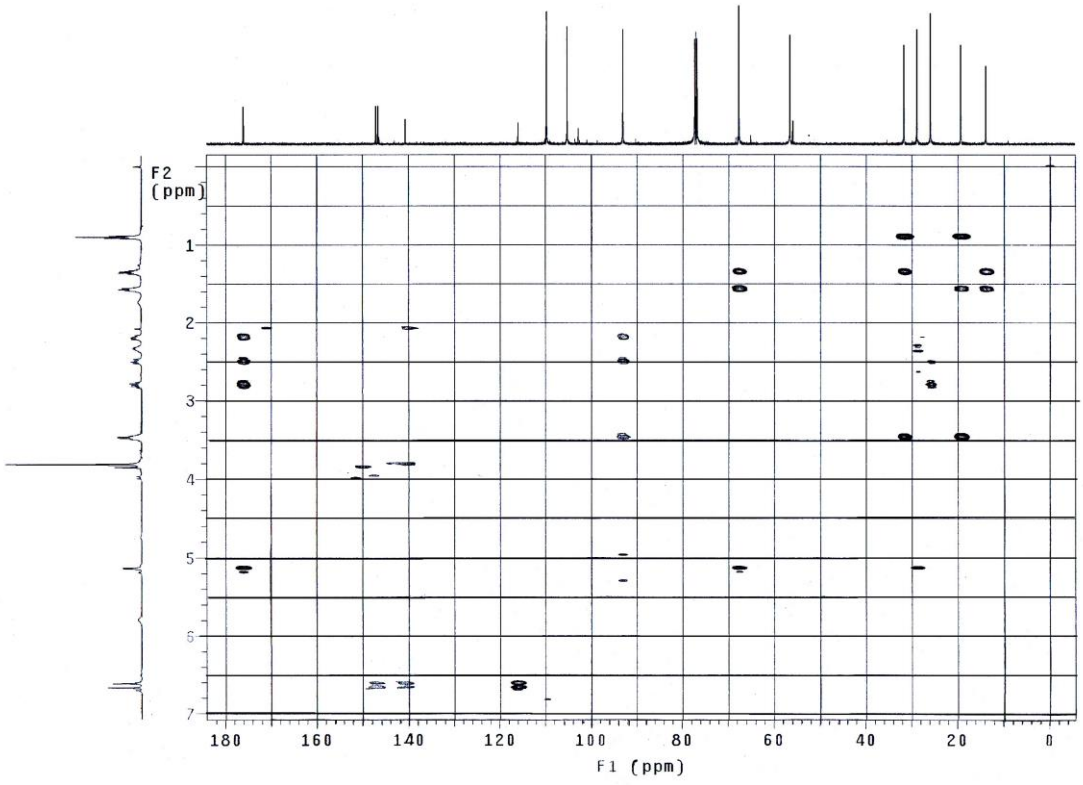

Figure A.25 HMBC spectrum of compound **PLBU_2_5_5** (500 MHz, $CDCl_3$)

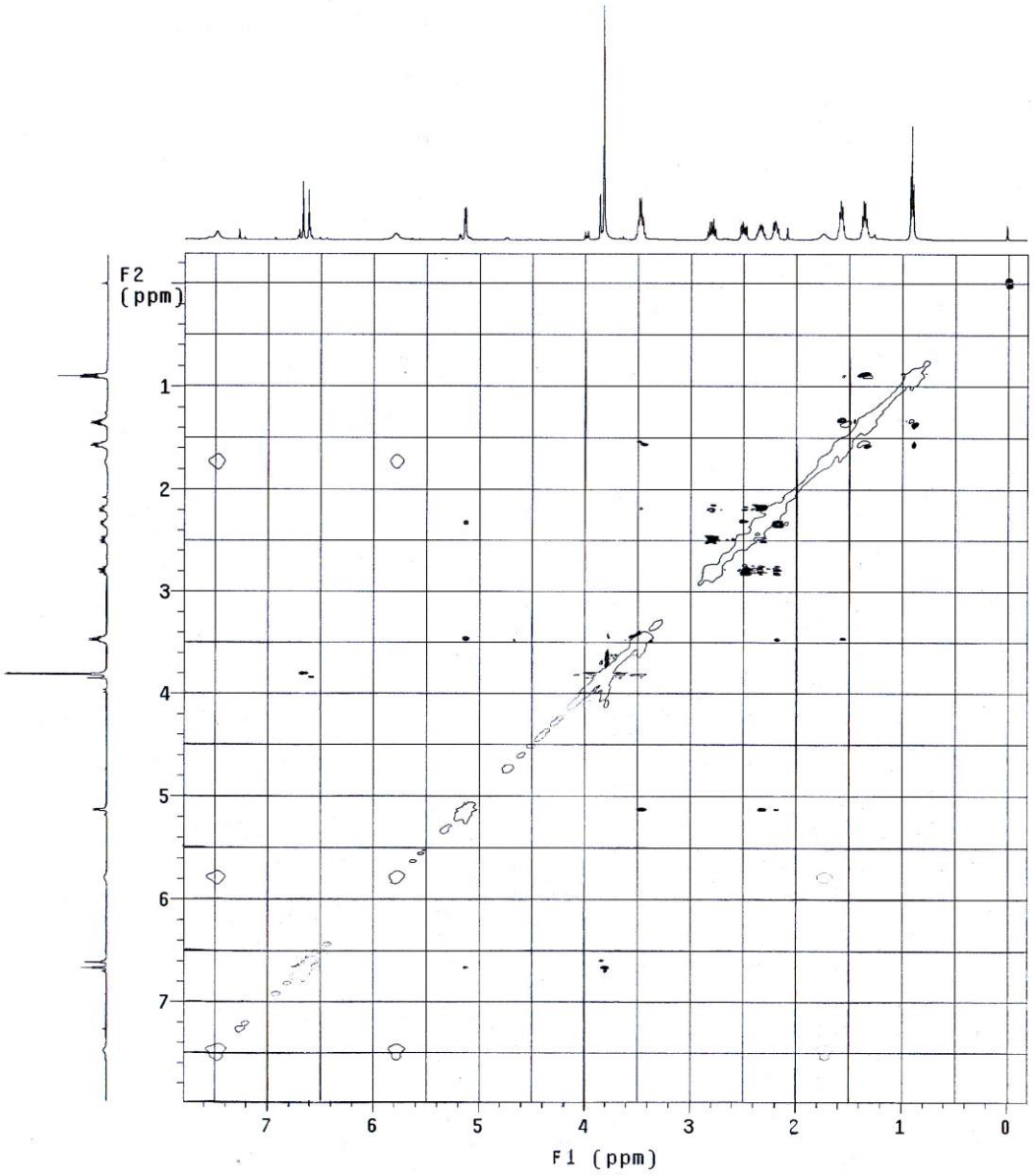

Figure A.26 NOESY spectrum of compound **PLBU_2_5_5** (500 MHz, $CDCl_3$)

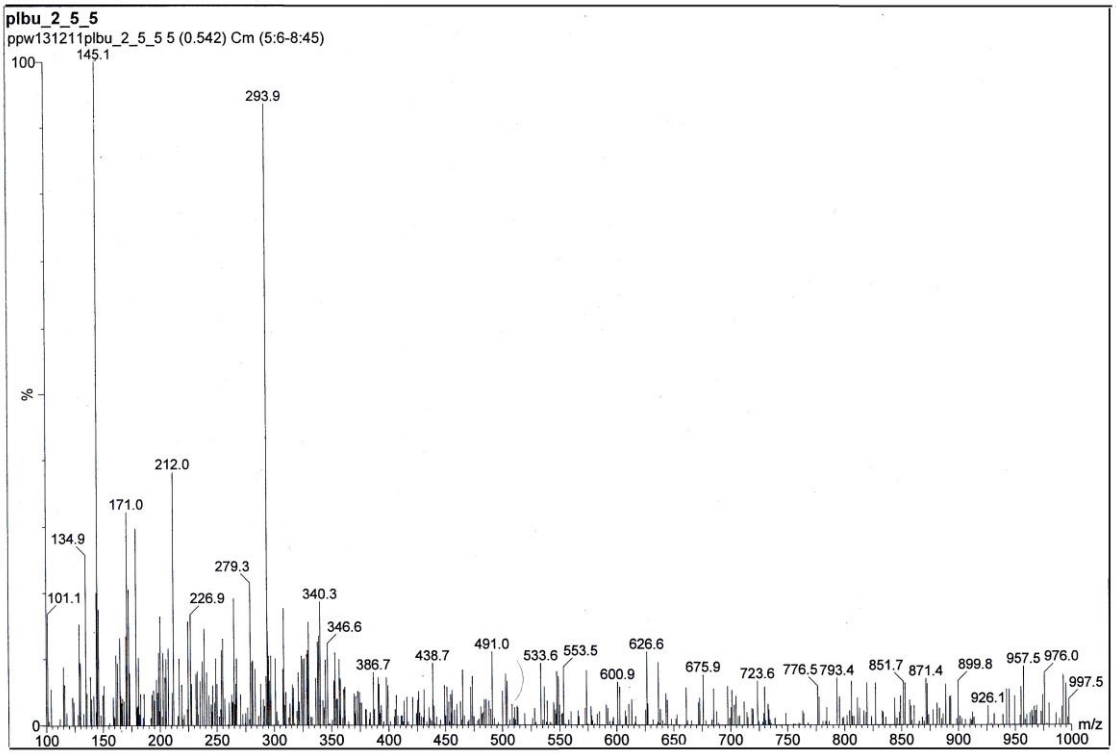

Figure A.27 Mass spectrum of compound **PLBU_2_5_5**

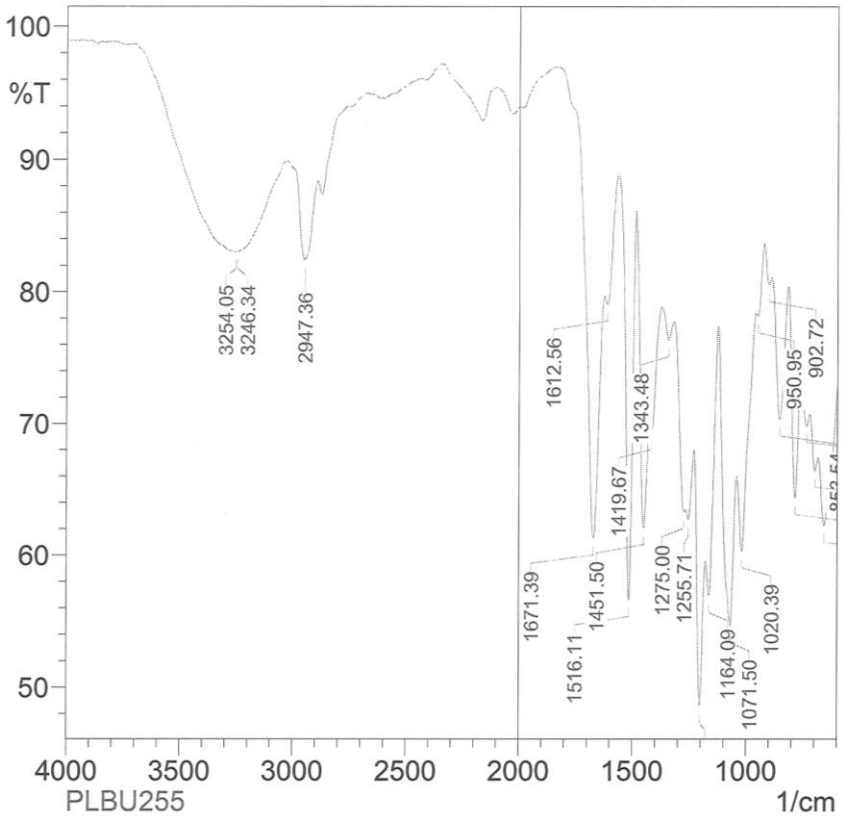

Figure A.28 IR spectrum of compound **PLBU_2_5_5**

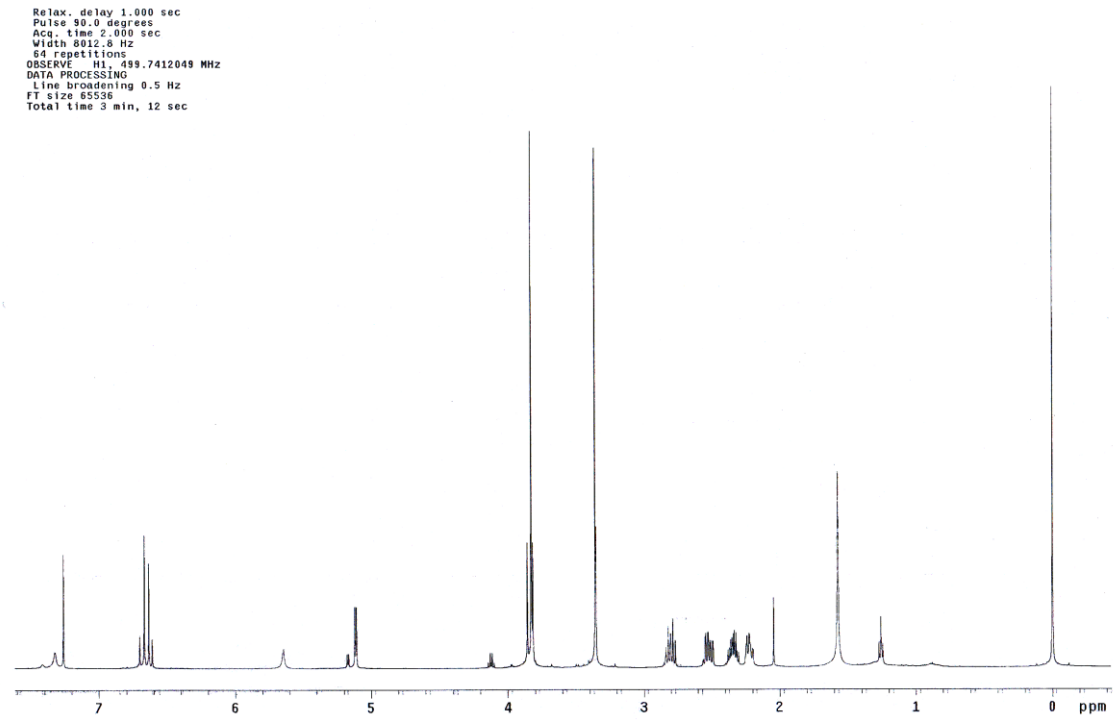

Figure A.29 ^{1}H NMR spectrum of compound **PLBU_2_5_5_m_2 (**500 MHz, $CDCl_3$)

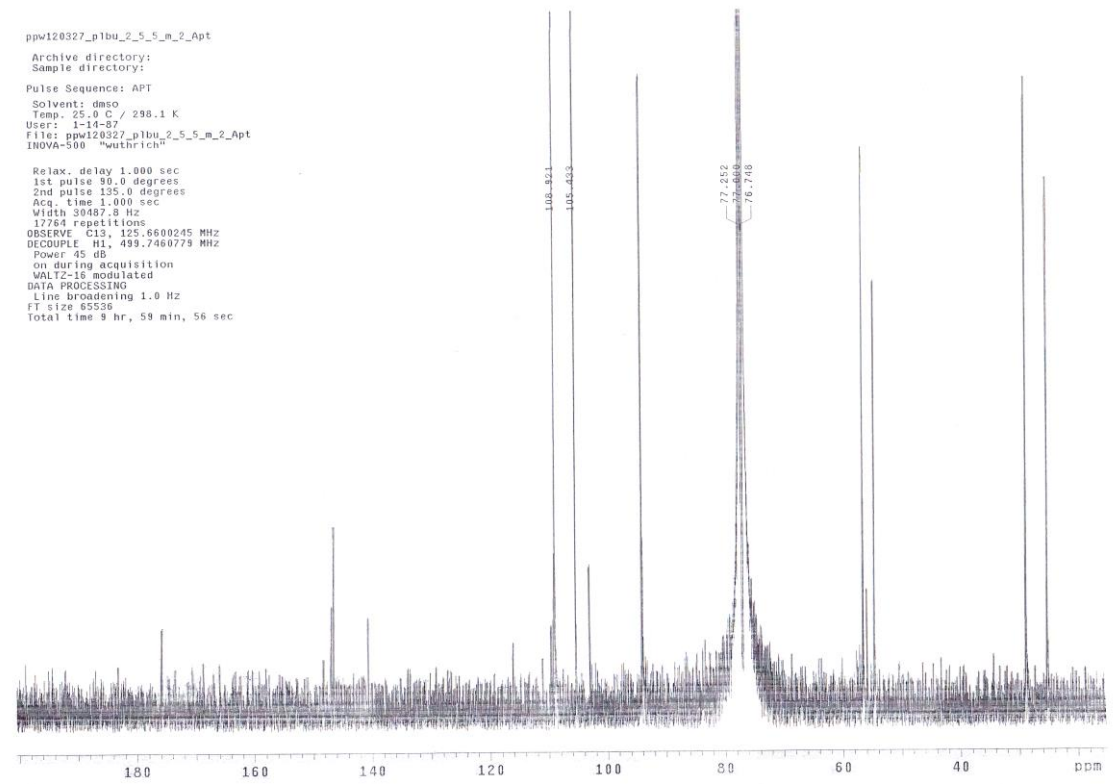

Figure A.30 ^{13}C NMR spectrum of compound **PLBU_2_5_5_m_2** (125 MHz, $CDCl_3$)

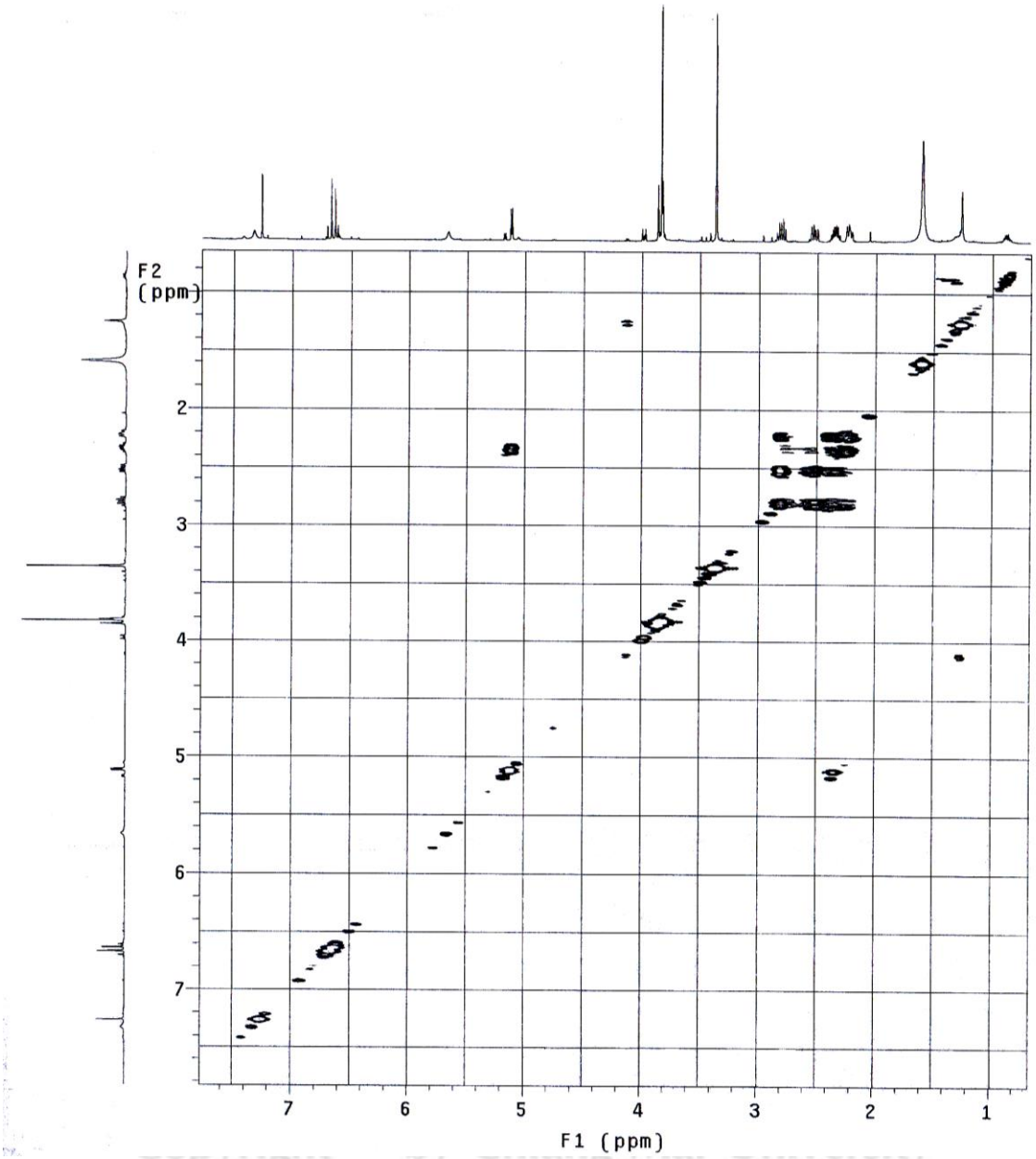

Figure A.31 COSY spectrum of compound **PLBU_2_5_5_m_2** (500 MHz, $CDCl_3$)

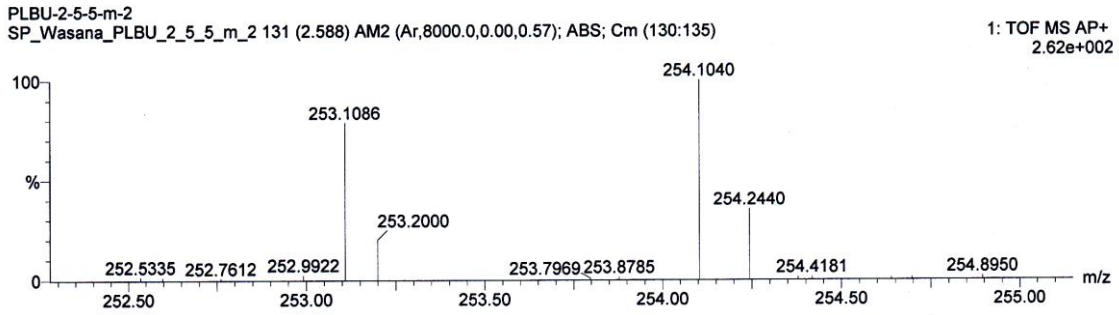

Figure A.32 HR-MS spectrum of compound **PLBU_2_5_5_m_2**

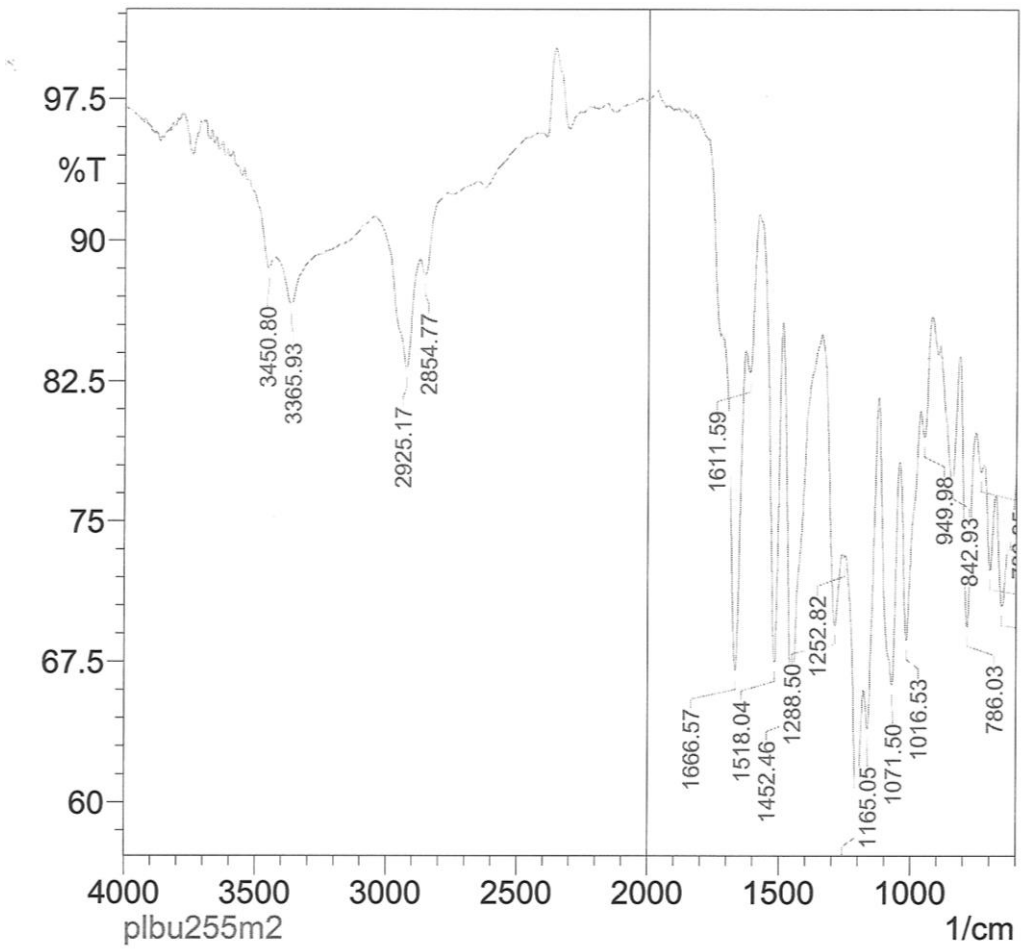

Figure A.33 IR spectrum of compound **PLBU_2_5_5_m_2**

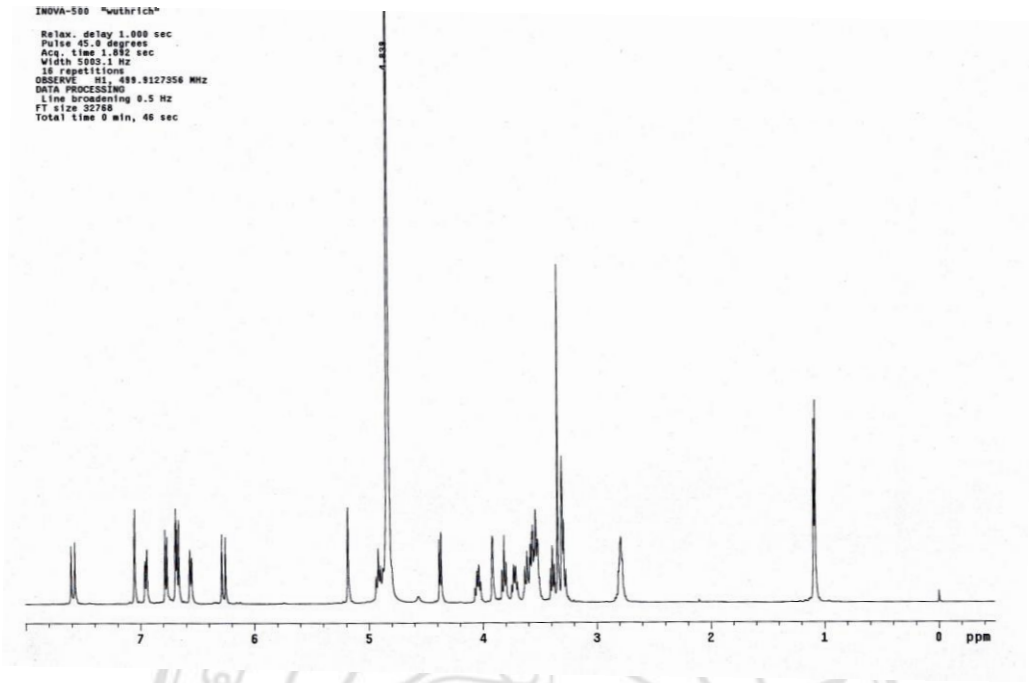

Figure A.34 ^{1}H NMR spectrum of compound **BSET_6_1_19a** (500 MHz, CD_3OD-d_4)

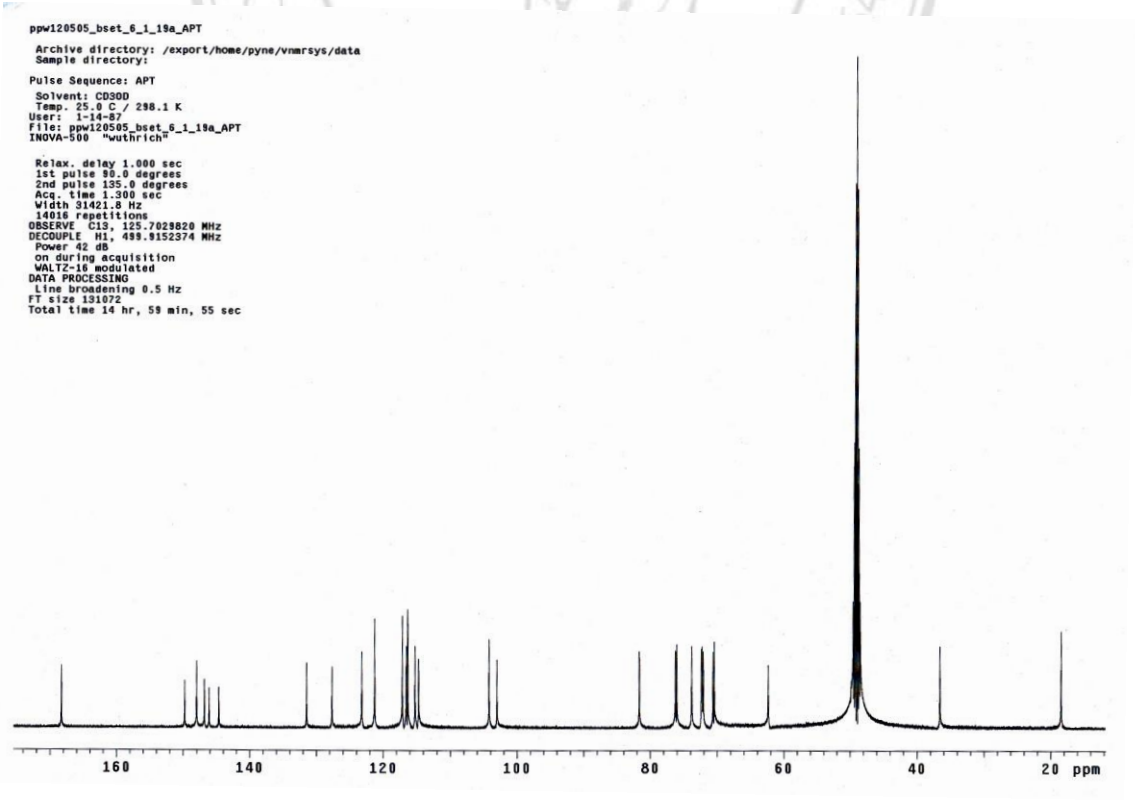

Figure A.35 ^{13}C NMR spectrum of compound **BSET_6_1_19a** (125 MHz, CD_3OD-d_4)

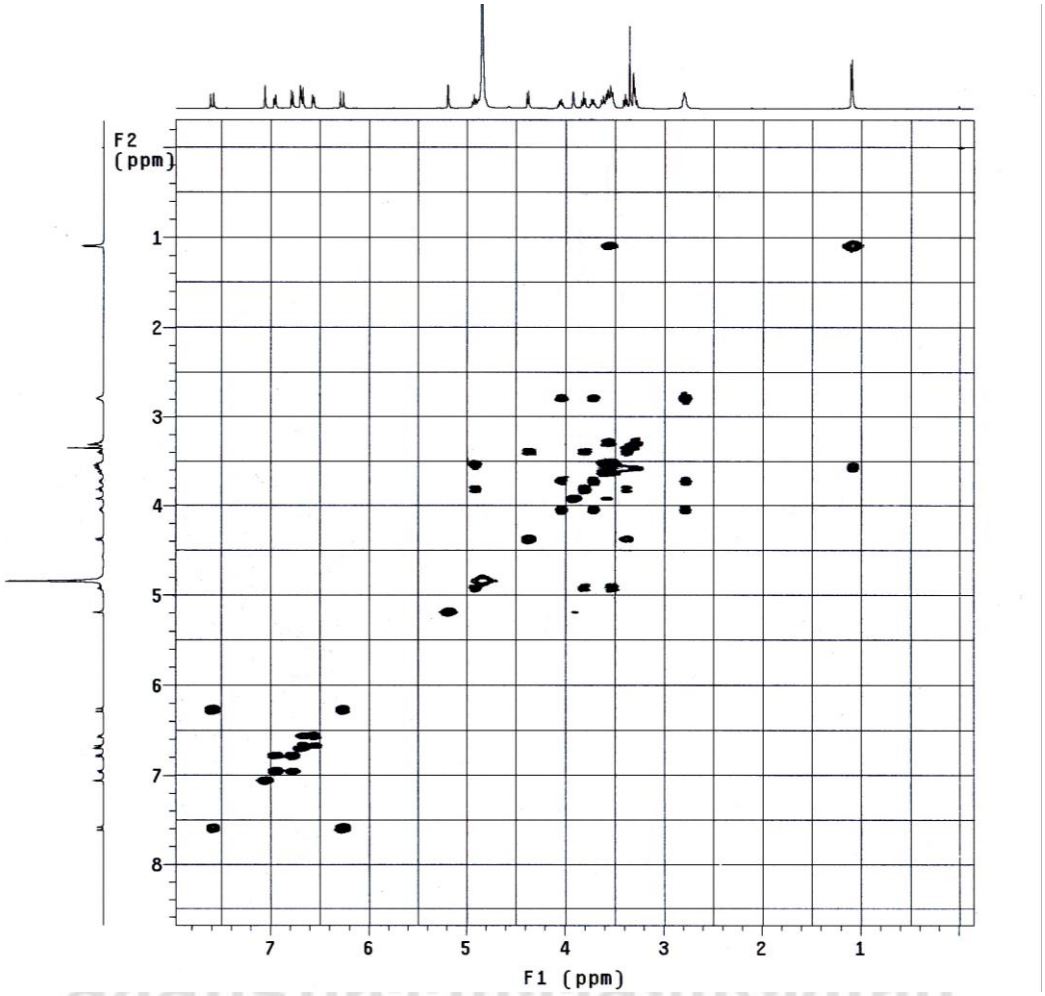

Figure A.36 COSY spectrum of compound **BSET_6_1_19a** (500 MHz, CD_3OD-d_4)

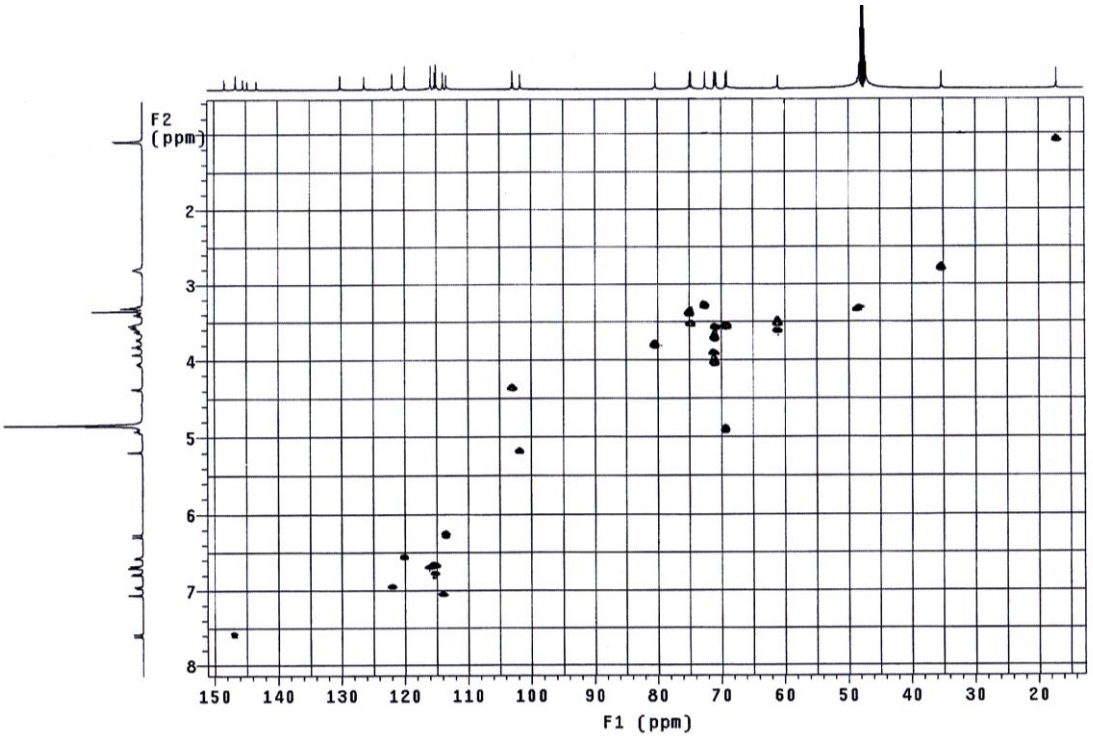

Figure A.37 HSQC spectrum of compound **BSET_6_1_19a** (500 MHz, CD_3OD-d_4)

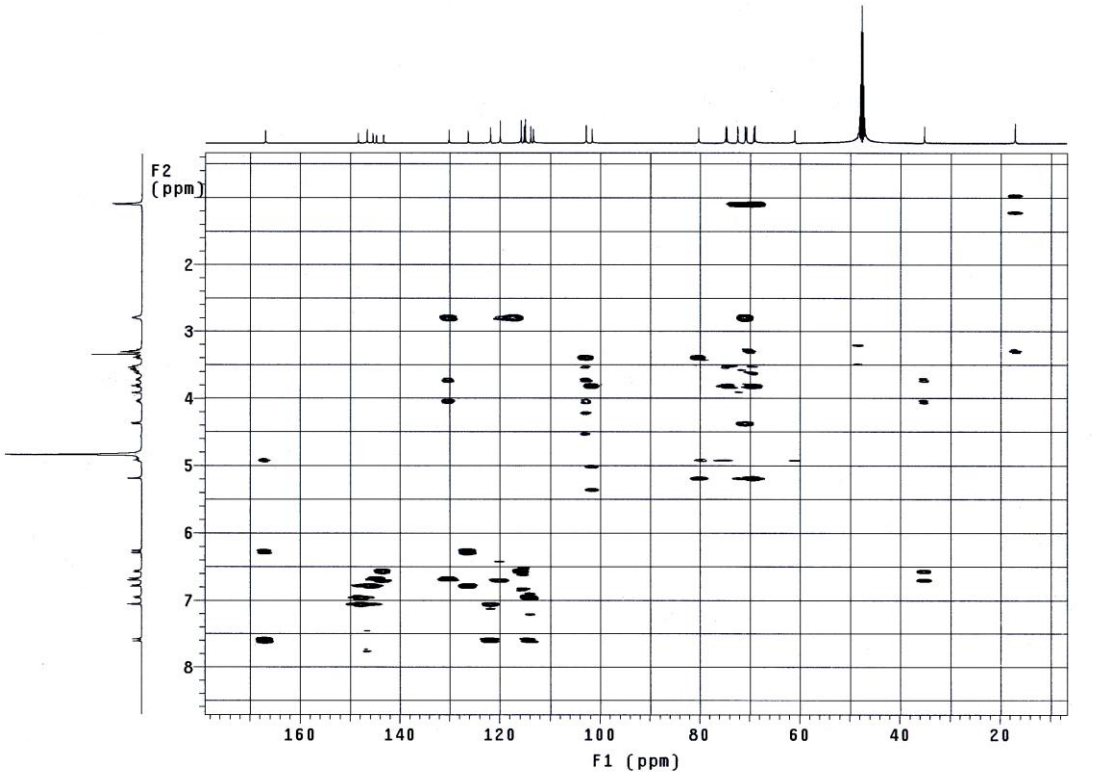

Figure A.38 HMBC spectrum of compound **BSET_6_1_19a** (500 MHz, CD_3OD-d_4)

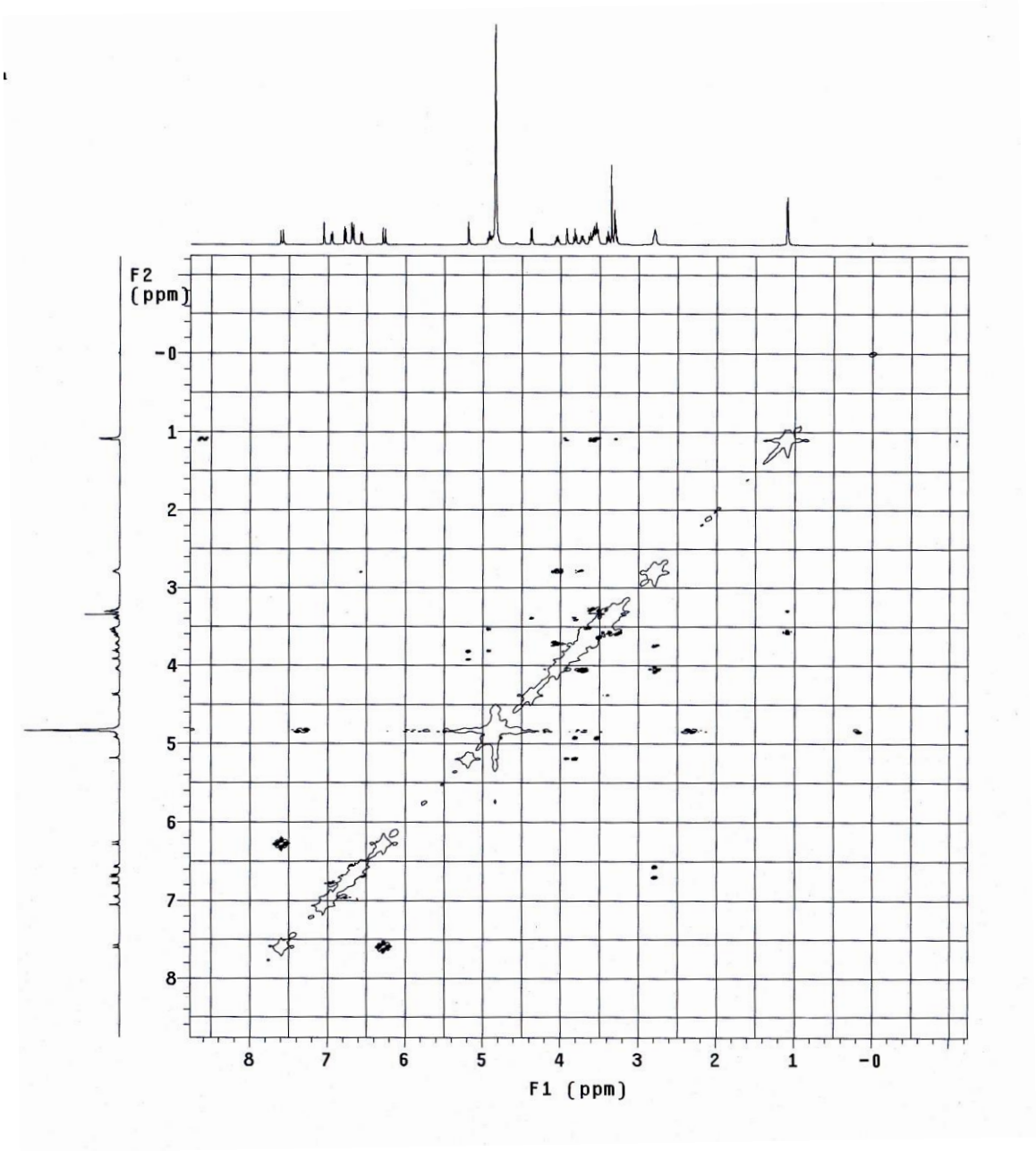

Figure A.39 NOESY spectrum of compound **BSET_6_1_19a** (500 MHz, CD_3OD-d_4)

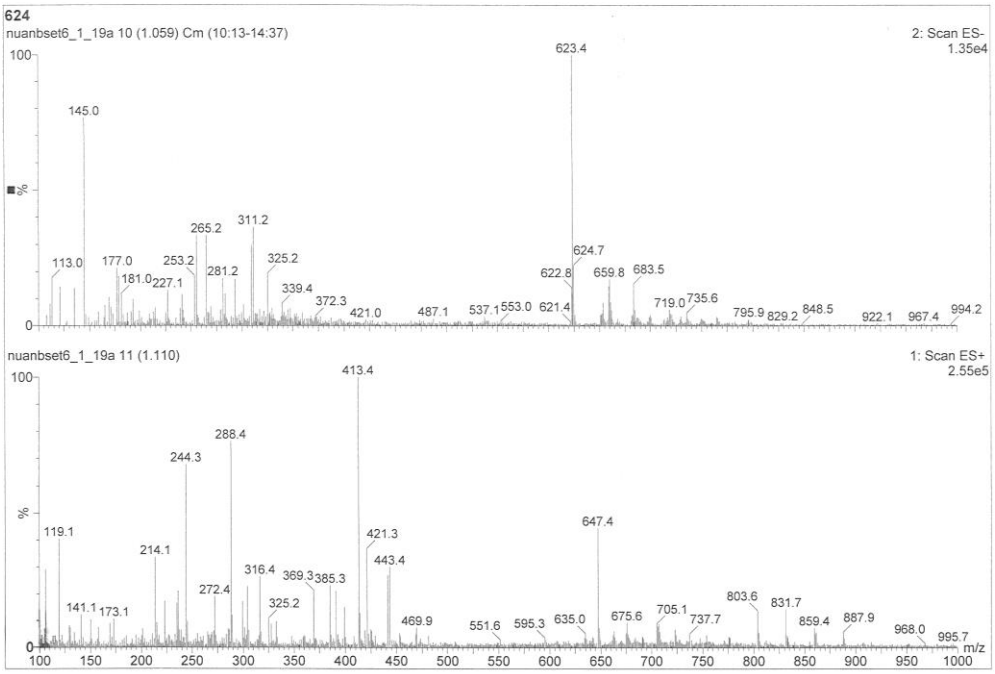

Figure A.40 Mass spectrum of compound **BSET_6_1_19a**

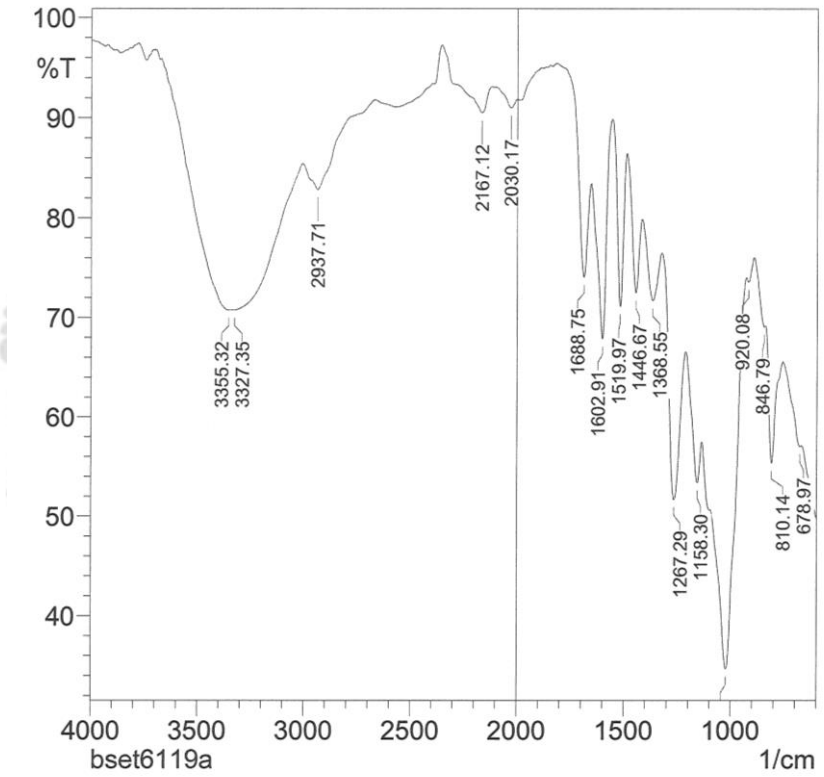

Figure A.41 IR spectrum of compound **BSET_6_1_19a**

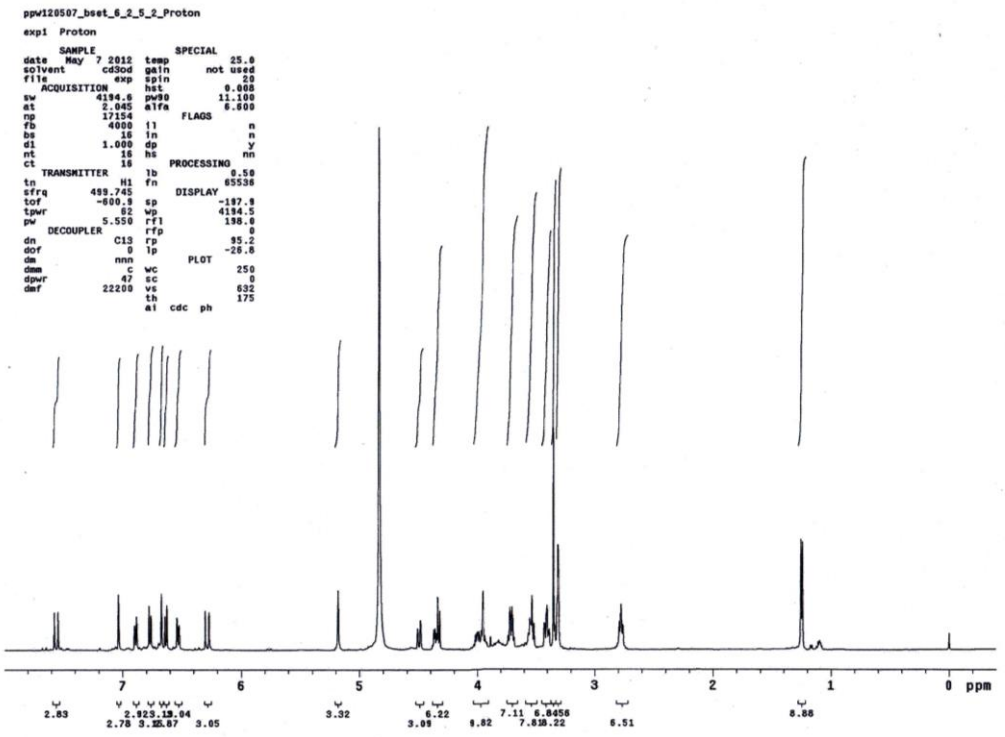

Figure A.41 ^{1}H NMR spectrum of compound **BSET_6_2_5_2** (500 MHz, CD_3OD-d_4)

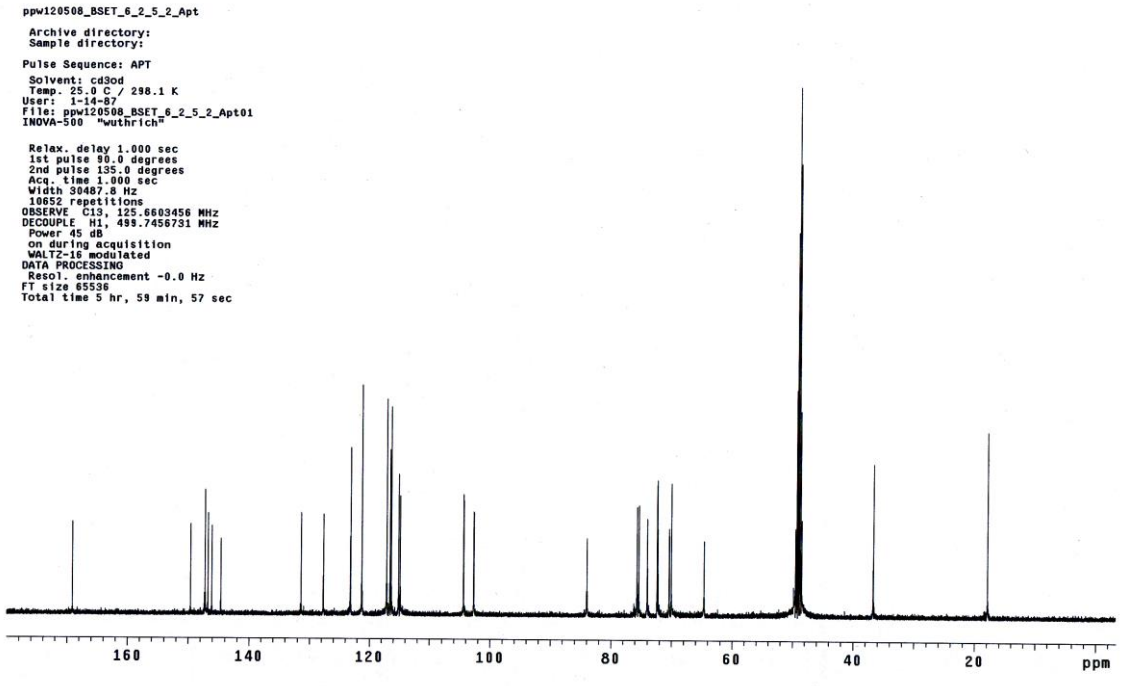

Figure A.42 ^{13}C NMR spectrum of compound **BSET_6_2_5_2** (125 MHz, CD_3OD-d_4)

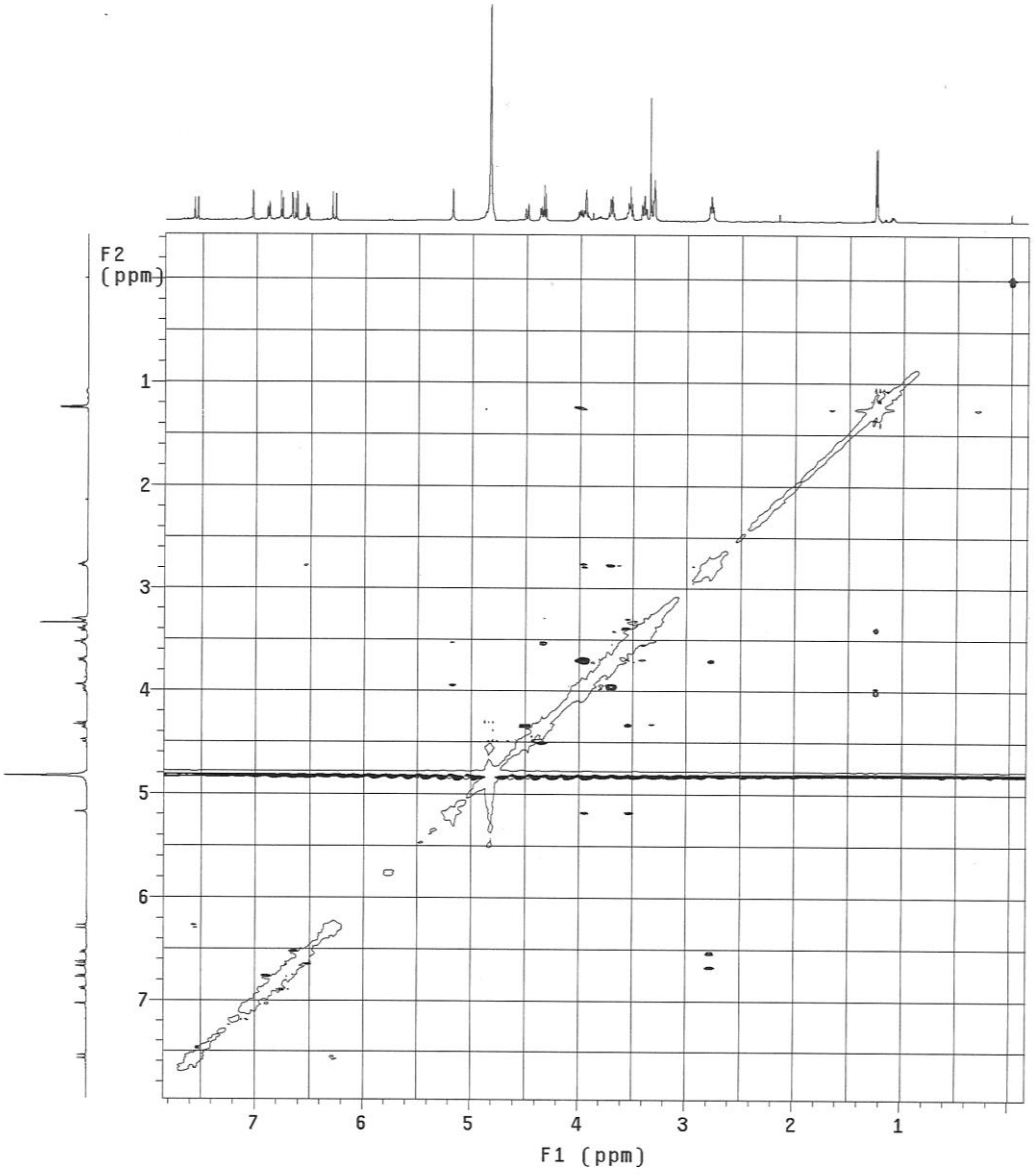

Figure A.43 COSY spectrum of compound **BSET_6_2_5_2** (500 MHz, CD_3OD-d_4)

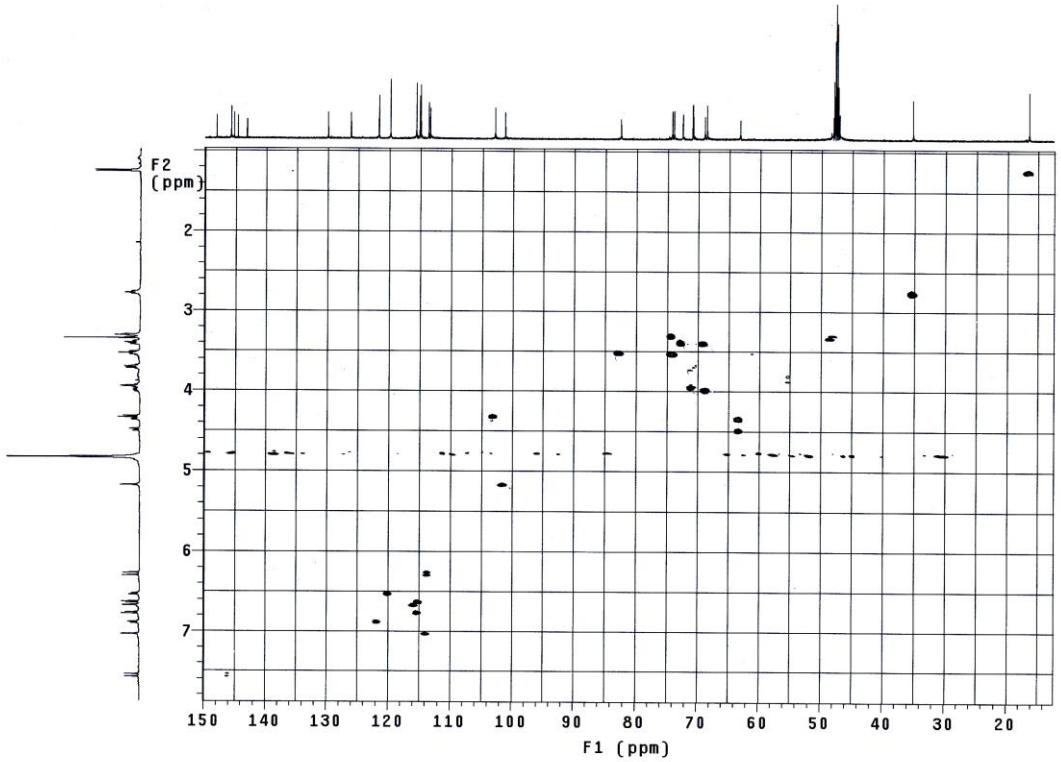

Figure A.44 HSQC spectrum of compound **BSET_6_2_5_2** (500 MHz, CD_3OD-d_4)

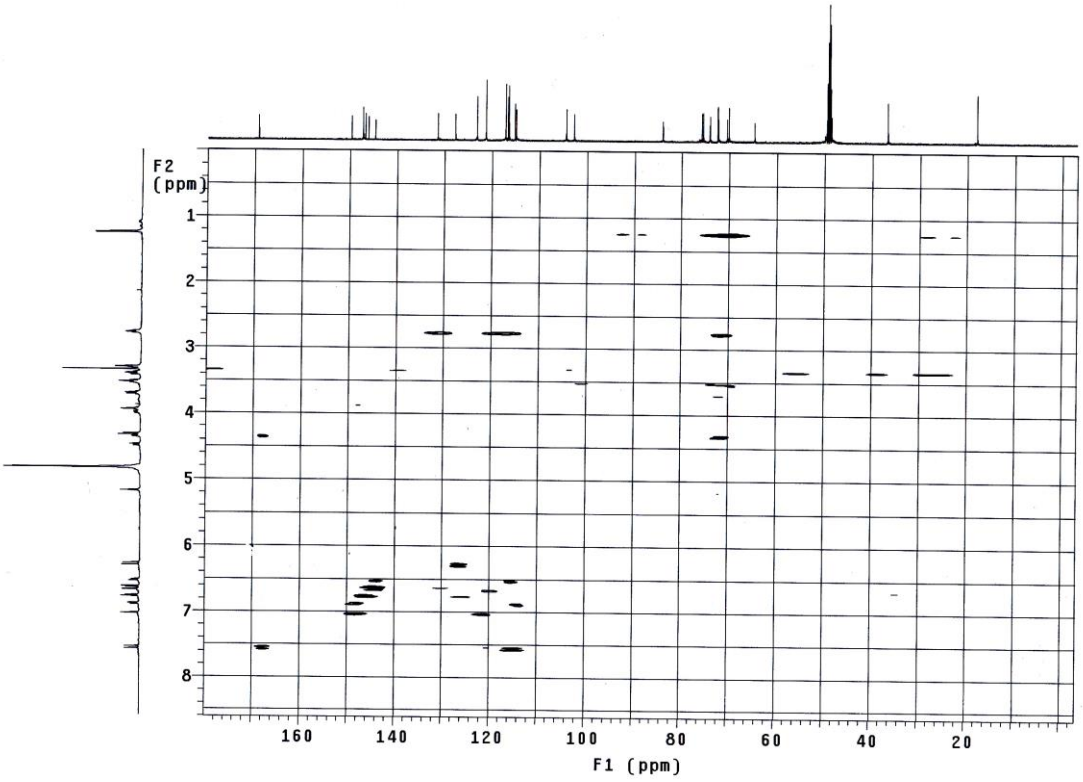

Figure A.45 HMBC spectrum of compound **BSET_6_2_5_2** (500 MHz, CD_3OD-d_4)

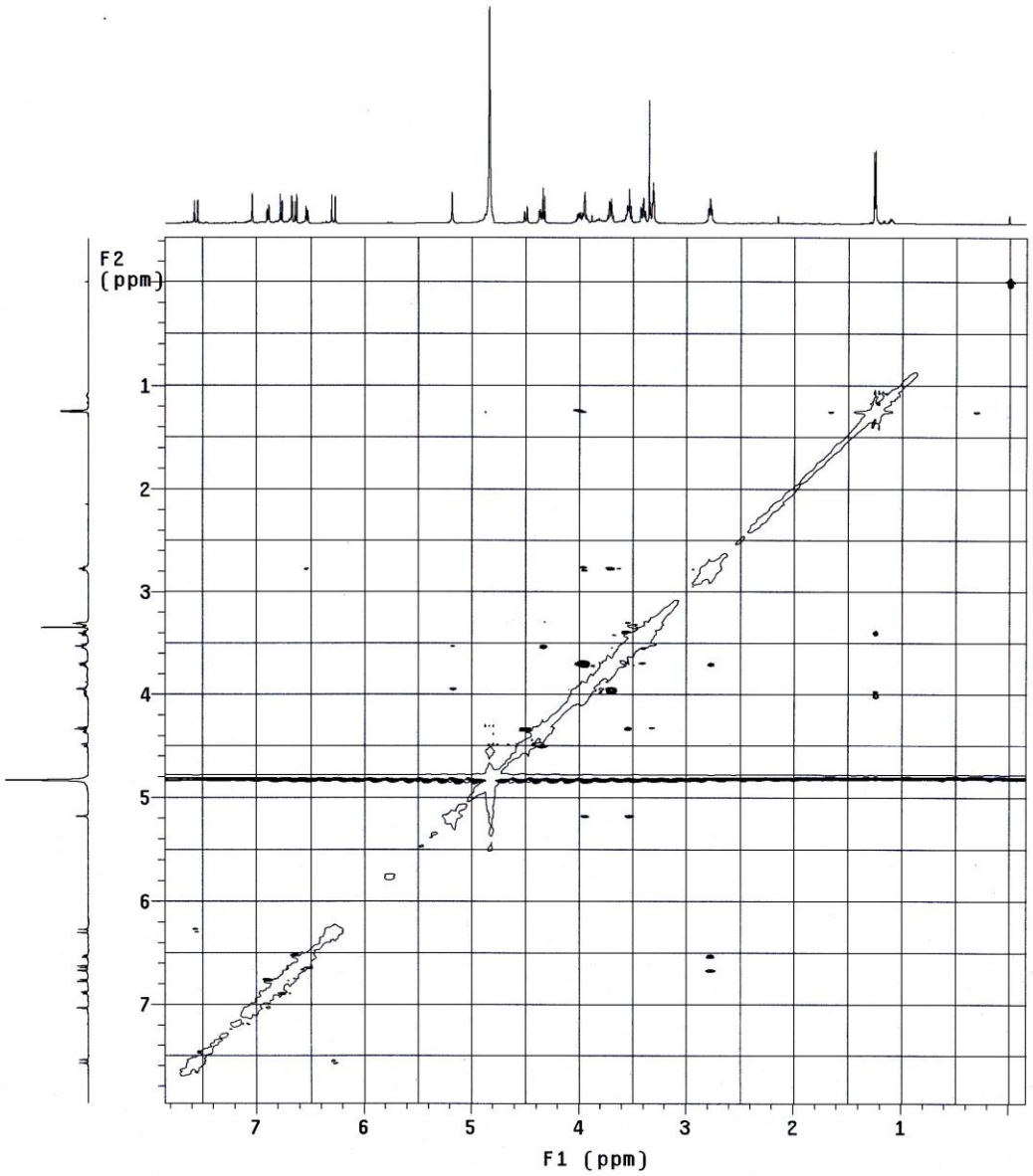

Figure A.46 NOESY spectrum of compound **BSET_6_2_5_2** (500 MHz, CD_3OD-d_4)

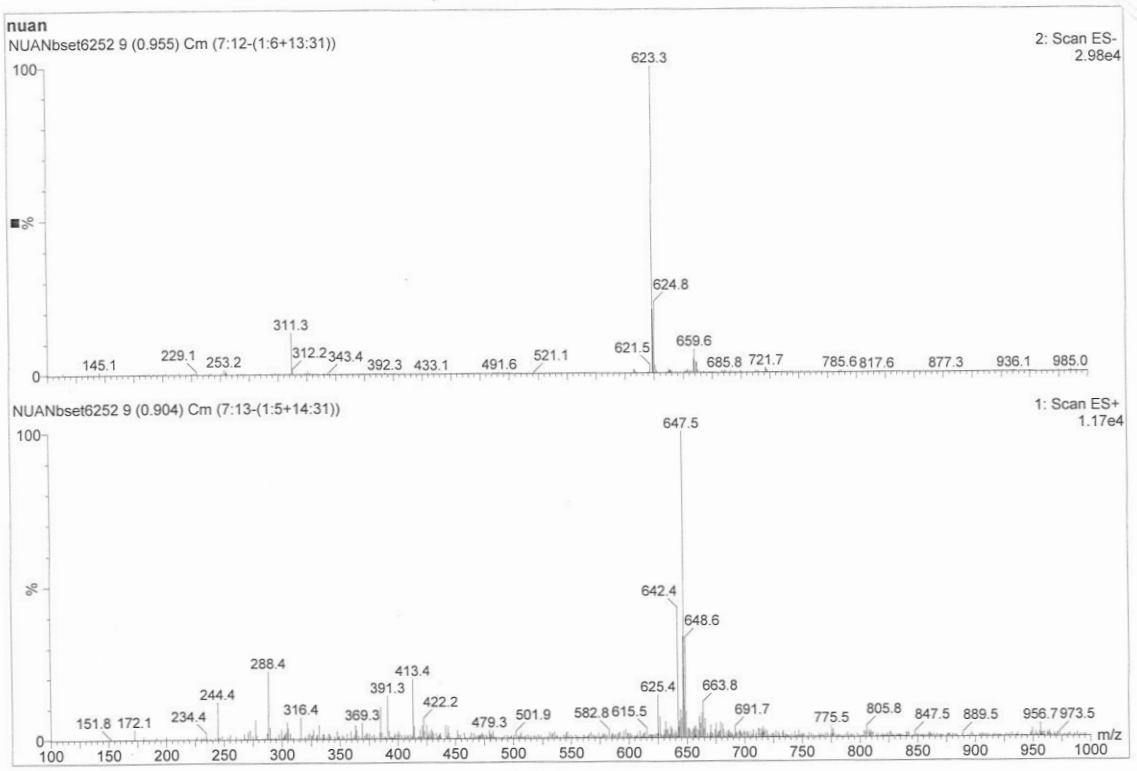

Figure A.47 Mass spectrum of compound **BSET_6_2_5_2**

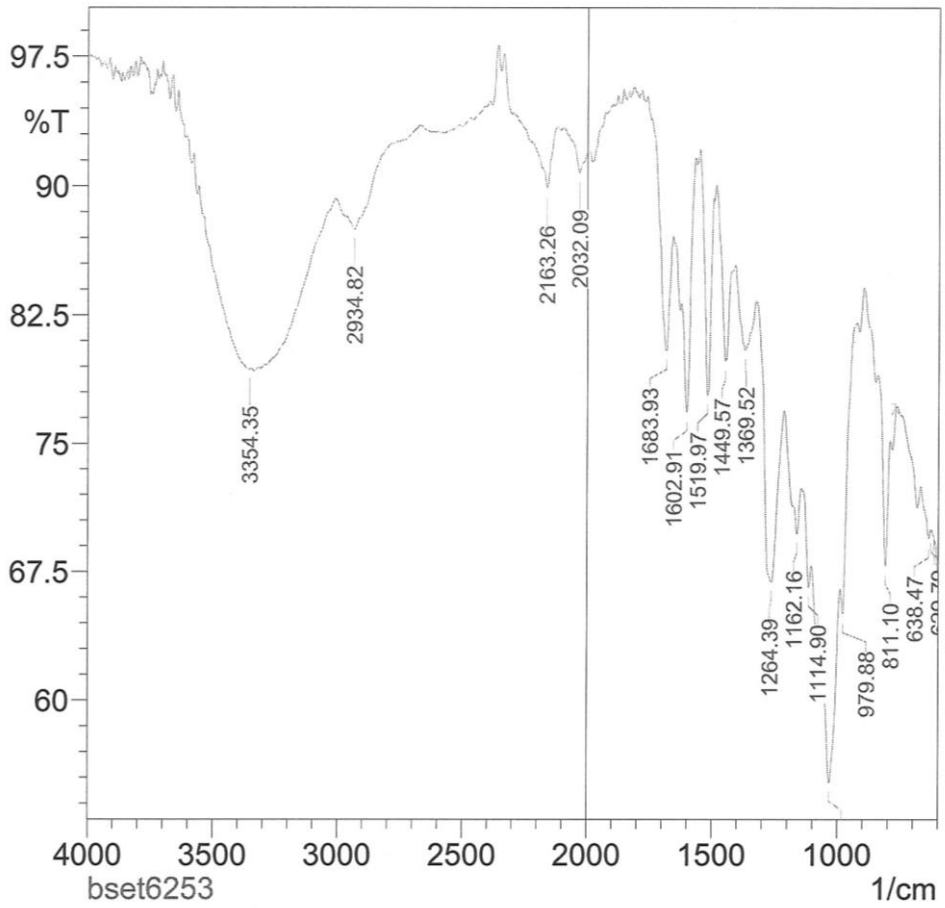

Figure A.48 IR spectrum of compound **BSET_6_2_5_2**

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