BIOLOGICAL ACTIVITIES OF MALE BUD EXTRACTS OF *Musa sapientum* LINN. (*Musa* ABB GROUP CV. 'NAM WA')

BY

MISS SOMJET KHONGKHON

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN APPLIED THAI TRADITIONAL MEDICINE

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OF *Musa sapientum* LINN.
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ABSTRACT

Musa sapientum Linn. (*Musa* ABB GROUP CV. 'NAM WA') is an affiliate of the MUSACEAE family and is ordinarily used in Thai traditional medicine and Thai health food as a preparation for postpartum breastfeeding care. The objectives of this research were to investigate the anti-oxidant activity by DPPH radical scavenging assay, determine total phenolic content and anti-inflammatory activity by NO inhibition from RAW264.7 cells. Thirty-one extract samples of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') male buds (blossom) were separated into two parts to use as dried and fresh. The dried part was dried in a hot air oven at 50 °C before use and the fresh was used as a fresh sample. The male buds (blossom) were divided into three parts. First, the outside of bracts from the male bud. Second, the flowers inside the bract with anthers and ovary in the flower. Last, the young male bud inside dark violet and red of male bud. There was extraction by decoction, maceration in 50% ethanol, residue 50% ethanol, maceration in 95% ethanol, residue of 95% ethanol and blending with water of fresh young male buds only. The ethanolic extracts were dried by rotary evaporator and water extracts were dried by freeze-dryer.

Among yields of the extracts, residue dry male flower maceration 50%EtOH (DMfr50) gave the highest yield (38.20%) followed by dry bract decoction

(DMbd) (34.83%) and residue dry young male bud maceration 95% EtOH (DMyr95) (25.97%).

Musa sapientum Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts were studied for anti-oxidant activities by DPPH radical scavenging assay. Fresh male flower decoction (FMfd) showed the highest DPPH radical scavenging, followed by fresh young male bud decoction (FMyd) and dry male flower decoction (DMfd) with EC_{50} values 5.78, 6.28 and 10.60 µg/ml, respectively. The positive standard was used BHT (Butylated hydroxytoluene) was tested in the same manner with EC_{50} values 14.12 µg/ml.

The content of total phenolic compounds ranged widely from 2.31-269.31 mgGAE/g (crude extract). Fresh male flower decoction (FMfd) showed the highest total phenolic contents, followed by fresh young male bud decoction (FMyd) and dry male flower decoction (DMfd) with values 269.31, 201.16 and 146.85 mg GAE/g (crude extract), respectively.

For anti-inflammatory activity of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts was tested by determining their effect on inhibition of LPS induced NO production by macrophages RAW 264.7 cell lines. Dry male flower decoction (DMfd) showed the highest potency for NO inhibitory activity, followed by dry young male bud decoction (DMyd) and residue dry male flower maceration 50% EtOH (DMfr50) with IC_{50} values 5.98, 20.91 and 31.17 µg/ml, respectively. The positive standard was used prednisolone with IC_{50} values 0.16 µg/ml.

The stability of the water extract of dry male flower decoction (DMfd) was investigated under accelerated condition at 40±2 ºC with 75±5% RH for six months. After that, this extract was tested for anti-oxidant activity by DPPH radical scavenging assay, total phenolic contents and anti-inflammatory activity by NO inhibition from RAW264.7 cells. Dry male flower decoction (DMfd) was showed DPPH radical scavenging assay, total phenolic contents of property and anti-inflammatory activity by NO inhibition which after Day 30 showed poor (40±2ºC, 75±5% RH for 6 months).

Bioassay guided fractionation was used to isolate active compounds from the water extract of dry male flower decoction (DMfd) by using column chromatography found pure compound which elucidated structure to be

(2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol. It showed no active in NO inhibitory activity and DPPH radical scavenging assay.

The finding indicates that the dry male flower decoction (DMfd) extract showed the highest anti-inflammatory activity but main active components of (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol did not anti-inflammatory effect. Thus, the decoction extracts should be studied extensively in animal models for anti-inflammatory activity and continuously isolated active anti-inflammatory compounds because the decoction extract of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') as food method and it has the potential for development as a health food to prevent many diseases causes from inflammation.

Keywords: *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA'), Male bud, Anti-oxidant activity, Anti-inflammatory activity

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บทคัดย่อ

หัวปลีกล้วยน้ำว้า *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') อยู่ในวงศ์ MUSACEAE ส่วนของหัวปลีอ่อนนิยมนำมาเป็นส่วนประกอบในอาหารและเป็นส่วนประกอบของยาพื้นบ้านของประเทศไทยเพื่อใช้ในการขับน้ำนม,บำรุงน้ำนมในมารดาหลังคลอด วัตถุประสงค์ในการศึกษาครั้งนี้ เพื่อศึกษาฤทธิ์ต้านอนุมูลอิสระโดย DPPH radical scavenging assay, วิเคราะห์ปริมาณสารกลุ่มฟีนอลรวมและฤทธิ์ต้านการอักเสบโดยการยับยั้งการหลั่งไนตริกออกไซด์ของเซลล์ RAW 264.7 โดยนำหัวปลีของกล้วยน้ำว้ามาสกัดได้เป็น 31 ตัวอย่าง แบ่งเป็นสมุนไพรสดและสมุนไพรแห้ง จากนั้นแยกหัวปลีกล้วยน้ำว้าเป็น 3 ส่วน คือ กาบหัวปลี, หัวปลีอ่อน และดอกหัวปลี แล้วนำไปสกัดด้วยวิธีการต้ม, หมักด้วย 50% เอทานอล, กาก 50% เอทานอลนำไปต้ม, หมักด้วย 95% เอทานอล, กาก 95% เอทานอลนำไปต้มและหัวปลีอ่อนสดนำมาปั่นแล้วกรองเอาแต่น้ำ สารสกัดชั้นเอทานอลจะนำไประเหยให้แห้งด้วยเครื่องระเหยแห้ง Rotary evaporator ส่วนสารสกัดชั้นน้ำจะทำให้แห้งด้วยวิธีการฟรีซดราย (Freeze-dryer)

พบว่ากากจากการหมักด้วย 50% เอทานอลของสารสกัดดอกหัวปลีแห้ง (DMfr50) ให้ปริมาณผลผลิต % yield สูงที่สุด (38.20%) รองลงมาคือ สารสกัดของกาบหัวปลีแห้งต้ม (DMbd) (34.83%) และ กากจากการหมักด้วย 95% เอทานอลของสารสกัดหัวปลีอ่อนแห้ง (DMyr95) (25.97%) นำสารสกัดทั้งหมดมาศึกษาฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH radical scavenging assay พบว่า สารสกัดของดอกหัวปลีสดต้ม (FMfd) มีฤทธิ์ในการต้านอนุมูลอิสระปฏิกิริยาออกซิเดชั่นสูงที่สุด รองลงมาคือ สารสกัดของหัวปลีอ่อนสดต้ม (FMyd) และ สารสกัดของดอกหัวปลีแห้งต้ม (DMfd) มีค่า EC_{50} เท่ากับ 5.78, 6.28 และ 10.60 µg/ml ตามลำดับ

ผลการศึกษาหาปริมาณสารในกลุ่มฟีนอลด้วยวิธี Folin-Ciocalteu's reagent ผลการศึกษาพบว่า หัวปลีกล้วยน้ำว้า มีปริมาณสารกลุ่มฟีนอลอยู่ในช่วง 2.31-269.31 mgGAE/g โดยสารสกัด ของดอกหัวปลีสดต้ม (FMfd) มีปริมาณสารในกลุ่มฟีนอลสูงที่สุด รองลงมาคือ สารสกัดของหัวปลีอ่อนสดต้ม (FMyd) และ สารสกัดของดอกหัวปลีแห้งต้ม (DMfd) มีค่าเท่ากับ 269.31, 20.116, 146.85 mgGAE/g ตามลำดับ

ผลการศึกษาฤทธิ์ต้านการอักเสบ โดยการยับยั้งการหลั่งไนตริกออกไซด์ในเซลล์ RAW 264.7 พบว่า สารสกัดของดอกหัวปลีแห้งต้ม (DMfd) มีฤทธิ์ในการยับยั้งการหลั่งไนตริกออกไซด์สูงที่สุด รองลงมาคือ สารสกัดของหัวปลีอ่อนแห้งต้ม (DMyd) และกากจากการหมักด้วย 50% เอทานอลของสารสกัดดอกหัวปลีแห้ง (DMfr50) มีค่า IC_{50} เท่ากับ 5.98, 20.91 และ 31.17 µg/ml ตามลำดับ

สารสกัดของดอกหัวปลีแห้งต้ม (DMfd) มีฤทธิ์ต้านการอักเสบดีที่สุด และมีฤทธิ์ต้านอนุมูลอิสระที่ดี จึงเลือกสารสกัดของดอกหัวปลีแห้งต้ม (DMfd) มาทดสอบความคงตัวและหาสารที่เป็นองค์ประกอบ เมื่อทำการทดสอบความคงตัวภายใต้สภาวะเร่ง อุณหภูมิ 40±2 ºC ความชื้นสัมพัทธ์ 75±5% เป็นเวลา 6 เดือน แล้วนำมาทดสอบฤทธิ์ต้านการอักเสบ ฤทธิ์ต้านอนุมูลอิสระ และปริมาณสารกลุ่มฟีนอลิก พบว่าหลังจากเก็บรักษานาน 30 วัน สารสกัดของดอกหัวปลีแห้งต้ม (DMfd) มีฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ต้านการอักเสบลดลง

การแยกองค์ประกอบทางเคมีของสารสกัดของดอกหัวปลีแห้งต้ม (DMfd) โดยอาศัย Bioassay guided fractionation ทำการแยกสารด้วยคอลัมน์โครมาโทกราฟี ได้สารบริสุทธิ์ชื่อ (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol เมื่อนำมาทดสอบฤทธิ์ทางชีวภาพ พบว่าไม่มีฤทธิ์ต้านการอักเสบด้วยวิธีไนตริกออกไซด์และไม่มีฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH radical scavenging

สรุปได้ว่าสารสกัดของดอกหัวปลีแห้งต้ม (DMfd) ของกล้วยน้ำว้า *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') มีฤทธิ์ต้านการอักเสบดีที่สุด แต่สารที่แยกได้ คือ (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol ไม่มีฤทธิ์ต้านการอักเสบ ดังนั้นจึงควรมีการนำสารสกัดของดอกหัวปลีแห้งต้มของกล้วยน้ำว้า *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') มาศึกษาฤทธิ์ต้านการอักเสบทางคลินิกหรือในสัตว์ทดลอง และควรมีการศึกษาต่อเพื่อนำมาแยกหาสารบริสุทธิ์ที่เป็นสารสำคัญที่ออกฤทธิ์ต้านการอักเสบของสารสกัดดอกหัวปลีต้มซึ่งนำมาใช้เป็นอาหารและมีศักยภาพพัฒนาเป็นผลิตภัณฑ์อาหารสุขภาพที่ใช้ป้องกันโรคที่เกิดจากการอักเสบต่อไป

คำสำคัญ: หัวปลีชนิด *Musa sapientum* Linn. (*Musa* ABB GROUP CV. ‘NAM WA’), หัวปลี, ฤทธิ์ต้านอนุมูลอิสระ, ฤทธิ์ต้านการอักเสบ

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
%	Percent
&	And
/	Per
<	Less than
≤	Less than or equal
>	More than
≥	More than or equal
=	Equal
BHT	Butylated hydroxytoluene
$CHCl_3$	Chloroform
cm	Centimeter
cm^2	Square centimeters
CO_2	Carbon dioxide
conc.	Concentrated
DMSO	Dimethylsulfoxide
DPPH	1,1-Diphenyl-2-picrylhydrazyl
EC_{50}	Concentration causing 50% effective activity
et al.	Et alii, and colleagues
EtOH	Ethanol
FBS	Fetal bovine serum
g	Gram
g/ml	Gram per milliliter
h	Hour
H_2O	Water
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid

LIST OF ABBREVIATIONS (CONTINUED)

Symbols/Abbreviations	Terms
IC_{50}	Concentration of extract that causes a 50% growth inhibition compared with control
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
m	Meter
M	Molar or mole per liter (concentration)
MCF-7	Human breast cancer cell line
MeOH	Methanol
mg	Milligram
mg/g	Milligram per gram
mg/ml	Milligram per milliliter
min	Minute
ml	Milliliter
mm	Millimeter
mM	Millimolar
MTT	Thiazolyl blue tetrazolium bromide or 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl2H-tetrazolium bromide
N_2O_3	Nitrous anhydride
Na_2CO_3	Sodium carbonate
ng	Nanogram
ng/ml	Nanogram per milliliter
NMR	Nuclear magnetic resonance
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NO_2^-	Nitrite

LIST OF ABBREVIATIONS (CONTINUED)

Symbols/Abbreviations	Terms
• NO_2	Nitrogen dioxide
NOS	Nitric oxide synthase
O_2	Oxygen
ºC	Degree Celsius
OD	Optical density
P/S	Penicillin-Streptomycin
PBS	Phosphate buffered saline
pH	Log concentration of (H^+)
ppm	Parts per million
RAW 264.7	Murine macrophage leukemia
RH	Relative humidity
RNS	Reactive nitrogen species
RO•	Alkoxyl
ROO•	Peroxyl
ROS	Reactive oxygen species
rpm	Revolutions per minute
RPMI 1640	Roswell Park Memorial Institute 1640
SD	Standard deviation
SEM	Standard error of mean
std	Standard
TLC	Thin layer chromatography
UV	Ultraviolet
w/w	Weight per weight
α	Alpha
β	Beta
µg	Microgram
µl	Microlitre

LIST OF ABBREVIATIONS (CONTINUED)

Symbols/Abbreviations	Terms
μg/ml	Microgram per milliliter

CHAPTER 1
INTRODUCTION

1.1 Background

Maternal milk is vital to growth and to enhancing long-term health of the child. The breastfeeding mother also makes a positive impact on her own health and that of her child. The World Health Organization has recommended that birth mothers be breastfeeding mothers until infants reach 6 months without eating anything else (WHO and UNICEF, 1990) . The Ministry of Public Health in the 4th Thailand National Economic and Social Development plan recommended that infants drink their mother's milk from birth onwards. In the 9th plan, the policy stated that infants are to drink their mother's milk for at least the first 6 months. Currently, women have a problem with insufficient milk production. There is not enough milk. Increasing milk production is a top priority. This is consistent with studies of Gatti (Gatti, 2008), who found that 3 5 percent of postpartum women stopped breastfeeding during the first week after birth. This has resulted in postpartum women producing too little milk for the child. Solutions include monitoring the initial breastfeeding posture which should be practiced properly. The frequency of breastfeeding should be increased to once every hour or more often in order to stimulate the flow of breast milk. Also switching the baby to the breast on both sides when breast-feeding each time for continuous stimulation of breast milk flow. Mothers should have enough rest. At present, Thailand has brought the wisdom of the ancients by cooking banana blossom to help production. Male bud (blossom, banana flower) has long been used as a component in Thai functional food and traditional medicine. It is also used in postpartum care for breastfeeding and helps maintain health. Lactation is the most important element in breastfeeding. Early breast development in girls is stimulated by the hormones estrogen and progesterone which produce mammary ducts and supporting tissues. During pregnancy there is an increase in hormones, namely estrogen, progesterone, human placental lactogen and prolactin to stimulate breast growth. The size and number of mammary ducts increases. Estrogen and progesterone

hormone will inhibit lactation (lactogenesis) until postpartum when they are reduced to normal levels because the placenta is eliminated. The production and secretion of milk starts when the baby begins to suckle. Ways to prevent breastfeeding problems that fast of breastfeeding, frequency of breastfeeding, correct posture during breastfeeding complete elimination of breast milk (Thai Breastfeeding Center Foundation, 2016).

Free radicals are the reactive oxygen species (ROS) and reactive nitrogen species (RNS) a cause many pathological conditions including aging, atherosclerosis and inflammatory diseases (Bor *et al*., 2006). Oxidative stress results from a difference between the generation of ROS, free radicals and anti-oxidants to scavenge them. Anti-oxidants prevent first-chain of initiation or chain-breaking by scavenging of free radicals. Overproduction of RNS is associated with oxidative stress and chronic inflammation involved in pathophysiology of various diseases such as inflammation and atherosclerosis. Nitric oxide (NO) is derived from peroxynitrite ($ONOO^-$), which is produced from NO and superoxide and are the main sources of RNS *in vivo*. Nitric oxide has several biological roles as well as modulation of vascular tone, memory formation and inflammation (Darley-Usmar *et al*., 1995)

The inflammatory response has several characteristic features and is a complex process that includes activation of monocytes, granulocytes and lymphocytes that release inflammatory mediators, humoral mediators and complementary systems. The inflammation process begins with stimulus causing release of prostaglandins from cells such as lipopolysaccharides. These can induce nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) to produce NO and symptoms of injury agents, infection, burn etc. The inflammation brings restoration of homeostasis which leads to interaction between macrophage and lymphocyte with activation and release of inflammatory mediators. Phagocytic cells and macrophages are concerned in immunopathological disorders related to oxidative stress, diseases and inflammation. Macrophages are sensitive to changes in the oxidant and anti-oxidant balance, because they produce ROS and RNS as part of their normal function. So, macrophages are a good model system for studying the NO inhibitory activity (Bor *et al*., 2006).

Since breastfeeding mitigates oxidant and inflammatory conditions, this study aims to investigate the anti-oxidant and anti-inflammatory effect of male bud which helps to increase breast milk postpartum. The researchers were banana keen to study the biological activity of male bud for use in women after childbirth, especially anti-inflammatory, anti-oxidant activities. Results will serve to guide further study.

1.2 Research question

1.2.1 Is there any antioxidant activity in male bud extracts of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA')?

1.2.2 Is there any anti-inflammatory activity in male bud extracts of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA')?

1.2.3 Are there active compounds to isolate from *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') for anti-inflammatory activity?

1.2.4 How stable are male bud extracts?

1.3 Aims of this study

1.3.1 Overall aims

This research is to investigate the antioxidant and anti-inflammatory activities in male bud extracts of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA').

1.3.2 Specific aims

1.3.2.1 To study the antioxidant activity of male bud extracts.

1.3.2.2 To study the anti-inflammatory activity of male bud extracts.

1.3.2.3 To study the isolated compounds from *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA').

1.3.2.4 To study the stability of male bud extracts.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Literature Review

Male bud Young male bud

Figure 2.1

Musa (ABB GROUP) (MUSACEAE)

2.1.1 Botanical characteristics

Scientific name : *Musa sapientum* Linn.

Family : MUSACEAE

English name : Male bud, banana blossom, banana flower

Genome : ABB Group

Thai name : Huaplee

Strain : Khao nuan

Descriptions:

The male bud is an inflorescence appearing at any time of the year. It is enclosed by many curved bracts, which are reddish brown in color. The male bud has an ovary in the base of the flower and has anthers in the top of the flower. The male bud grows from the top of the parent plant like a dome. The bracts unfold from the base to the top and fall off (Tepsorn, 2009).

2.1.2 Uses

In Indian folk medicine *Musa sapientum* Linn. has been used to treat diabetes mellitus because the unripe pulp has tannin (Matsuo and Itoo, 1981)**,** used to treat diarrhea (Reynolds, 1989), leucocyanidins are part of flavonoid group (Lewis *et al*., 1999), used to treat peptic ulcers (Goel *et al*., 2001.; Pannangpetch *et al*., 2001) Unripe pulp and unripe peel has been used for wound healing (Agarwal *et al*., 2009.; Phuaklee, 2012). Ripe pulp has been used as a laxative, root used to treat muscle sprains, burns and scald. Banana sap has been used for haemostasis, to treat thrush from moniliasis, to treat insect stings and female flowers have been used for post partum care. Fruit peel has been used as an antibacterial agent and astringent. Leaves have been used to protect from allergies and to prevent burns (Sabchalern, 2005.; Yuenyongsawat and Kongthong, 1994.; Panyoyai, 2008.; The National Archives of Thailand, 1999).

2.1.3 Phytochemical screening tests of *Musa sapientum* Linn.

Phytochemicals found as constituents in *Musa paradisiacal* flowers include alkaloids, tannins, saponins, glycosides, flavonoids, steroids (Mahmood, 2011) and anthocyanins have been found as constituents in *Musa X paradisiaca* bracts (Alexandra Pazmiño-Duran *et al*., 2000).

2.1.4 Biological activities of *Musa sapientum* Linn.

2.1.4.1 Anti-oxidant activity

Decoction extract of the fresh flower showed the EC_{50} was 5.16±0.34 µg/ml, followed by the fresh flower water extract (24-hr soaking) having the EC_{50} of 5.82±0.28 µg/ml (Phuaklee, 2012). The decoction extract of the fresh bract had EC_{50} of 18.92±1.51 µg/ml, followed by the water extract of the fresh bract with EC_{50} of 33.06±3.18 µg/ml (Itharat and Sakpakdeejaroen, 2010).

2.1.4.2 Total phenolic contents

The water extract of the fresh flower had total phenolic contents of 218.75 mg GAE/g, followed by the fresh flower decoction extract with 185.86 mg

GAE/g (Phuaklee, 2012). The fresh bract of the decoction extract had total phenolic contents of 73.00 ±0.45 mg GAE/g, (Itharat and Sakpakdeejaroen, 2010).

2.1.4.3 Anti-inflammatory activity

The water extract of the dried flower exhibited NO-inhibitory activity of IC_{50} of 9.75±0.93 µg/ml. The cytotoxic effects of extracts were investigated using MTT assay (Phuaklee, 2012). The dried bract of the 95% ethanol extract exhibited NO-inhibitory activity with IC_{50} of 93.93±3.88 µg/ml. The cytotoxic effects of extracts were determined using MTT assay (Itharat and Sakpakdeejaroen, 2010).

2.1.4.4 Others

Musa x paradisiaca (*M. x paradisiaca*) has been tested for galactagogue activity. Dried flower aqueous extract produced more milk in treated rats than control and ethanol groups. Aqueous extract increased milk production by 25%, petroleum ether extract by 18%. The aqueous, petroleum ether, ethanol and control extracts showed 4.62±2.45, 4.37±1.93, 3.65±1.89 and 3.69±1.79 g/pub/day, respectively (Mahmood *et al*., 2012).

The 95% ethanol extract of dried flowers of the ‘Khao nuan’ variety showed the highest *in vitro* anticancer effect against lung cancer cells with IC_{50} of 4.14 µg/ml. The fresh flower decoction extract of ‘Khao nuan’ showed the highest inhibition on HIV-1integrase with IC_{50} of 8.7µg/ml. The peel from the end of flower stalk exhibited the highest inhibition on HIV-1 protease with IC_{50} of 26.3 µg/ml. The fresh flower extract by 95% ethanol showed the highest anti-allergic activity by inhibitory effects on the release of β-hexosaminidase with IC_{50} of 15.587µg/ml. The fresh flower extract by 95% ethanol of the ‘La-ong nam’ variety showed the highest anti-acetylcholinesterase activity % inhibition of 5.53±1.85 µg/ml. The fresh flower by decoction extract showed the highest MIC by Disc diffusion against *S.aureus* of 7.00±0.00 1,250 µg/ml (Itharat and Sakpakdeejaroen, 2010).

2 . 1 . 5 Causes and problems in breastfeeding (National Statistical Office, 2006)

- Working 33.15%
- Food supplementary 19.10%
- No breastfeeding 11.31%
- Mother has health problem 5.64%
- Child has health problem 2.85%
- Husband and family do not support 1.03%

2.1.6 Free radicals

2.1.6.1 Overview

Free radicals are the result of an atomic orbital of any group of atoms or an atom that contains one or more unpaired electrons (Fang *et al*., 2002). They can give an electron or take an electron from other molecules. The action of oxidants reductants cause high reactivity, appointing of other free radicals (Lobo *et al*., 2010). The main effect of free radicals is damage to biological systems. There are many types of freeradicals are which formed inside the body. The free radicals containing oxygen are superoxide anion radical, hydroxyl radical, oxygen singlet, hydrogen peroxide, hypochlorite, peroxynitrite radical and nitric oxide radical. The highly reactive species can enter the nucleus and membranes of cells which damages all components of cell containing proteins, lipids and DNA. This lead leading to several chronic diseases such as Alzheimer's disease, aging diabetes, Parkinson's disease, cancer, atherosclerosis, cardiovascular diseases and inflammatory diseases (Fernández, 2009).

Table 2.1 Reactive radical species (Cadenas and Packer, 2002)

Radicals	Nonradicals
Reactive oxygen species (ROS)	
Hydroxy, $OH^{\bullet}$	Hydrogen peroxide, H_2O_2
Hydroperoxyl, $HO_2^{\bullet}$	Hypobromous acid, HOBr
Lipid alkoxyl, $LO^{\bullet}$	Lipid peroxide, LOOH
Lipid peroxyl, $LO_2^{\bullet}$	Ozone, O_3
Superoxide, $O_2^{-\bullet}$	Singlet oxygen, O_2^{-}
Reactive nitrogen species (RNS)	
Nitric oxide, $NO^{\bullet}$	Nitrosyl anion, NO^{-}
Nitrogen dioxide, $NO_2^{\bullet}$	Peroxynitrite, $ONOO^{-}$

2.1.6.2 Source

The endogenous and exogenous cellular factors produce reactive oxygen species and their products. Endogenous sources include cytochrome P450, peroxisomes, mitochondria and active inflammatory cells activation (Valko *et al*., 2006). Mitochondria initiate quantities of H_2O_2 that use about 90% of cellular O_2. The process of mitochondrial action reduces oxygen for production of water, $O_2^{-\bullet}$, H_2O_2, $OH^{\bullet}$ and several short-lived groups. $O_2^{-\bullet}$ and $OH^{\bullet}$ are poisonous to cells. The cause of cell annihilation is free radical generation (Wickens, 2001). Other sources of endogenous cellular ROS are macrophages, neutrophils and eosinophils. Macrophages are activated by respiratory burst and an increase in oxygen uptake and give rise to types of ROS, such as H_2O_2, NO and O_2 (Conner and Grisham, 1996). Free radicals from exogenous sources are pollutants (ozone, cigarette smoke, vehicle exhaust, heavy metals, industrial exhaust and pesticides), radiations (UV, X-rays and gamma-rays), drugs (doxorubicin, gentamycin, tacrolimus, bleomycin, vinblastine and cyclosporine), chemotherapeutic agents (quinones), dietaries (alcohol, fat, oils and smocked meat), aromatic hydroxylamines, aromatic intro compounds and pesticides (e.g.paraquat). In of sources in free radicals, enzymatic and nonenzymatic metabolisms have the ability to cause oxidative damage on a wide range of biological macromolecules (Mateos and Bravo, 2007).

Table 2.2 Reactive oxygen and nitrogen species generation in the cell (Bolisetty and Jaimes, 2013)

Cellular oxidants	Source	Oxidative species
Endogenous	Mitochondria	O_2^-, H_2O_2, OH
	Cytochrome P450	O_2^-, H_2O_2
	Macrophage/inflammatory cells	O_2^-, H_2O_2, $^{\bullet}NO$, OCl^-
	Peroxisomes	H_2O_2
Exogenous	Redox cycling compounds	O_2^-
	Metals (Fenton reaction)	$^{\bullet}OH$
	Radiation	$^{\bullet}OH$
	Ultraviolet light	
	Environmental pollutants	

Table 2.3 Pathways of intracellular oxidant generation (Valko *et al*., 2007)

1.Generation of ROS via reduction of molecular oxygen
$O_2 + e^- \longrightarrow O_2^-$ (superoxide anion)
$O_2^- + H_2O \longrightarrow HO_2^{\bullet}$ (hydroperoxyl radicals)
$H_2O + e^- + H \longrightarrow H_2O_2$ (hydrogen peroxide)
$H_2O_2 + e^- \longrightarrow HO^- + {}^{\bullet}OH$ (hydroxyl radical)
2.Production of RNS
L-ARGININE + $O_2 \longrightarrow {}^{\bullet}NO$ (nitric oxide) + L-CITRULLINE
$O_2^{-\bullet} + {}^{\bullet}NO \longrightarrow$ ONOO-(peroxynitrite)
ONOO- + $CO_2 \longrightarrow ONOOCO_2^-$ (nitrosoperoxy carbonate)
$ONOOCO_2^- \longrightarrow NO_2^{\bullet}$ (nitrogen dioxide) + $CO_3^{-\bullet}$
3.Fenton reaction
$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^-$

2.1.7 Oxidative stress

Oxidative stress is caused by an imbalance between production and elimination of oxidants that cause many degenerative diseases such as cancer, coronary heart disease, diabetes, rheumatoid arthritis and cataracts (Cooke *et al*., 2003). Oxidative stress damages of many biomolecules such as lipids, proteins and

DNA. It is not clear that the first point of attack which injury of mechanisms for overlap widely. DNA is an important in first target of damage (Guetens *et al*., 2002).

2.1.7.1 Lipid peroxidation

Lipid peroxidation is a three-stage process which polyunsaturated fatty acids pass through. The initiate step is propagation step to finally of termination step. The propagation step is specific damage begins since it concerns a chain reaction until the final step of termination occurs.

(1) Initiation

Lipid peroxidation on biomembranes is introduced by interaction of sufficient reactive oxidant for instance hydroxyl radical ($HO^{\bullet}$) and fatty acid (RH) to produce a fatty alkyl of a free radical.

$$RH + HO^{\bullet} \longrightarrow R^{\bullet} + H_2O$$

(2) Propagation

The fatty alkyl of free radical ($R^{\bullet}$) reacts vary quickly with molecular oxygen to form of a fatty peroxyl radical ($ROO^{\bullet}$). This species has enough oxidizing power to attack an adjoining of unsaturated fatty acid (RH) in the membrane to produce of hydroperoxides and a new alkyl radical ($R^{\bullet}$).

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

(The reaction of alkyl radical in O_2)

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$$

(The reaction of peroxyl radical in nearly fatty acid)

The development of autocatalytic cycle or chain reaction begins a process that will spread until the free radical chain terminates.

(3) Termination

The autocatalytic chain is eventually terminates by the conflict of two radical species to react with nonradical products. The success of the termination reaction outlined depends on concentration of intracellular oxygen.

$$R^{\bullet} + R^{\bullet} \longrightarrow R\text{-}R$$

$$R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR$$

$$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOOOR$$

Oxidative degradation in biomembranes of lipoproteins are noviced of complex cascade event which formation of reactive, unstable oxidants, long lived of toxic or biologically active of inflammatory mediators. These are capability of spreading damage further the cell in question. Free radicals cause the destruction of unsaturated fatty acids in the lipoproteins of the membranes. These are important function changes which result in cell loss of function or cell death (Srikwan, 2013).

2.1.8 Anti-oxidant

The antioxidant is any substance that when show at low concentrations compared become oxidizable substrate and preclude oxidation of substance (Kohen and Hyska, 2002). The term of oxidation in substance is consisting of lipid, proteins, carbohydrates and DNA. The antioxidant in the body are classified to four type based of function.

2.1.8.1 Prevention

These are keeping formation in reactive species of minimum (enzyme: selenoprotein, catalase, transferring, ferritin, peroxidase, glutathione, lactoferrin, carotenoids etc.).

2.1.8.2 Interception

The antioxidants are scavenged of reactive species to suppress of chain induction and destroy of chain propagation reactions by using catalytic and non-catalytic.

2.1.8.3 Repair

These repair target molecules in damaged cells.

2.1.8.4 Adaptation

These are the signals of reactions and production of free radicals which induce the transport and formation of suitable antioxidants to the right sites.

An antioxidant describes the actions of radical scavenger, electron donor, hydrogen donor, enzyme inhibitor, synergist, peroxide decomposer, singlet oxygen quencher and metal-chelatating agents. Both enzymatic and nonenzymatic in antioxidant are present in the intracellular and extracellular environment to detoxify the ROS (Frie *et al*., 1988).

Reactive oxygen species (ROS) reactive nitrogen species (RNS), include both radical and non-radical molecules with unpaired orbital electrons devived from nitrogen such as nitric oxide and peroxyl radical. Roles of ROS and RNS are killing foreign organisms and in acute inflammation. However, their over production many cause tissue damage and vascular leakage in septicaemia, rheumatoid arthritis and inflammatory bowel disease (Darley-Usmare *et al*., 1995). In addition, the interaction of ROS and RNS are lead to the highly reactive non-radical species for example the peroxynitrite, ordinarily generated by macrophages beneath pathological conditions and the product of nitric oxide and superoxide (Ischiropoulos *et al*., 1992).

Free radicals or oxidants are highly dangerous molecules and unstable. Oxidants are produced from many sources such as heavy metals, rancid fatty acids, pollution, smoke, UV radiation etc. Molecule oxygen is lead to electrons from another species after oxidizing agent. In good health of an aerobic organisms are produced of reactive oxygen species which approximately balanced by anti-oxidant defence system in the body. The endogenous of anti-oxidants can protect from harmful molecules. The body is development from own natural free radical

scavengers, anti-oxidant vitamins (vitamin C and E), anti-oxidant minerals (selenium and zinc) and anti-oxidant enzyme systems (superoxide dismutase and glutathione peroxidase). They are backbone of cellular anti-oxidant defence system (Itharat, 2003).

Anti-oxidant is a molecule capable of preventing the oxidation and slowing of the other molecules. Chemical reaction of oxidation can transfers electrons from substance of oxidizing agent. Oxidation reactions are produce free radicals of start chain reactions by damage cells. Anti-oxidant are terminate of chain reactions when removing free radical intermediates, inhibit other oxidation reactions from oxidized by themselves (Sies, 1997).

DPPH (1,1-diphenyl-2-picrylhydrazyl) is study of stable free radical which the paramagnetism conferred by odd electron. The solution of absolute ethanol is showed of deep violet color and showed a strong absorption at 520 nm. DPPH radical can affirm of electron or hydrogen radical come to be stable diamagnetic molecule that absorption vinishes. The result of decolorization is stomachiometric that the number of electrons raises. The solution showed pale violet color (Blois, 1958). DPPH solution has concentration of $6x10^{-5}$ M. The substance was tested by DPPH solution which gives rise to pale violet. It was showed this substance has an anti-oxidant effect which showed the mechanism of free radical scavenging activity. The follow assay of procedure was described by Yamasaki *et al*., 1994.

2.1.8.5 Endogenous antioxidants

(1) Superoxide dismutase (SOD)

The superoxide dismutases (SODs) include metal enzymes which are active of the rapid dismutation of $O_2^{-\bullet}$ to O_2 and H_2O_2. 10,000-fold are the rate of reaction higher than the spontaneous of dismutation. Then, H_2O_2 are generated is removed by catalase and glutathione systems.

$$2O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \text{ enzymic dismutation } 10^9\ M^{-1}\ S^{-1}$$

$$2O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2 \text{ non-enzymic dismutation } 10^5\ M^{-1}\ S^{-1}$$

The enzyme has been found in all aerobic organisms where it has a crucial role in the defense against of toxicity in oxygen species. This is generated to produce of many biological in oxidations. In mammalian tissues are differences to isoenzymes of SOD. The mitochondrial can divided in Mn^{2+} dependent and show in cytoplasm of Cu dependent. The report is showed an extracellular in Cu-Zn dependent of enzyme (Cristiana *et al*., 2014).

2.1.8.6 Exogenous antioxidants

(1) Phenolic compounds

The anti-oxidant in plant extracts are present in large part in phenolic compounds. The phenolic compounds are many, and are secondary in metabolites and show in high concentrations in vegetables, many fruits and beverages (wine, tea). Moreover, they are plentiful in agricultural products such as peanut skins, hulls, roots, grape seeds and many herbs. Phenolic compounds are reducing agents hydrogen donors and singlet oxygen of quenchers (Dutra *et al*., 2008). Phenolic compounds are present to terminate oxidation of reaction by participating through resonance steady in free radical forms that act of free radical scavengers (Flora, 2009). The simple of phenolics range are low molecular weight, derived polyphenols, large of single aromatic-ringed compounds and complex of tannins. These can be divided into two groups that flavonoids and non-flavonoids (Beecher, 2003).

(1.1) Flavonoids

Flavonoids are classified into flavanols or flavan-3-ols, flavones, flavonols, flavanones, isoflavones and anthocyanins (Crozier, 2006).

(1.2) Flavan-3-ols

Flavanols are complex subclass in flavonoids ranging from the simple (+)-catechin and isomer (-)-epicatechin into the oligomeric and straight chain polymeric of procyanindins or condensed tannins (Balentine *et al*., 1977).

(1.3) Flavonols

Quercetin is the most common flavonol, kaempferol, myricetin and isorhamnetin are found as o-glycosides. The conjugation occurs the most frequently at the 3 position of the C-ring, but replacement at the 7, 3′, 4′ and 5′ also occurs (Hermann, 1976).

(1.4) Flavones

Flavones have structure similar to flavonols, but they do not have hydroxylation in the C-ring for 3-position (Manach *et al*., 2004).

(1.5) Flavanones

The main of structural has two main of features characterize in the flavanones, but it is absenced of the C2-C3 double bond, and presence of the chiral centre at C_2. The general of flavononeses are glycosylated of disaccharide at position 7 and typically of neohesperidose that communication of bitter taste or rutinose, which is flavourless (Kannes *et al*., 2004).

(1.6) Isoflavones

The structures of isoflavones are diverged from other flavonoids in the orientation of the B ring. The most of isoflavones are daidzein and genistein (Scalbert and Williamson, 2000).

(1.7) Anthocyanidins

Anthocyanins are different of chemical forms which both coloured, colourless and according to pH. Anthocyanins are highly of unstable in the aglycone form (anthocyanidins), but they are resistant of light in plant and oxidation conditions which likely to appear in degradation. Degradation is precluded of glycosylation and glucose at position 3 (Crozier, 2003).

(2) Non-flavonoids

Non-flavonoid compounds are composed of one or two benzene rings and source of benzoic acid, cinnamic acid their specific of aldehydes.

(2.1) Stilbenes

Stilbenes are resveratrol (3, 5, 4′ - trihydroxystilbene) of phytoalexin that found in wine which one of the most widely phenolic studied. They are potential effects on human health. Stilbene is the δ-viniferin showed the result of oxidation by fungus (Vitrac *et al*., 2005).

(2.2) Phenolic acids and related compounds

Phenolic acids are derivative of gallic acid, ellagic acid and hydroxybenzoates (Gross, 1992). The base units of gallotannins are gallic acid. Gallic acid and ellagic acid are the both of subunits in the ellagitannins. Ellagitannins are usually in glucose that present in polyol and esters of hexahydroxydiphenic acid. When they are exposed to bases or acids, the ester bonds are hexahydroxydiphenic acid and hydrolysed of spontaneously rearranges forming in water-insoluble of ellagic acid (Srikwan, 2013).

2.1.9 Inflammatory activity

2.1.9.1 Inflammation

The inflammation was described a injured tissue, a localized, trauma, microbial of destroys and dilutes of injurious agent (Gallin and Snyderman, 1999). The part of inflammation is non specific immune response which showed reaction any type of physical injury in which the cardiac signs of inflammation can be described by increased blood flow, vasodilation, cellular influx, exalted cellular metabolism, extravasation of the fluids and liberate of soluble mediators (Ferrero-Miliani *et al*., 2006). Acute and chronic inflammations are classification based of duration on inflammation.

2.1.9.2 Acute inflammation

Acute inflammation is early or almost immediate response to tissue injury. It is nonspecific and evoked by any injury short of immediately lethal (Chandrasoma and Taylor, 1998) . Acute inflammation follows typical process in vascularized tissues where interstitial fluid and white blood cells accumulate on site of injury. Microvascular permeability increases early and leads to edema formation

during inflammation. Many other mechanisms are activated, contributing to the amplification of inflammatory response and tissue damage (Bucci *et al*., 2005) . Cardinal signs are redness, heat, swelling, pain and loss of the function in acute inflammation (Gallin and Snyderman, 1999). Heat and redness bring increased blood flow the inflamed area, swelling by accumulation of fluid and pain is the release of chemicals that stimulate nerve endings and loss of function which is caused a combination of factors (Chandrasoma and Taylor, 1998).

2.1.9.3 Chronic inflammation

Chronic inflammation is a prolonged process of a week to a month of in active inflammation with mononuclear cells. Attempts at healing occur simultaneously with tissue destruction (Kumar *et al*., 2005). Chronic inflammation is characterized by the presence of macrophages in the injured tissue. These cells are powerful defensive agents in the body, but the toxins released include reactive oxygen species which are injurious to the organism's own tissues as invading agents. The results in chronic inflammation are almost always accompanied by tissue destruction. Inflammation is mediated by complex pathophysiological process with a variety of signaling molecules produced by leukocytes, macrophages, mast cells etc. Macrophages produce key pro-inflammatory molecules like nitric oxide (Saha *et al*., 2004).

2.1.10 Biological significance of nitric oxide

Nitric oxide as and inflammatory mediator is found in many organs. Nitric oxide is involved as and inorganic free radical in various physiological and pathological processes such as vasodilation, acute or chronic inflammation of non-specific host defense in the organ systems (Aktan, 2004.; Tewtrakul and Itharat, 2007). NO of low concentration has been shown to have a role as neurotransmitter. NO production at high has been implicated to have a role in the pathogenesis of stroke, septic stroke and other inflammatory diseases (Kim *et al*., 2000) . NO is synthesized enzymatically by the oxidation of L-arginine by nitric oxide synthase on NOS family which become inducible iNOS or constitutive cNOS. NOS of three isoforms are encoded by distinct genes. NOS-I is known as brain NOS (nNOS) or

neuronal, can be found in high concentrations in some nonneuronal tissue and neuronal tissue. NOS-II is known as macrophage NOS or inducible NOS (iNOS), can be found in macrophages. Moreover, it also exists in vascular smooth muscle cells and in a variety of cell types or hepatocytes, fibroblasts and in epithelial cells. NOS-III is known as endothelial NOS (eNOS), which is identified in the enzyme which produces endothelium derived relaxing factor. Both NOS-I and NOS-III are often grouped together as constitutive NOS (cNOS and their activities are regulated by intracellular calcium concentration via calmodulin (Davis *et al*., 2001.; Hobbs *et al*., 1999.; MacMicking *et al*., 1997). NOS-II and inducible NOS (iNOS) are not present in resting cells but can be induced by immunostimulatory cytokines, bacterial products or infection in a number of cells, endothelium, mast cells, monocytes, hepatocytes, macrophages and smooth muscle cells. It can generate NO independently of intracellular calcium concentrations (Aktan, 2004.; Hobbs *et al*., 1999.; MacMicking, *et al*., 1997.; Tezuka *et al*., 2001; Makchuchit, 2010). The isoforms of nitric oxide synthase, major physiological functions and implications in various diseases are summarized graphically in Figrue 2.2.

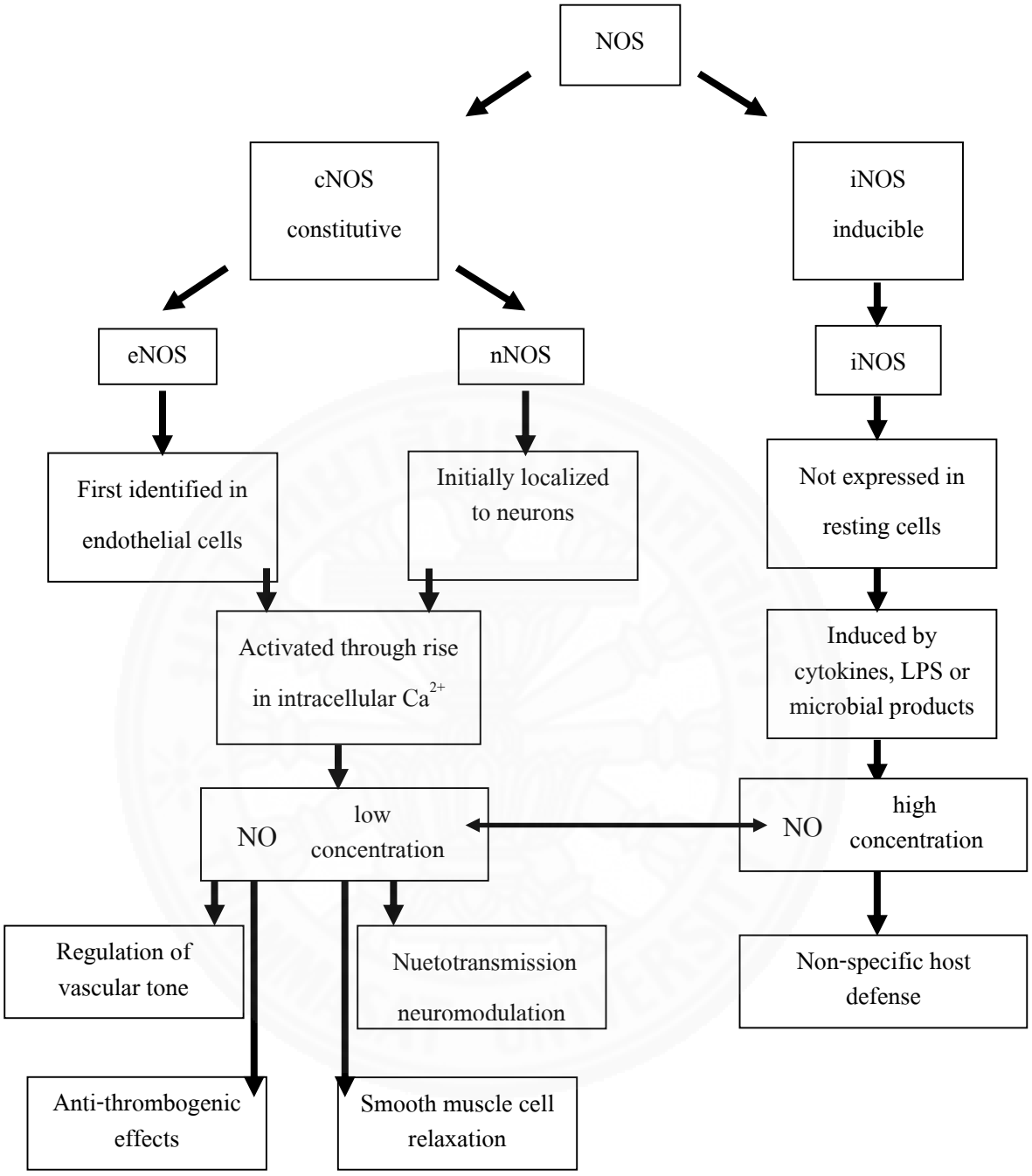


Figure 2.2

Isoforms of nitric oxide synthases, two cNOS of enzymes (eNOS, nNOS) are contrasted by a third that is inducible NOS (Wibuloutai, 2006.; Makchuchit, 2010).

The nitric oxide (NO) is produced by oxidation of L-arginin producing inducible nitric oxide synthase (iNOS) in cells (Tezuka *et al*., 2001). The whole reaction consists of two step oxidative conversion of L-arginine to NO and L-citrulline via N^{W}-hydroxy-L-arginine (NOHarginine) are intermediated by monooxygenase I and monooxygenase II. Each step is a model of mixed-function oxidation (Aktan, 2004).

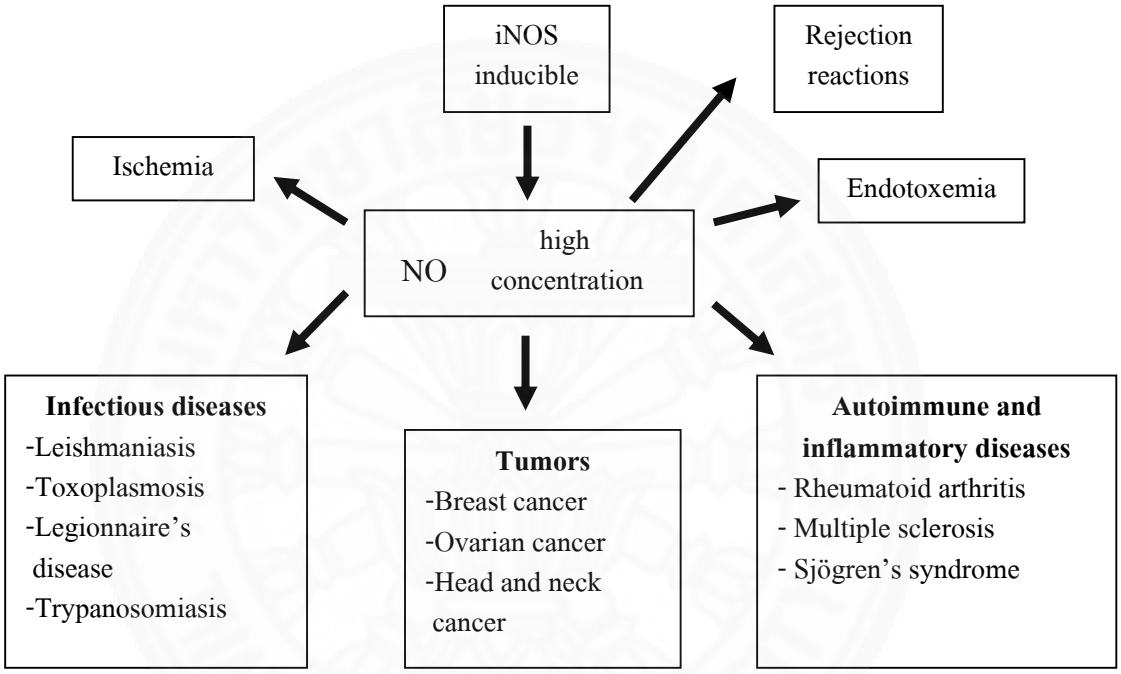


Figure 2.3

Various human diseases with implication of iNOS-derived NO. The key roles of NO are demonstrated in the human defense against adverse factors from environment. Also, several chronic inflammatory diseases are associated with sustained iNOS expression (Wibuloutai, 2006.; Makchuchit, 2010).

2.1.11 Monitoring of NO production by spectroscopic methods

The most common method of detecting NO from a wide variety of samples and matrices of diazotization assay is known as the Griess assay. The NO_2^- is reacted with sulfanilamide (SA) and *N*-(1 -Naphthyl) ethylenediamine Dihydrochloride (NED) under acidic (phosphoric acid) conditions to yield an azo dye, the indicator of NO_2^- and NO concentration in the sample. This method can be widely

used today (Hetrick and Schoenfisch, 2009) . After stimulation with lipopolysaccharide (LPS) , Gram-negative bacteria of major component out of membranes (Kim *et al*., 2004) , the iNOS was expressed to many cells including macrophages which is responsible for the production of large many NO. This inducible enzyme is part of essential components of inflammatory response and implicated in pathogenesis of several inflammatory diseases (Saha *et al*., 2004). The Griess assay is used to determine NO metabolites in RAW 264.7 cells induced by LPS. The Griess reaction in first reacted NO_2^- with sulfanilamide (SA) under acidic conditions to form a diazonium salt intermediate. The diazonium salt intermediate then is coupled to *N*-(1-Naphthyl) ethylenediamine dihydrochloride (NED) to form the stable water-soluble azo dye. The NO_2^- concentration is determined by comparing the absorbance in the azo dye solution to calibration curve prepared with known concentrations of NO_2^- (Ketrick and Schoenfisch, 2009.; Makchuchit, 2010).

CHAPTER 3
RESEARCH METHODOLOGY

3.1. Materials

3.1.1 Plant materials

Male bud (blossom) of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA'), (Thai: huaplee) was collected from Pathumthani province in Thailand in October, 2013. The blossom was divided into three parts. First, the outside bracts from the male bud, having dark violet and red color. Second, the flowers inside the bract, with anthers and ovary in the flower. Last, the young male bud inside dark violet and red of male bud. The male buds were separated into two parts to use as dried and fresh. The dried part was treated in a hot air oven at 50 °C before use and the fresh was used as a fresh sample.

Table 3.1 The ethnobotanical data of male bud (banana flower)

Species	Family	Places for specimen collection	Voucher specimen number	Thai name	Part used
Musa sapientum Linn.	MUSACEAE	Pathumthani	SKP 119 13 19 01	Huaplee	Male flower, Bract, Young male bud

3.2 List of chemicals and reagents

3.2.1 Preparation of extracts

Name	Source
95% ethanol, commercial grade	C.M.J. Anchor company, Thailand
Distilled water	Milford, USA

3.2.2 DPPH radical scavenging assay

Name	Source
Absolute ethanol, (C_2H_5OH) Analytical grade	RCI labscan, Thailand
Butylated hydroxytoluene (BHT)	Fluka, Germany
1,1-diphenyl-2-picrylhydrazyl	Fluka, Germany

3.2.3 Total phenolic content assay

Name	Source
Folin-Ciocalteu's reagent	Fluka, Germany
Gallic acid ($(HO)_3C_6H_2CO_2H$)	Sigma-Aldrich, USA
Sodium carbonate (Na_2CO_3)	Merck, Germany

3.2.4 NO inhibitory effect assay and MTT assay

Name	Source
Dimethyl sulfoxide [$(CH_3)_2SO$)] (DMSO)	RCl Labscan, Thailand
Fetal bovine serum (FBS)	Biochem, Germany
Hydrochloric acid (HCL)	Univar, Australia
Isopropanol	RCI Labscan, Thailand
N-(1-Naphthyl)ethylenediamine dihydrochloride	Sigma, USA
Lipopolysaccharide from *E.coli* O55:B5 (LPS)	Sigma-Aldrich, USA
Penicillin-Streptomycin (P/S)	Gibco, USA
Phosphate buffered saline (PBS)	Amresco, USA
Phosphoric acid solution	Sigma, USA
RPMI medium 1640	Gibco, USA
Sodium bicarbonate ($NaHCO_3$)	BHD, England
Sodium hydroxide (NaOH)	Univar, Australia
Sulfanylamide	Sigma, USA
Thiazolyl blue tetrazolium bromide (MTT)	Sigma, USA
Trypan blue stain 0.4%	Gibco, USA
Trypsin-EDTA	Univar, Australia

3.2.5 Phytochemical investigation of compounds isolated

3.2.5.1 Column chromatography (CC)

Name	Source
Acetone (CH_3COCH_3)	RCI Labscan, Thailand
Anisaldehyde ($C_8H_8O_2$)	Fluka, Switzerland
Chloroform (CHCl3) Analytical grade	RCI Labscan, Thailand
Diaion® HP-20	Supelco, USA
Ethyl acetate ($CH_3COOC_2H_5$) Analytical grade	RCI Labscan, Thailand
Hexane ($CH_3(CH_2)_4CH_3$) Analytical grade	RCI Labscan, Thailand
Methanol (CH3OH) Analytical grade	RCI Labscan, Thailand
Silica Gel 60 (0.040-0.063 mm)	Merck, Germany
Silica Gel 60 (0.063-0.200 mm)	Merck, Germany
Sulfuric acid (H_2SO_4)	Merck, Germany
TLC silica gel 60 F254	Merck, Germany

3.2.6 List of Instruments

Name	Source
25 cm^2 plastic tissue culture flasks	Costar Corning, USA
75 cm^2 plastic tissue culture flasks	Costar Corning, USA
96-well plate flat-bottom with lid	Costar Corning, USA
96-well plate flat-bottom without lid	Costar Corning, USA
Analytical balance	Mettler Toledo, Switzerland
Autoclave	Hirayama, Japan
Biomedical freezer	Thermo Scientific, USA
Centrifuge machine	Boeco, Germany
Centrifuge tube 15, 50 ml	Costar Corning, USA
CO_2 humidified incubator	Shel lab, USA
Column (5.5 x 90 cm, glass)	Becthai, Thailand
Crucibles	Coorstex, USA
Cryogenic tube 2 ml	Costar Corning, USA
Disposable pipette 25 mL	Costar Corning, USA

3.2.6 List of instruments (Continued)

Name	Source
25 cm^2 plastic tissue culture flasks	Costar Corning, USA
75 cm^2 plastic tissue culture flasks	Costar Corning, USA
96-well plate flat-bottom with lid	Costar Corning, USA
96-well plate flat-bottom without lid	Costar Corning, USA
Analytical balance	Mettler Toledo, Switzerland
Autoclave	Hirayama, Japan
Biomedical freezer	Thermo Scientific, USA
Centrifuge machine	Boeco, Germany
Centrifuge tube 15, 50 ml	Costar Corning, USA
CO_2 humidified incubator	Shel lab, USA
Column (5.5 x 90 cm, glass)	Becthai, Thailand
Crucibles	Coorstex, USA
Cryogenic tube 2 ml	Costar Corning, USA
Disposable pipette 25 mL	Costar Corning, USA
Eppendorf	Costar Corning, USA
Erlenmeyer flasks	Schott Duran, Germany
Examination glove	Sritrang gloves, Thailand
Filter paper no.1 (125 mm, diameter)	Whatman, USA
Filter paper no.40 (125 mm, diameter)	Whatman, USA
Filter unit (0.22 µm, radio-sterilized)	Milipore, Ireland
Freezer (-20 °C)	Sanyo, Japan
Glass bottles 50, 250, 500, 1000 mL	Schott Duran, Germany
Glass column	Becthai, Thailand
Glasswares	Schott Duran, Germany; Pyrex, USA
Haemocytometer	Boeco, Germany
Hot air oven	Memmert, Germany
Hot plate	Thermolyne, USA
Incubated tabletop orbital shaker	Thermo Scientific, USA

3.2.6 List of instruments (Continued)

Name	Source
Inverted microscope	Nikon, USA
Laminar air flow	Boss tech, Thailand
Liquid nitrogen tank	Taylor-Wharton, USA
Litmus paper pH-fix 4.5-10.0	Macherey-Nagel, Germany
Lyophilizer	Lyophilization Systems Inc, USA
Membrane filters with pore-size rating of 0.22 microns	Sartorius, Germany
Micropipettes 1-20 µL, 20-200 µL, 100-1,000 µL	Gilson, USA
Microplate reader	Biotek, USA
Multi-channel pipette	Costar Corning, USA
Moisture analyzer	Scaltec instrument, Germany
Muffle furnace	Nabertherm, Germany
Multi-channels pipette	Costar Corning, USA
pH buffer	Thermo Scientific, USA
pH meter	WTW inolab, Germany
Pipette tips	Costar Corning, USA
Pipette boy	Integra biosciences, Switzerland
Reagent reservoir (sterile)	Costar Corning, USA
Rotary evaporator	Buchi, Switzerland
Shaking incubator	Vision Scientific, Korea
Sonicator	Elma, Germany
Stability incubator	Termarks, Norway
Sterile water for injection	Thai Nakorn Pattana, Thailand
Syringe	Nipro, Thailand
Syringe filters	Nipro, Thailand
Syringe filter nylon (13 mm, 0.45 µm)	GAT, Thailand
TLC plate heater	Canmax, Switzerland

3.2.6 List of instruments (Continued)

Name	Source
Vacuum pump	Rocker, Taiwan
Vortex mixer	Scientific industries, USA
Water bath	Memmert, Germany
Water purification machine	Elga, UK
Vacuum Desiccator	Simax, USA

3.3 Methods

3.3.1 Preparation of crude extracts

Musa sapientum Linn. (*Musa* ABB GROUP CV. 'NAM WA') male bud (blossom) was collected from Pathumthani province in Thailand in October, 2013. Three parts of male bud (blossom) were bract, young male bud and male flower. These were cleaned with water, cut into small pieces, and divided in two parts. One part was dried using a hot air oven at temperature 50 °C and crushed into powder to be used as a dried sample. The second part was used as a fresh sample. Three parts of the dried and of the fresh were extracted using thirty-one samples. There were extraction by decoction, maceration in 50% ethanol, residue 50% ethanol, maceration in 95% ethanol, residue 95% ethanol and blending with water of fresh young male buds only. The ethanol extracts were dried by rotary evaporator and water extracts were freeze-dried.

Maceration: Bracts, young male buds and male flowers both in fresh and dried condition were macerated with 50% ethanol and 95% ethanol for 3 days, then filtered and concentrated to dryness by rotary evaporator. Then, the residue was macerated 2 more times and dried using a rotary evaporator and kept in a freezer at -20°C until used. After that, percentages of yield were calculated.

Decoction: Bracts, young male buds and male flowers in both fresh and dried condition and their residues from 50% ethanol and residue 95% ethanol were decocted by boiling for 15 minutes. Then, they were boiled 2 more times,

filtered and dried with a lyophilizer. After that, they were freeze-dried and kept in a freezer at -20°C until used. After that, percentage yield was calculated.

Blend: Fresh young male bud only, were cut into small pieces and blended in water at room temperature, filtered and dried with a lyophilyzer and kept in a freezer at -20°C until used. After that, percentage of yield was calculated.

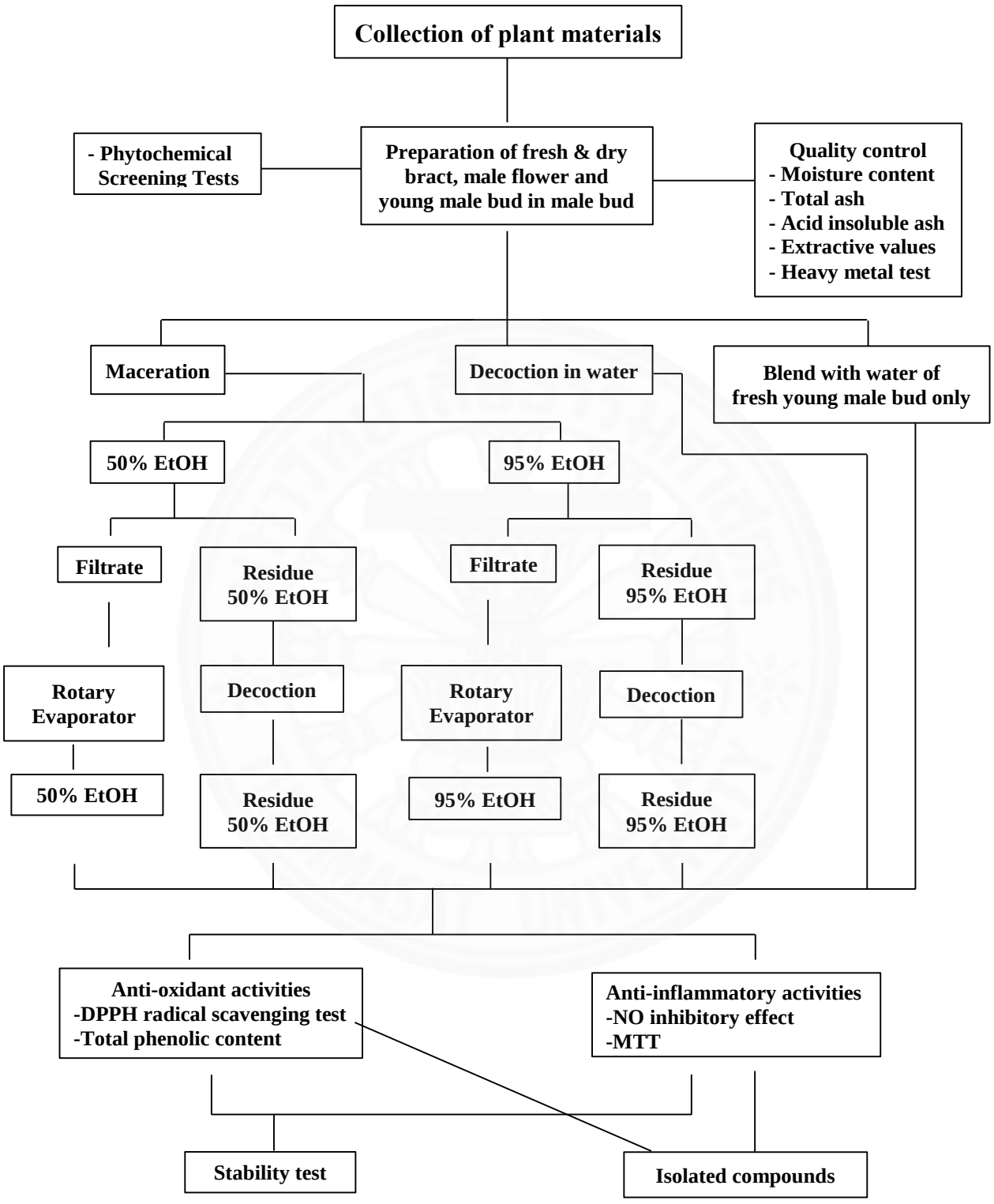


Figure 3.1

Conceptual framework of research

3.3.2 Assay for quality control of male bud

Quality control of plant materials follows the Thai Herbal Pharmacopoeia protocols. Extractive value, moisture content, total and acid insoluble ash were determined and subjected to physical standardization (Ministry of Public Health, 2000).

Extractive value

Ethanol soluble extractive value

5 grams of dried powder were put in 100 ml of 95% ethanol in a flask closed with foil for 24 hours, and shaken frequently during the first 6 hours then allowed to stand for 18 hours. Contents then were filtered and 20 ml filtrate placed in a shallow bowl. Put in a hot air oven at 105°C to reach constant weight. Finally, the percentage of ethanol soluble extract was calculated using the equation:

$$\% \text{ ethanol extractive value} = \left\{ \left\{ \frac{\text{Weight of the extract (g)}}{\text{Weight of dried powder of plant (g)}} \right\} x5 \right\} x\ 100$$

Water soluble extractive value

The procedure as detailed in ethanol soluble extract was repeated but using 0.25% chloroform in water instead of ethanol in volumetric flask.

Loss on drying

In this study, loss on drying was determined by electronic moisture analyzer using 2 grams sample. After switching on place the sample in the test tray and wait 60 second while the loss on drying is automatically recorded. Repeat twice to obtain 3 readings. The following formula is applied manually to the data.

$$\%\text{Loss on drying} = \frac{\text{Weight of sample before} - \text{Weight of sample after}}{\text{Weight of sample before}} \times 100$$

Total ash

Ash and acid-insoluble ash are determined by placing a crucible in a hot air oven at 105°C until weight of crucible was stable. Then, 2 grams of prepared powder were put in to the crucible and put in a muffle furnace at 450°C to burn the crucible for 9 hours. Next, allow crucible to cool in a desiccator and note weight of ash. After that, put ash into muffle furnace at 450°C for 5 hours. Allow crucible to cool in a desiccator and note weight of ash. When the crucible's weight is stable, calculate total ash using the following equation:

$$\%\text{ total ash} = \frac{\text{Stable weight after burning (g)}}{\text{Weight of before burning (g)}} \times 100$$

Acid insoluble ash

When weight of total ash was constant total ash, then put in beaker, adds 10%HCl and boil for 5 minutes. Then, filter using Whatman paper No.42 and wash the sample paper until pH 7 using distilled water. Dry, and then place filter paper in crucible and burn in a muffle furnace at 450°C for 9 hours. Cool in a desiccator and weigh. When the crucible's weight is stable, acid insoluble ash is calculated using the following equation:

$$\%\text{ acid insoluble ash} = \frac{\text{Stable weight after burning (g)}}{\text{Weight of ash before burning (g)}} \times 100$$

3.3.3 Heavy metal test of plants

The method was used to analyse the heavy metal content of aliquot portion of stabilized hot nitric acid extract by Atomic Absorption Spectrophotometry Technique (AAS). 1 gram of sample in 100 ml beaker was dissolving in 20 ml of 65% Nitric acid and the beaker closed with a watch glass for 24 hours. Then, it was heated

in a hot plate for 5-8 hours until dissolved, then filtered with Whatman paper No.42. The standard was measured by Atomic Absorption Spectrophotometry Technique (AAS). Stand and solutions using iron (Fe) (0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25 and 50 ppm) were prepared and a calibration curve generated. Results of samples were expressed on a weight basis as parts per million (ppm) using the standard calibration graph. The standard of heavy metal content of plants are Arsenic (As) less than 4 ppm, Lead (Pb) less than 10 ppm and Cadmium (Cd) less than 0.3 ppm (World Health Organization, 2007).

3.3.4 Phytochemical screening test of plants

The objective of phytochemical screening test was to know chemical groups present in the plant. There are 3 main chemical groups to be found in the plant. There are glycosides, alkaloids and primary metabolites such as vitamin, hormone, resin etc. They were observed routinely by basic tests. Even though specific reagents are used, false positive or false negative results are possible.

3.3.4.1 Cyanogenic glycosides test

Cyanogenetic glycoside is a glycoside. After it is hydrolysed, it becomes hydrocyanic acid (HCN) or prussic acid such as amygdalin (Bitter almond). Plants in this group have enzymes for the hydrolysis reaction by themselves, but they were in a different location in the cell. So, it was necessary to break the cells and try to catch the hydrolyzing enzyme of cyanogenetic glycosides and call it autohydrolysis.

Experimental procedures

The three parts used were bract, young male bud and male flower. These were cut into small pieces (of about 5 grams) and put in to tube with water and 3 drops of $CHCl_3$ added to increase reaction rate. After that, the tube was closed by cork with sodium picrate paper hanging inside or guaiac-copper sulphate paper and placed in a water-bath at 50°C and observed in 1 hour. Positive results show on the paper depending on the chemical used. Brick red of sodium isopurpurate

shows on sodium picrate paper. Blue shows on guaiac-copper sulphate paper. It is not difficult to test for these basic chemicals, but false positive and negative indications can be obtained even when the correct specific reagent is used. Phytochemical screening follows well-tried basic tests (Farnsworth, 1966).

3.3.4.2 Coumarin tests

Coumarin is in the benzo-α pyrone group. Use heat to volatilize coumarin from the dried ground plant part. The fluorescence process begins with sodium hydroxide reacting with coumarin to form *cis*-o-hydroxycinnamic acid when in acid conditions. Under UV light, this compound changes to the *trans*-type which emits light proving the presence of coumarin.

Experimental procedures

The bracts, young male buds and male flowers are cut into 5 gram pieces and put into a tube with water. The tube is closed with a cork, having paper soaked in 10% NaOH hanging in it. It is heated in a boiling water-bath for 3-5 minutes. The paper is then held under UV light of 365 nm wavelength. Positive results of a green or green and blue, or blue show the presence of coumarin. Positive results will show by color on the paper, which will turn green, green with blue or blue in fluorescence (Farnsworth, 1966).

3.3.4.3 Anthraquinone glycosides test

These are detected by the Borntraeger reaction. This is test for O-glycosides. First hydrolyse the anthraquinone glycoside in acid (add hydrogen peroxide to oxidize any anthracene derivatives to anthraquinone) . In alkaline conditions (NaOH, KOH) the structure of anthraquinone (or anthracene) will break down to an aglycone of K or Na which a pink-red color.

Experimental procedures

The bracts, young male buds and male flowers are cut into 5 gram pieces and put in a tube with 20 ml 1% H_2SO_4 , then filtered, chloroform added

to the filtrate and shaken. Remove the upper layer of $CHCl_3$, place into a new tube and add 1% NaOH and shake vigorously. A pink-red color will show the presence of anthraquinone glycoside (Farnsworth, 1966).

3.3.4.4 Iridoids

Strong anti-inflammatory and defense against herbivores: monoterpenes which occur widely.

Experimental procedures

The bracts, young male buds and male flowers are cut into 5 gram pieces and put into an Erlenmeyer flask with 20 ml 2% H_2SO_4 for 24 hours, then filtered with Wieffering's reagent. Then, added to the filtrate, and the tube heated in a boiling water bath for 30 secs. Positive results are show by a blue or green-blue color (Farnsworth, 1966).

3.3.4.5 Cardiac glycosides test

Experimental procedures

The bracts, young male buds and male flowers were cut into small 5 gram pieces and put Erlenmeyer flask with 20 ml 95% ethanol for 24 hours, then filtered. 10% ethanol and chloroform is added to the filtrate. The chloroform is withdrawn and the residue evaporated to dryness in a shallow bowl. The residue is used for the following tests:

Tube 1, add 2-3 drops of Kedde's reagent, and 2-3 drops of 5% alcoholic KOH. Positive results were show a violet with red color immediately in the shallow bowl.

Tube 2, dissolve in a little chloroform in a shallow bowl. Transfer to a tube and add $FeCl_3$ reagent and shake. Then slowly add conc. H_2SO_4 down the side of the tube. Positive result is a brown ring at the interface of the 2 liquids (Farnsworth, 1966).

3.3.4.6 Saponin glycosides

Experimental procedures

The bracts, young male buds and male flowers are cut into 5 gram pieces and added to 10 ml water in a tube. The tube is heated in a water-bath at 50-60°C for 10 min and filtered. The filtrate is divided between two tubes. Tube 1 is shaken vigorously for 3-5 min and allowed to stand for 30 min. Positive results are hexagonal bubbles in the tube. Tube 2-dilute H_2SO_4 is added and the tube boiled, followed by shaking as for tube 1. No bubbles observed means that the saponin glycoside was hydrolysed by the acid into the aglycone form. Small bubbles reveal the presence of plant acids. Add Na_2CO_3 until basic and shake. Bubbles indicate a form of soap between base and plant acids (Sabri, 2012).

3.3.4.7 Flavonoids

Experimental procedures

The bracts, young male buds and male flowers are cut into 5 gram pieces in an Erlenmeyer flask with 95% ethanol for 24 hours and filtered. 50% ethanol is added to the filtrate, and then divided between 2 tubes. Tube 1 is control. 3-5 drops of conc.HCl and 2-3 pieces of magnesium ribbon are added to tube 2. After the vigorous reaction, positive results are shown by a pink-red color after 1-2 min (Sabri, 2012).

3.3.4.8 Tannins

Experimental procedures

The bracts, young male buds and male flowers are cut into 5 gram pieces and put into a tube with 10 ml water and boiled for 15 min and filtered. The filtrate is divided between 4 tubes. Tube 1 is control. Tube 2 receives 2-3 drops of gelatin solution. A dirty white color is a positive result. Tube 3 receives 2-3 drops of 1% $FeCl_3$ solution. Positive results are shown by a blue color for hydrolysable tannin,

and green color for condensed tannin. Tube 4 receives formalin-HCl then boiled. Positive result will show by red color of condensed tannin (Sabri, 2012).

3.3.4.9 Alkaloids

Alkaloids are tested by Mayer's reagent and Dragendorff's reagent, which are easy to use, and give consistent and clear results.

Experimental procedures

The bracts, young male buds and male flowers are cut into 20 gram pieces and put into Erlenmeyer flask with 95% ethanol for 24 hours. Then, it is filtered and the filtrate evaporated to dryness. It is dissolved in 10 ml 20% H_2SO_4 and filtered. The filtrate is divided into 3 tubes. Tube 1 is control. Tube 2 will receive Mayer's reagent and a positive result is a dirty white color. Dragendorff's reagent is added to Tube 3. Positive result is shown by orange sediment (Sabri, 2012).

3.3.5 Anti-oxidant activity

3.3.5.1 *In vitro* test for anti-oxidant activity by DPPH radical scavenging assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay was described by Yamasaki *et al* (1994). The crude extracts will be prepared for testing by dissolving in absolute ethanol to final concentrations of 1, 10, 50 and 100 µg/ml. First, 100 µl of sample solutions are added to 96-well micro plates. 100 µM $6x10^{-5}$ mM DPPH solution (in absolute ethanol) is added to the 96-well microplate. They are incubated for 30 minutes in the dark at room temperature. The absorbance is measured at 520 nm by spectrophotometer. The positive standard is to use BHT (Butylated hydroxytoluene) tested in the same manner. All tests will be performed in triplicate and the results reported as mean±SEM of three replicates. The percentage inhibition is calculated as follows:

$$\% \text{ inhibition} = [(OD_{control}-OD_{sample})/OD_{control}) \times 100] \quad \text{where OD = absorbance}$$

Effective concentration of sample required to scavenge DPPH radical by 50% (EC_{50}) is calculated by Prism program.

3.3.5.2 Total phenolic contents by Folin-Ciocalteu's method

The total phenolic content procedure is described by Miliauskus (2004) but is to be determined here using Folin-Ciocalteu's method. Prepare a 10 mg sample ethanol extract in 10,000 µl and 10 mg absolute ethanol, sample water extract in 20 µl distilled water of extract solution was added to 96-well microplates, and 100 Folin-Ciocalteu's reagent added and mixed. After standing 5 min, 80 µl of (7.5% w/v) sodium carbonate solution is added. The samples are mixed and incubated for 30 minutes at room temperature. Then, the absorbance is measured at 765 nm by spectrophotometer. Standard solutions using gallic acid (12.5, 25, 50, 100, 200 and 400 µg/ml) are prepared and a calibration curve generated. Results of samples are expressed on a weight basis as milligram gallic acid equivalents/gram of sample (mg GAE/g) using the standard calibration graph.

3.3.6 Anti-inflammatory activity (Tewtrakul and Itharat, 2007)

3.3.6.1 *In vitro* assay of NO inhibitory effect

Animal cell lines

Murine macrophage leukemia (RAW 264.7) cell line is the most commonly used cell line in medical research. RPMI 1640 medium (BIOCHROM^{AG}) is used to culture the cell line. It is supplemented with 10% heated fetal bovine serum, 50 IU/ml penicillin and 50 µg/ml streptomycin. The cells are maintained at 37°C in 5% CO_2 atmosphere with 95% humidity and the culture medium is changed twice per week.

Preparation of sample solution

First, the samples for ethanolic extracts are dissolved in a quantity of sterile dimethylsulfoxide (DMSO) at concentration of 10 mg/ml. The sample for water extracts are dissolved in sterile distilled water and filtered by 0.22 µl

and concentration of 50 mg/ml. Next, the extracts are diluted in medium to produce required concentrations of 1, 10, 30, 50 and 100 µg/ml.

NO inhibitory effect test

Raw 264.7 cells are cultured at $1x10^5$ cells/well, and they are accorded growth profiles in the optimal plating densities. Cells grow as a monolayer in 75 cm^3 flask in this assay. They are washed twice with 3 ml phosphate-buffered saline (PBS) free of magnesium and calcium. The PBS is decanted and cells detached by 3ml of 0.25% trypsin-EDTA to produce a single cell suspension. Next, medium is added to the 3ml in flask to stop trypsin-EDTA working, and the cell plates are centrifuged at 1,500 rpm for 5 minutes. The supernatant is removed and then 10 ml of fresh medium added and mixed with the cells to make a single cell suspension. The viable cells are counted by Trypan blue exclusion in haemocytometer. Raw 264.7 cells are diluted with medium to give a final concentration of $1x10^6$ cells/ml. These cell suspensions are seeded in 96-well plate at 100 µl/well and incubated at 37°C, 5% CO_2 atmosphere with 95% humidity for 24 hours. After that, the medium is replaced with fresh medium containing 10 ng/ml of LPS (Lipopolysaccharide) together with test samples at various concentration levels and incubated at 37°C, 5% CO_2 atmosphere with 95% humidity for 24 hours. Finally, NO production is determined from nitrite in the culture supernatant using the Griess reagent for measuring the accumulation. 100 µl of supernatant is removed from the well plates and 100 µl Griess reagent added in 96 well plates for NO determination. The absorbance using a spectrophotometer is measured at 570nm. Finally, the % inhibition and IC_{50} is calculated by Prism program.

Cytotoxicity is determined using 3-(4,5-dimethyl-2-thiazolyl) -2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric method after incubation with test sample for 24 hours. 10 µl MTT solution with samples is put into plates and incubated at 37°C, 5% CO_2 atmosphere with 95% humidity for 2 hours. Next, the medium is removed and isopropranol containing 0.04 M HCl added to dissolve the formazan production in the cells. Formazan solution enables measurement of the optical density with a microplate reader at 570 nm. Prednisolone is used as positive control. If surviving cells ≥30% it means no cytotoxicity, then it is

recorded as no inhibition of cell growth. Finally, the % inhibition and IC_{50} values are calculated by Prism program.

$$\% \text{ inhibition} = \frac{\text{A-B x 100}}{\text{A-C}}$$

A-C : NO_2^- concentration (μM)

A: LPS (+), sample (-)

B:LPS(+), sample (+)

C: LPS (-), sample (-)

3.3.7 Bioassay guided fractionation of isolated compounds

3.3.7.1 Column chromatography (CC)

Bioassay guided fractionation is performed to find active compounds in dried male flower extract by decoction. Column chromatography is the beginning process, and the extract is isolated using Diaion® HP-20 (about 400 g) as stationary phase and using solvents in gradient from high-polarity to low polarity as mobilephase; 100% water, 25% methanol, 50% methanol, 75% methanol, 100% methanol and ethyl acetate. The derived fractions are evaporated and tested for NO inhibitory activity. After that, the fraction that presents the best activity is further separated using normal phase column chromatography. Silica gel 60 (0.040-0.063 mm) about 30 g is used as stationary phase and using solvents in a gradient from low polarity to high-polarity as mobile phase; 100% Chloroform , 10% methanol, 30% methanol, 50% methanol, 70% methanol, 100% methanol, 5% water: 95% methanol. The structure of the isolated pure compounds is identified by nuclear magnetic resonance spectroscopy method (NMR). Finally, the pure compounds are tested for NO inhibitory activity and used as marker of the crude extract.

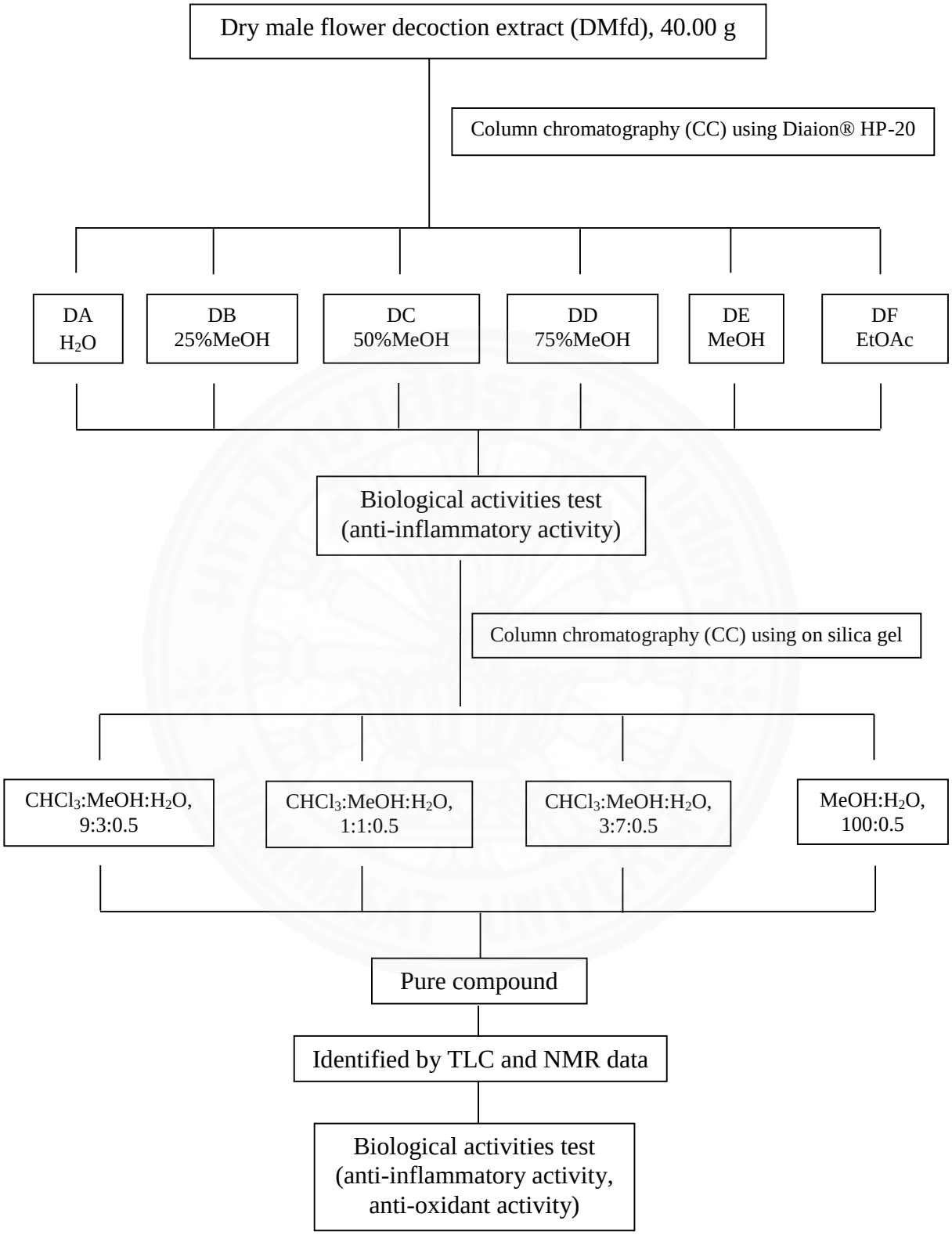


Figure 3.2
Bioassay-guided fractionation of dry male flower decoction extract of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA')

3.3.8 Stability test

The stability of crude extracts were carried out in triplicate using transparent vials with screw cap according to the Thai Food and Drug Administration (Thai FDA) guideline (WHO, 2016). These were kept at 40ºC with 75±5% RH and samples taken and tested on days 15, 30, 60, 90, 120, 150 and day 180 for a 6 month period. They were tested for DPPH radical scavenging activity, total phenolic contents, total flavonoid contents, NO inhibitory effect. The sample results were compared with control sample (Kept at -20 ºC and taken as day 0).

3.3.9 Statistical analysis

All data analyse of the mean of three replications. Analysis of values different parameters as the mean±standard error of mean and statistical analysis of all data by SPSS statistical software.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Preparation of plant extracts

The % yield of water and ethanol extracts of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') (male buds) are shown as percentage by weight in table 4.1. Water extracts gave yield more than ethanol extracts. All of them, residue dry male flower maceration 50%EtOH (DMfr50) gave the highest yield (38.20%) followed by dry bract decoction (DMbd) (34.83%) and residue dry young male bud maceration 95% EtOH (DMyr95) (25.97%).

Table 4.1 The % yield of the aqueous extracts and ethanolic extracts of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') preparation

Part of male bud	Extract	Code	% yield (w/w)
Male flower	Fresh male flower decoction	FMfd	3.91
	Fresh male flower 50%EtOH	FMf50	1.88
	Residue fresh male flower 50%EtOH	FMfr50	0.48
	Fresh male flower 95%EtOH	FMf95	3.50
	Residue fresh Male flower 95%EtOH	FMfr95	0.82
	Dry male flower decoction	DMfd	23.30
	Dry male flower 50%EtOH	DMf50	23.28
	Residue dry male flower 50%EtOH	DMfr50	38.20

Table 4.1 The % yield of the aqueous extracts and ethanolic extracts of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') preparation (Continued)

Part of male bud	Extract	Code	% yield (w/w)
Male flower	Dry male flower 95%EtOH	DMf95	8.97
	Residue dry male flower 95%EtOH	DMfr95	23.43
Bract	Fresh bract decoction	FMbd	2.35
	Fresh bract 50%EtOH	FMb50	1.50
	Residue bract 50%EtOH	FMbr50	0.27
	Fresh bract 95%EtOH	FMb95	1.55
	Residue fresh bract 95%EtOH	FMbr95	0.69
	Dry bract decoction	DMbd	34.83
	Dry bract 50%EtOH	DMb50	23.71
	Residue dry bract 50%EtOH	DMbr50	6.22
	Dry bract 95%EtOH	DMb95	12.13
	Residue dry bract 95%EtOH	DMbr95	16.78
Young male bud	Fresh young male bud decoction	FMyd	3.02
	Fresh young male bud 50%EtOH	FMy50	0.75
	Residue young male bud 50%EtOH	FMyr50	0.34
	Fresh young male bud 95%EtOH	FMy95	1.62
	Residue fresh young male bud 95%EtOH	FMyr95	0.80

Table 4.1 The % yield of the aqueous extracts and ethanolic extracts of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') preparation (Continued)

Part of male bud	Extract	Code	% yield (w/w)
Young male bud	Fresh young male bud Blend with water	FMyb	1.35
	Dry young male bud decoction	DMyd	18.07
	Dry young male bud 50%EtOH	DMy50	16.09
	Residue dry young male bud 50%EtOH	DMyr50	12.85
	Dry young male bud 95%EtOH	DMy95	4.92
	Residue dry young male bud 95%EtOH	DMyr95	25.97

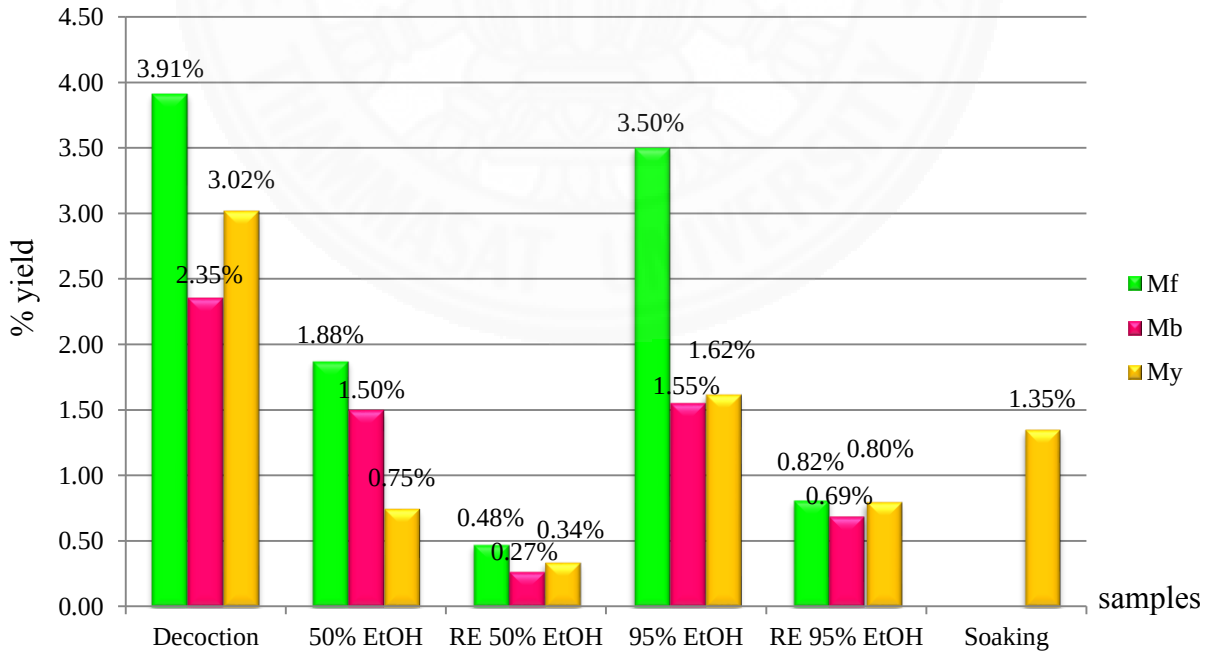


Figure 4.1

The % yield of fresh male flower (Mf), fresh bract (Mb) and fresh young male bud (My) of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA')

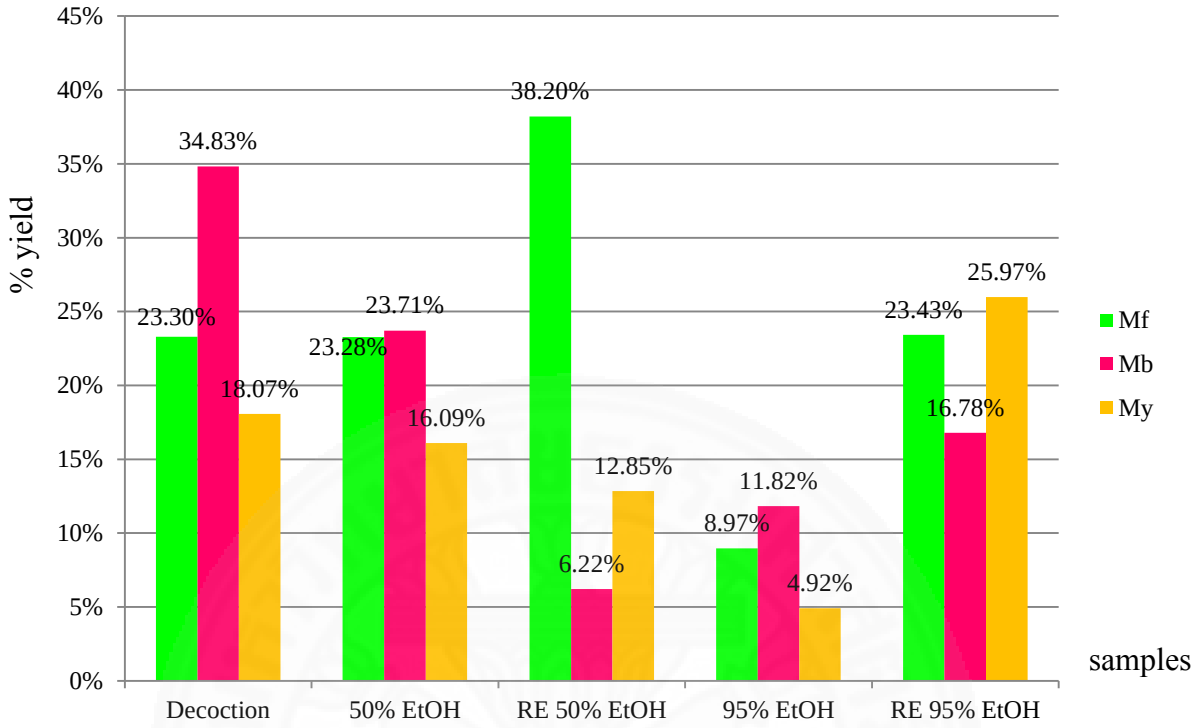


Figure 4.2

The % yield of dried male flower (Mf), dried bract (Mb) and dried young male bud (My) of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA')

4.2 Quality control of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA')

The quality standard parameters were water soluble extractive, ethanol soluble extractive, loss on drying, total ash and acid insoluble ash. Quality standard values investigation of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') are shown as percentage in the Table 4.2.

Table 4 .2 Quality control of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') (n=3)

Quality parameter	Standard value(%w/w) (Thai Herbal Pharmacopoeia, 2000)	*Musa sapientum* Linn. (%)		
		Male flowers	Bracts	Young male buds
Water extractive	-	23.94	28.94	24.91
Ethanol extractive	-	5.60	6.28	2.46
Loss on drying	< 10.00	7.54	8.69	8.93
Total ash	< 10.00	12.87	10.58	14.63
Acid insoluble ash	< 2.00	6.03	2.64	1.07

Table 4.2 shows all parameters of quality of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') is shown male flowers, bracts and young male buds are standard values of Thai Herbal Pharmacopoeia (THP) that the specify of loss on drying value less than 10%, total ash content value less than 10% and acid insoluble ash value less than 2%. The results are over limit standard values of Thai Herbal Pharmacopoeia (THP) because they may have sands or stones. Then, male flowers, bracts and young male buds are heavy metal test by Atomic Absorption Spectrophotometry Technique (AAS). The standard values of Thai Herbal Pharmacopoeia (THP) that the specify of heavy metal test of Pb value less than < 10 ppm, Cd value less than < 0.3 ppm and As value less than < 4 ppm.

The results are shown in the Table 4.3 found that they can not detected because they had heavy metal (Pb, Cd and As) less until not detectable. This results can conclude that they had no these three heavy metal and safe following standard of Thai herbal pharmacopoeia.

Table 4 .3 Heavy metal test of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') (n=3)

Atomic absorption spectrophotometer (AAS)				
Test	**Sample**			**Requiered standard**
	Male flower	**Bract**	**Young male bud**	
Pb	ND	ND	ND	< 10 ppm
Cd	ND	ND	ND	< 0.3 ppm
As	ND	ND	ND	< 4 ppm

ND = Not Detected

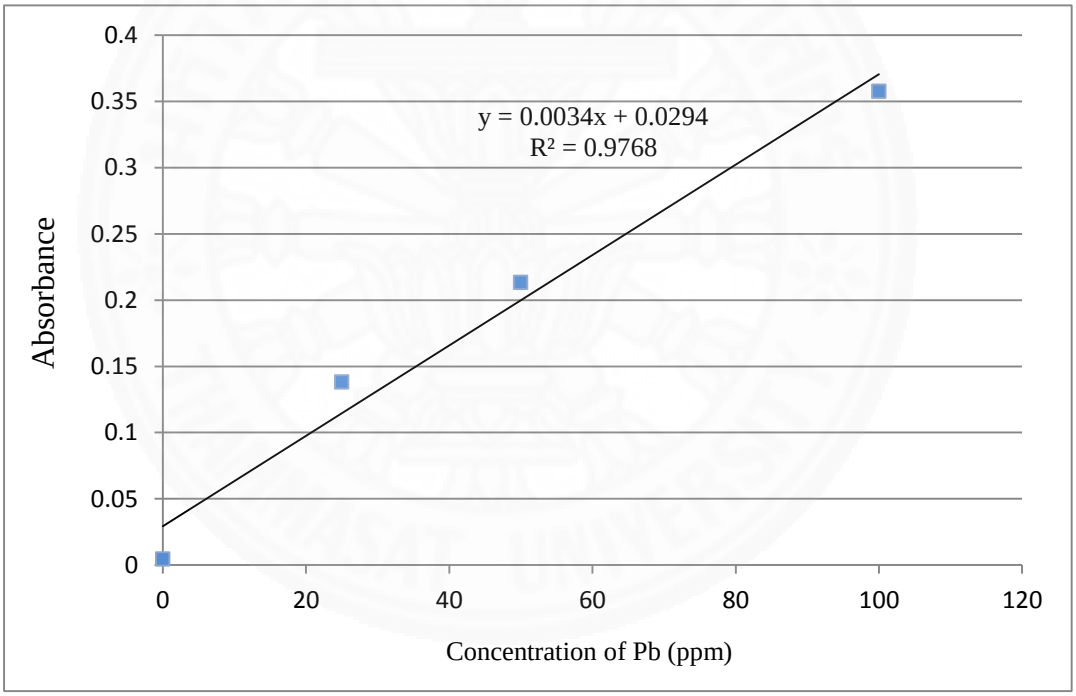


Figure 4.3
Standard calibration curve of Lead (Pb) (ppm)

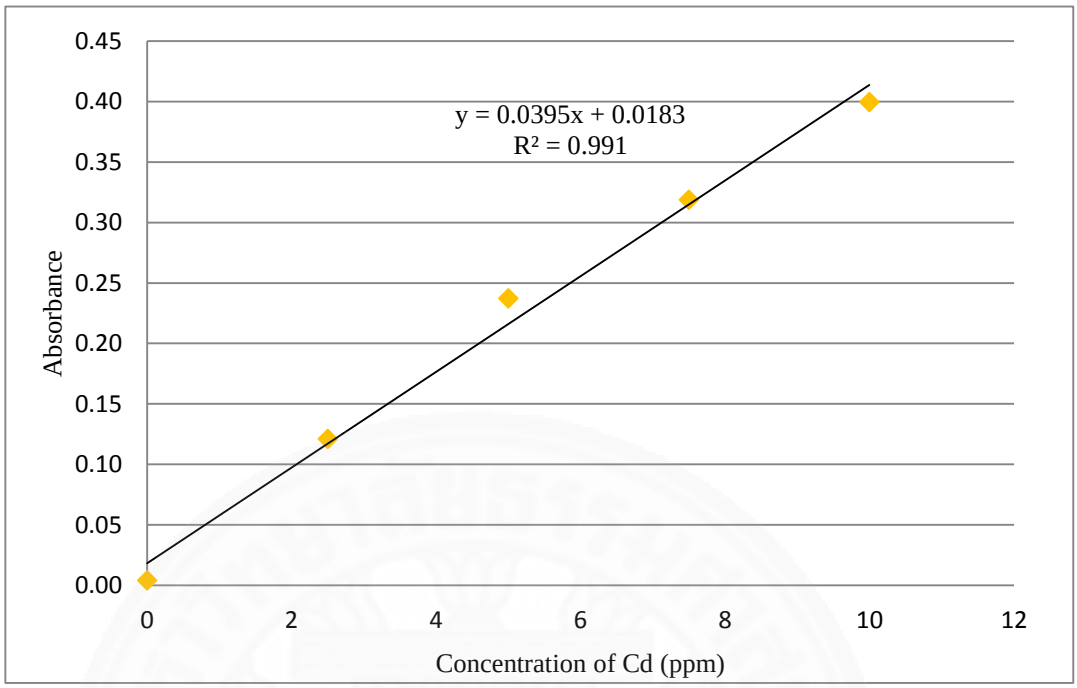


Figure 4.4
Standard calibration curve of Cadmium (Cd) (ppm)

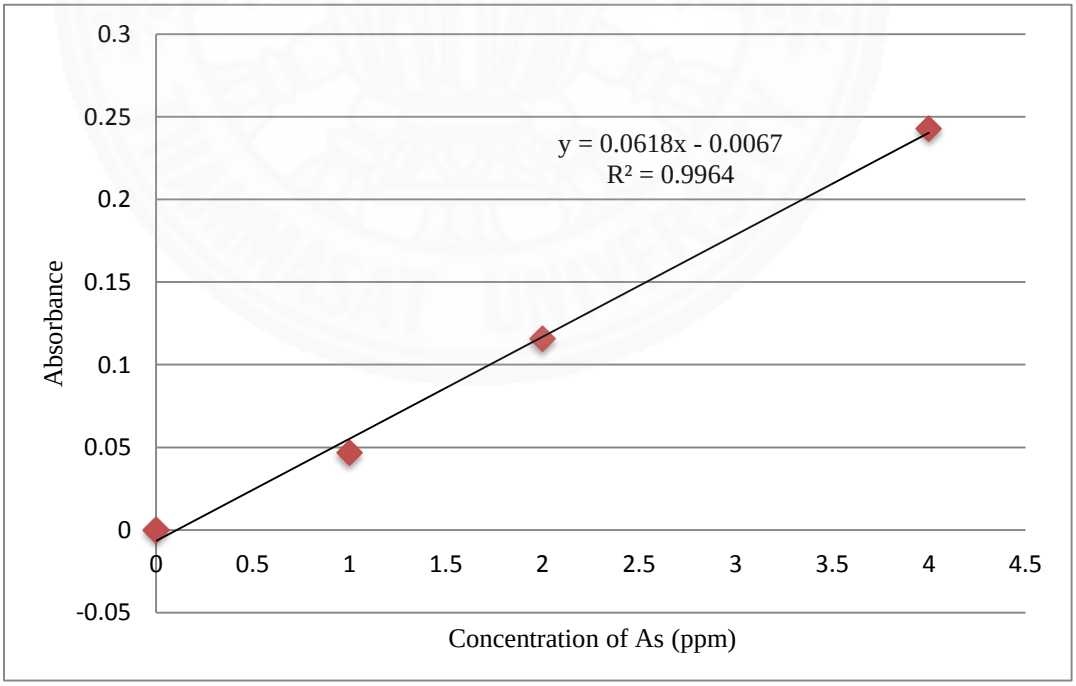


Figure 4.5
Standard calibration curve of Arsenic (As) (ppm)

4.3 Phytochemical screening tests of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA')

The phytochemical screening tests of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') (male buds) are shows as saponin glycosides by Froth test found in male flower, bract and young male bud. Flavonoids by Shinoda's test showed in male flower, bract and young male bud. Alkaloid by Mayer's&Dragendoff's reagent shows in male flower, bract and young male bud. Deoxysugar of Keller-killiani test be exhibited in male flower, bract and young male bud but had no cardiac glycoside because it showed only one part of cardiac glycoside test. Tannins by 1%$FeCl_3$ solution shows in male flower, bract and young male bud. Forrnalin-HCl shows bract (that indicate present (+), indicate absent (-)). The results can conclude that male flower, bracts and young male bud had the same manner compound such as saponin, flavonoid, anthocyanin, alkaloid and deoxysugar. However bract showed difference compound because it showed tannin which was both hydrolysable and condensed tannin but male flower and young male bud had only hydrolysable tannin. The phytochemical screening results of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') (male bud) are shown in Table 4.4.

Table 4.4 Phytochemical screening tests of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') male flowers, bracts and young male buds

Phytochemical groups	Testing/Reagents	Reaction of positive control	Results		
			Male flowers	Bracts	Young male buds
Cyanogenic glycosides	Grignard reaction	Red brick color	-	-	-
Coumarins		Green, green blue, blue color	-	-	-
Anthraquinone glycosides	Borntrager Test	Pink-red color	-	-	-
Iridoids	Wieffering's reagent	Green-blue, dark blue	-	-	-
Saponin glycosides	Froth Test	Hexagon froth	+	+	+
Flavonoids	Shinoda's Test	Pink-red color with froth	+	+	+
	Chalcones&aurones	Red color	-	-	-
	Anthocyanins	Orange-red to blue-red color	+	+	+
	Leucoanthocyanins	Violet-red color	+	+	+
	Catechin	Blue or green color	-	-	-
Alkaloid	Mayer's reagent	White precipitate	-	-	-
	Dragendoff's reagent	Orange precipitate	+	+	+

(+) = indicate present, (-) = indicate absent

Table 4.4 Phytochemical screening tests of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') male flowers, bracts and young male buds (Continued)

Phytochemical groups	Testing/Reagents	Reaction of positive control	Results		
			Male flowers	Bracts	Young male buds
Cardiac glycosides	Unsaturated lactone ring (Kedde's reagent)	Violet-red color	-	-	-
	Steroidal nucleus (Liebermann-Burchard Test)	Blue or green color	-	-	-
	Deoxysugar (Keller-killiani Test)	Brown ring	+	+	+
Tannins	10%gelatin solution	White turbid precipitate	-	-	-
	1%$FeCl_3$ solution	Blue color for hydrolysable tannin, Green color for condensed tannin	+	+	+
	Forrnalin-HCl	Red color	-	+	-

(+) = indicate present, (-) = indicate absent

Table 4 .4 Phytochemical screening tests of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') male flowers, bracts and young male buds (Continued)

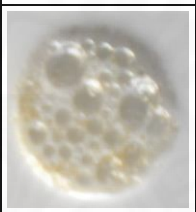
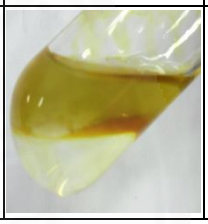
Phytochemical groups	Testing/Reagents	Results		
		Male flowers	Bracts	Young male buds
Saponin glycosides	Froth Test			
Flavonoids	Shinoda's Test			
	Anthocyanins			
	Leucoanthocyanins			

Table 4 .4 Phytochemical screening tests of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') male flowers, bracts and young male buds (Continued)

Phytochemical groups	Testing/Reagents	Results		
		Male flowers	Bracts	Young male buds
Alkaloid	Dragendoff's reagent			
Cardiac glycosides	Deoxysugar (Keller-killiani Test)			

4.4 Taste of male bud extract

The taste of male bud extracts test of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') (male bud) are shown as data of male flower(salt, low astringency), bract(mild salt, mild astringent) and young male bud(salt, strongly astringent). The taste of male bud decoction extracts of *Musa sapientum* Linn. (*Musa* ABB group cv. 'Nam Wa') (male buds) are shown in table 4.5

Table 4.5 Taste of male bud extract

Male flower	Bract	Young male bud
Salty Low astringency	Mild salt Mild astringent	Salt Strongly astringent

4.5 Assay of anti-oxidant activity and anti-inflammatory activity

Table 4.6 shows the EC_{50} values of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts. The FMfd was showed the highest antioxidant activity with EC_{50} values 5.78±0.12 µg/ml, followed by the FMyd and DMfd with EC_{50} values 6.28±0.09 and 10.60±0.98 µg/ml, respectively which these results exhibited higher than BHT (Butylated hydroxytoluene) the positive standard with EC_{50} values 14.12 µg/ml. These results related with the previously report which found that the fresh male flower decoction showed high antioxidant activity (Phuaklee, 2012).

Table 4.7 shows total phenolic contents values of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts. Fresh male flower decoction (FMfd) also show the highest total phenolic contents, followed by fresh young male bud decoction (FMyd) and dry male flower decoction (DMfd) with values 269.31, 201.16 and 146.85 mg GAE/g (crude extract), respectively.

Table 4.8 shows the IC_{50} values of anti-inflammatory effect on NO inhibitory activity of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts. Dry male flower decoction (DMfd) are show the highest potency for NO inhibitory activity, followed by dry young male bud decoction (DMyd) and residue dry male flower maceration 50%EtOH (DMfr50) with IC_{50} values 5.98, 20.91 and 31.17 µg/ml, respectively. All extract showed less anti-inflammatory than the positive standard as Prednisolone with IC_{50} values 0.16 µg/ml.

Table 4.6 EC_{50} of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts by DPPH assay (µg/ml, n=3)

Part used	Extract	Code	% inhibition(µg/ml) at various concentrations				EC_{50}±SEM (µg/ml)
			1	10	50	100	
Male flower	Fresh male flower decoction	FMfd	11.82±3.13	78.83±0.83	83.28±3.25	80.41±4.89	5.78±0.12
	Fresh male flower 50%EtOH	FMf50	-	-	-	32.56±0.40	>100
	Residue fresh male flower 50%EtOH	FMfr50	-	-	-	31.66±0.85	>100
	Fresh male flower 95%EtOH	FMf95	-0.79±0.30	16.31±1.66	64.53±1.69	88.22±0.55	33.76±0.05
	Residue fresh Male flower 95%EtOH	FMfr95	-	-	-	31.45±1.97	>100
	Dry male flower decoction	DMfd	5.48±0.57	48.43±4.05	84.68±2.07	86.51±1.53	10.60±0.98
	Dry male flower 50%EtOH	DMf50	-	-	-	42.58±1.46	>100
	Residue dry male flower 50%EtOH	DMfr50	3.46±0.93	30.72±0.36	85.44±0.85	89.17±0.24	18.04±0.14
	Dry male flower 95%EtOH	DMf95	-	-	-	43.25±1.08	>100
	Residue dry Male flower 95%EtOH	DMfr95	8.81±2.21	41.87±0.34	78.47±10.08	76.62±11.67	12.85±0.36

Table 4.6 EC_{50} of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts by DPPH assay (µg/ml, n=3) (continued)

Part used	Extract	Code	%inhibition (µg/ml) of various concentrations				EC_{50}±SEM (µg/ml)
			1	10	50	100	
Bract	Fresh bract decoction	FMbd	2.61±0.15	15.98±1.20	56.61±0.29	86.60±0.77	41.31±0.93
	Fresh bract 50%EtOH	FMb50	-	-	-	4.03±0.72	>100
	Residue bract 50%EtOH	FMbr50	-	-	-	19.12±0.93	>100
	Fresh bract 95%EtOH	FMb95	-	-	-	6.70±0.54	>100
	Residue fresh bract 95%EtOH	FMbr95	-	-	-	12.35±0.64	>100
	Dry bract decoction	DMbd	3.31±0.35	30.13±1.80	47.05±9.29	67.92±6.32	23.32±0.58
	Dry bract 50%EtOH	DMb50	-	-	-	21.39±0.90	>100
	Residue dry bract 50%EtOH	DMbr50	5.40±0.09	9.20±0.03	46.03±0.09	53.05±1.74	56.30±0.36
	Dry bract 95%EtOH	DMb95	-	-	-	47.78±0.82	>100
	Residue dry bract 95%EtOH	DMbr95	-1.75±0.61	10.48±0.62	54.52±0.22	84.43±0.29	44.85±0.18

Table 4.6 EC_{50} of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts by DPPH assay (µg/ml, n=3) (continued)

Part used	Extract	Code	%inhibition (µg/ml) of various concentrations				EC_{50}±SEM (µg/ml)
			1	10	50	100	
Young male bud	Fresh young male bud decoction	FMyd	10.68±7.72	74.14±5.30	90.70±4.39	87.26±3.27	6.28±0.09
	Fresh young male bud 50%EtOH	FMy50	-	-	-	5.82±0.21	>100
	Residue young male bud 50%EtOH	FMyr50	-	-	-	37.72±0.78	>100
	Fresh young male bud 95%EtOH	FMy95	3.80±2.80	16.34±1.98	62.80±2.00	89.12±3.44	36.89±1.37
	Residue fresh young male bud 95%EtOH	FMyr95	4.76±5.07	23.46±2.12	70.57±2.54	77.31±5.28	26.76±1.25
	Fresh young male bud Blend with water	FMyb	-	-	-	14.21±0.27	>100
	Dry young male bud decoction	DMyd	-0.05±1.07	9.09±1.35	45.02±0.80	59.52±2.30	59.16±0.04
	Dry young male bud 50%EtOH	DMy50	-	-	-	35.14±0.60	>100
	Residue dry young male bud 50%EtOH	DMyr50	-6.74±8.36	9.09±7.57	47.28±1.28	69.85±2.19	54.55±1.18
	Dry young male bud 95%EtOH	DMy95	0.58±1.88	12.85±2.41	60.42±1.29	92.33±0.66	39.70±1.26
	Residue dry young male bud 95%EtOH	DMyr95	-	-	-	31.69±1.12	>100
BHT (Positive control)			6.75±0.43	38.83±1.53	81.19±0.95	87.05±0.66	14.12±0.81

n = number of independent experiment

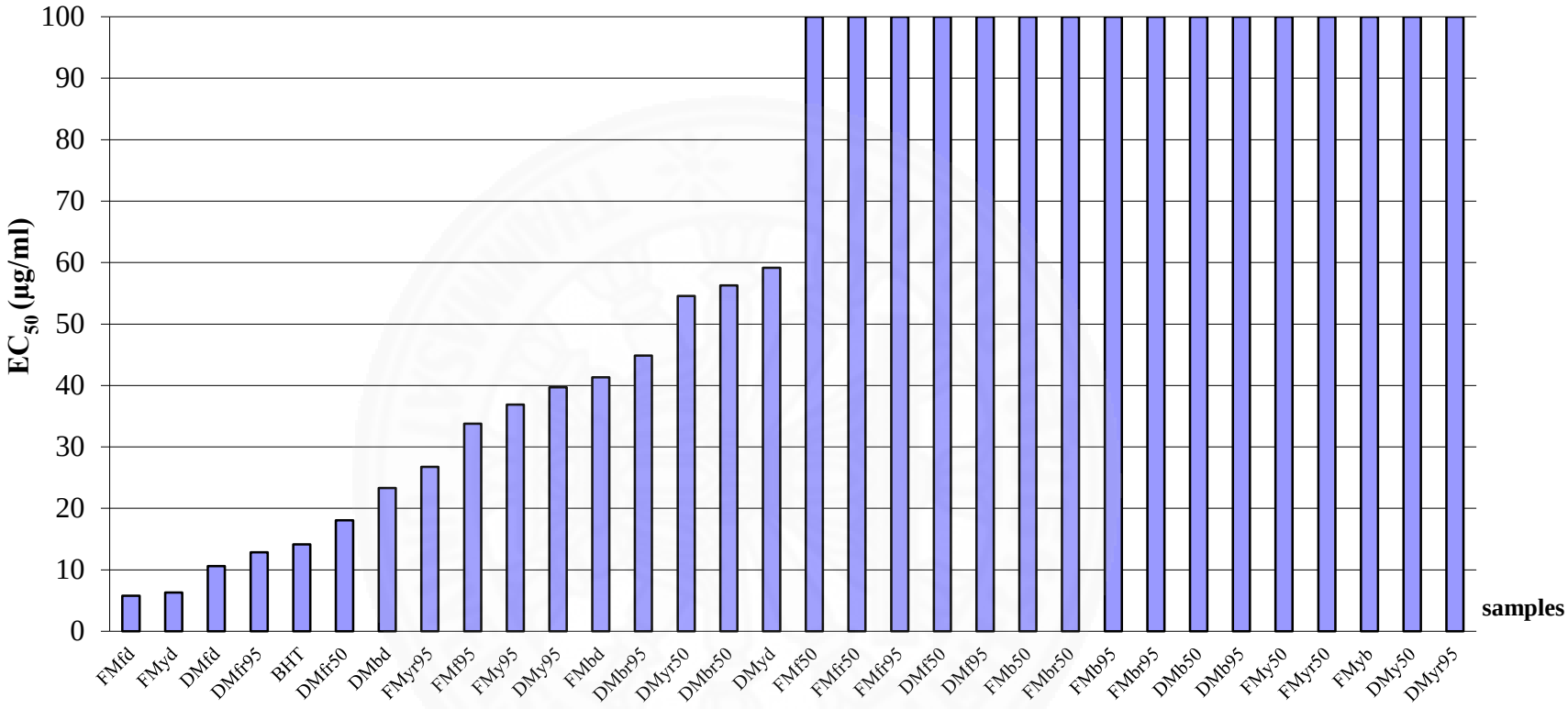


Figure 4.6

Anti-oxidant activity of crude extracts by DPPH radical scavenging assay (n=3)

Table 4.7 Total phenolic contents in *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts (mg GAE/g, n=3)

Musa sapientum Linn.condition	Part used	Total phenolic content (mg GAE/g, n=3)					
		Decoction	50%EtOH	Residue 50%EtOH	95%EtOH	Residue 95%EtOH	water
Fresh	Male flower	269.31±0.72	8.54±0.57	15.15±0.19	55.33±0.76	13.60±0.26	-
	Bract	42.96±0.74	3.27±0.92	8.90±0.34	9.67±0.63	45.96±0.96	-
	Young male bud	201.16±1.36	2.31±1.35	13.27±0.36	44.75±0.60	18.96±0.77	19.72±0.78
Dry	Male flower	146.85±0.66	11.67±0.37	91.13±1.45	3.60±1.16	123.74±0.76	-
	Bract	25.50±0.42	2.73±1.28	23.69±0.56	23.33±1.20	46.13±0.65	-
	Young male bud	36.25±0.56	27.90±1.40	51.27±0.43	58.79±0.75	75.58±0.67	-

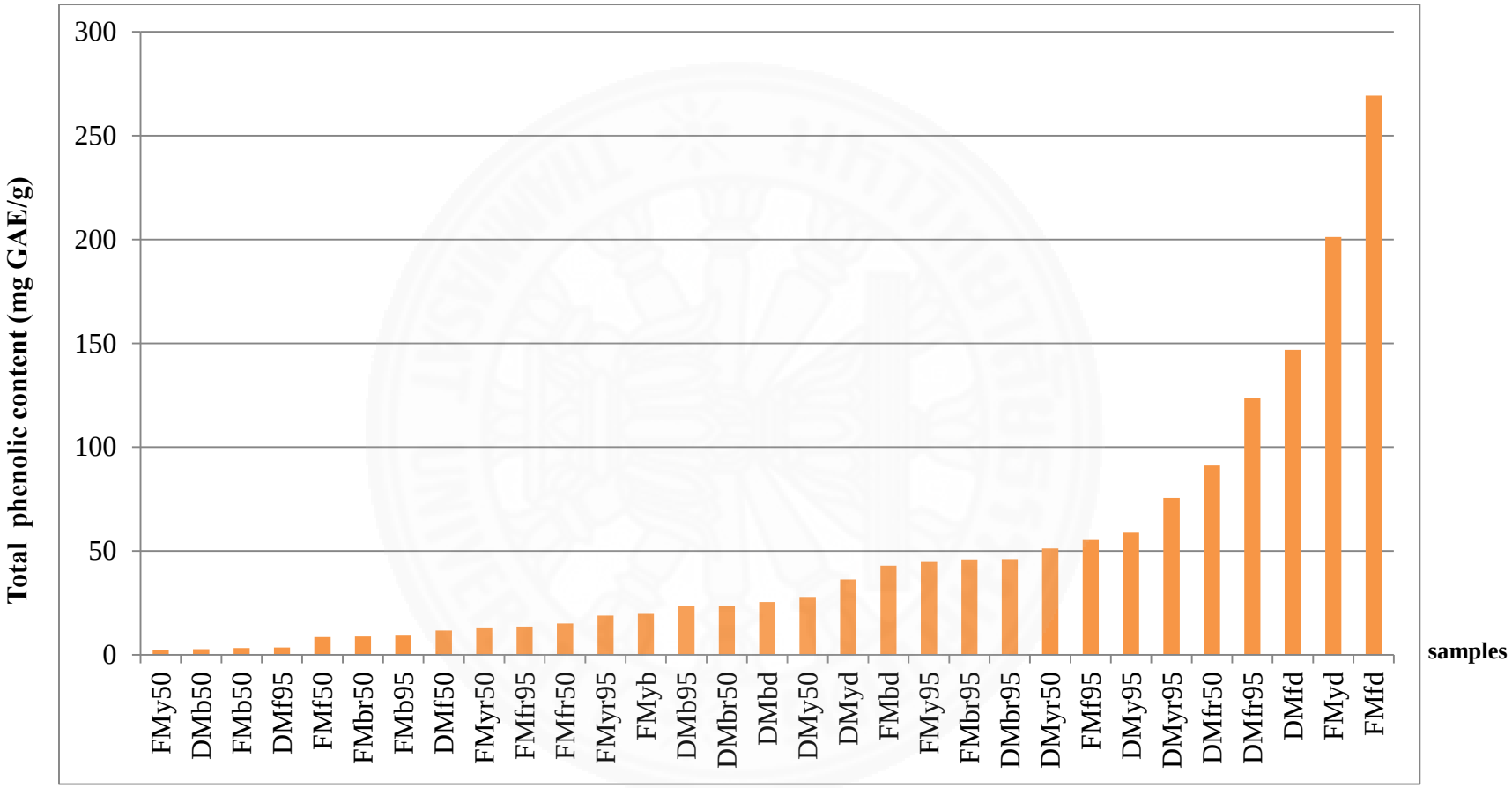


Figure 4.7

Total phenolic contents in *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts (mg GAE/g, n=3)

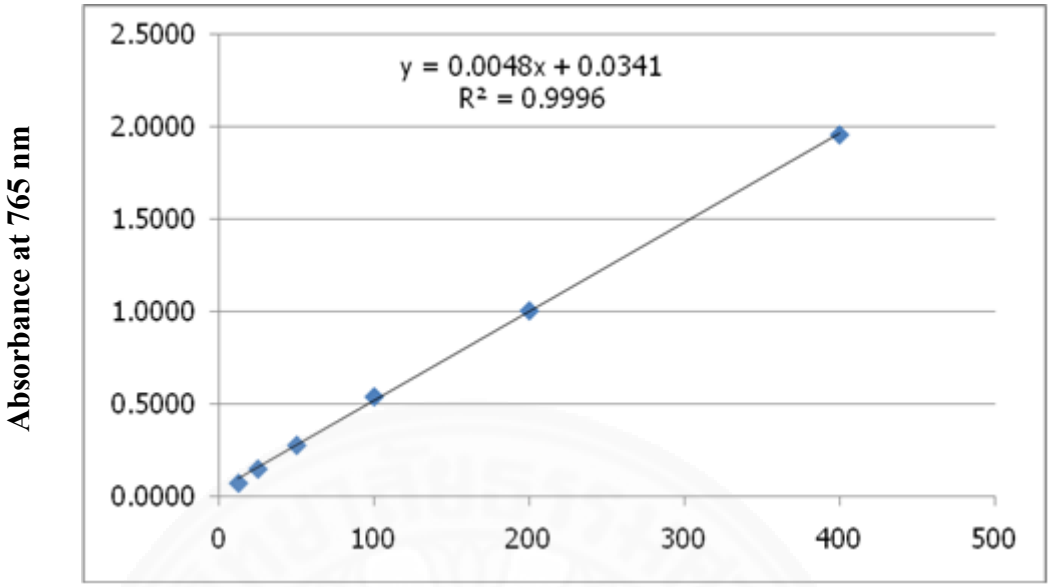


Figure 4.8

Standard calibration curve of gallic acid at 765 nm

Table 4.8 IC_{50} of NO inhibitory effect in *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts at various concentrations (µg/ml, n=3)

Part used	Extract	Code	%inhibition (µg/ml) (%survival of RAW264.7 cell)					IC_{50}±SEM (µg/ml)
			1	10	30	50	100	
Male flower	Fresh male flower decoction	FMfd	-	-	-	24.04±4.43 (22.66±4.00)	30.44±6.46 (21.70±2.52)	>100
	Fresh male flower 50%EtOH	FMf50	-	-	-	5.26±1.13 (5.60±1.18)	12.53±0.93 (-2.22±9.09)	>100
	Residue fresh male flower 50%EtOH	FMfr50	-	-	-	-7.81±0.26 (-0.08±9.20)	-9.51±0.14 (-8.72±2.23)	>100
	Fresh male flower 95%EtOH	FMf95	-	-	-	2.84±0.48 (-19.49±4.21)	14.55±2.43 (-6.34±5.21)	>100
	Residue fresh Male flower 95%EtOH	FMfr95	-	-	-	-14.34±2.08 (5.53±10.05)	-8.13±1.67 (-12.89±0.89)	>100
	Dry male flower decoction	DMfd	7.02±1.10 (-5.61±8.09)	77.32±1.99 (4.69±8.97)	90.80±3.30 (14.16±7.01)	95.94±3.03 (15.44±9.68)	94.00±3.64 (12.70±8.74)	5.98±0.19
	Dry male flower 50%EtOH	DMf50	-	-	-	7.67±11.04 (-3.16±6.45)	24.08±17.42 (1.82±13.14)	>100
	Residue dry male flower 50%EtOH	DMfr50	1.10±3.74 (3.38±5.18)	6.44±1.98 (4.13±6.18)	48.05±2.41 (-2.96±5.63)	70.63±2.00 (0.86±6.61)	83.89±4.92 (3.80±5.28)	31.17±1.37
	Dry male flower 95%EtOH	DMf95	-	-	-	6.50±1.45 (-5.07±3.72)	21.77±0.44 (-10.79±1.39)	>100
	Residue dry Male flower 95%EtOH	DMfr95	-10.45±2.36 (-4.69±5.81)	-5.02±1.29 (-9.83±3.33)	29.12±2.19 (1.22±2.20)	58.02±2.02 (-2.29±7.99)	82.12±0.53 (7.80±4.72)	43.46±0.72

Table 4.8 IC_{50} of NO inhibitory effect in *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts at various concentrations (µg/ml, n=3) (continued)

Part used	Extract	Code	%inhibition (µg/ml), (%survival of RAW264.7 cell)					IC_{50}±SEM (µg/ml)
			1	10	30	50	100	
Bract	Fresh bract decoction	FMbd	-	-	-	-5.97±2.99 (-3.96±1.14)	0.28±1.63 (-5.51±0.92)	>100
	Fresh bract 50%EtOH	FMb50	-	-	-	-13.28±7.34 (-12.41±11.57)	-7.04±4.40 (-30.90±4.58)	>100
	Residue bract 50%EtOH	FMbr50	-	-	-	-8.58±1.23 (-9.07±1.47)	-6.20±1.41 (-12.37±5.21)	>100
	Fresh bract 95%EtOH	FMb95	-	-	-	4.43±3.48 (-15.67±7.15)	27.60±8.56 (-35.16±7.86)	>100
	Residue fresh bract 95%EtOH	FMbr95	-	-	-	-9.33±0.86 (-2.31±13.53)	-7.85±0.62 (3.62±10.08)	>100
	Dry bract decoction	DMbd	-	-	-	16.85±4.17 (-5.85±4.95)	43.81±5.74 (0.14±0.28)	>100
	Dry bract 50%EtOH	DMb50	-	-	-	-0.87±0.64 (-23.81±12.45)	6.77±4.06 (-29.30±16.06)	>100
	Residue dry bract 50%EtOH	DMbr50	-	-	-	-1.34±0.50 (-32.52±8.99)	18.71±1.70 (-21.39±0.21)	>100
	Dry bract 95%EtOH	DMb95	-	-	-	14.07±1.26 (-29.49±13.85)	35.36±0.93 (-43.61±11.34)	>100
	Residue dry bract 95%EtOH	DMbr95	-	-	-	-6.12±3.25 (-23.94±1.00)	-3.86±4.09 (-31.54±6.69)	>100

Table 4.8 IC_{50} of NO inhibitory effect in *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts at various concentrations (µg/ml, n=3) (continued)

Part used	Extract	Code	%inhibition (µg/ml), (%survival of RAW264.7 cell)					IC_{50}±SEM (µg/ml)
			1	10	30	50	100	
Young male bud	Fresh young male bud decoction	FMyd	-	-	-	16.58±3.77 (-3.23±11.60)	24.76±6.90 (-3.32±9.20)	>100
	Fresh young male bud 50%EtOH	FMy50	-	-	-	0.63±1.48 (-7.49±9.04)	2.21±1.23 (-8.18±2.16)	>100
	Residue young male bud 50%EtOH	FMyr50	-	-	-	0.75±4.78 (3.56±12.85)	0.28±4.49 (4.40±13.98)	>100
	Fresh young male bud 95%EtOH	FMy95	-	-	-	19.00±2.18 (-2.61±5.74)	35.19±4.72 (0.86±1.75)	>100
	Residue fresh young male bud 95%EtOH	FMyr95	-	-	-	-5.74±3.68 (-16.93±6.28)	-1.71±12.74 (-10.54±8.15)	>100
	Fresh young male bud Blend with water	FMyb	-	-	-	-8.80±6.88 (-13.12±7.57)	-9.44±9.53 (-16.56±7.12)	>100
	Dry young male bud decoction	DMyd	11.71±0.53 (-6.70±2.09)	17.16±7.54 (-10.90±2.50)	71.25±0.59 (4.83±1.70)	86.12±0.96 (4.58±6.39)	89.48±0.85 (9.69±2.70)	20.91±0.23
	Dry young male bud 50%EtOH	DMy50	-	-	-	-4.988±2.546 (-0.818±4.874)	-6.98±4.79 (-10.98±3.32)	>100
	Residue dry young male bud 50%EtOH	DMyr50	-	-	-	-9.013±9.301 (-24.185±9.617)	-8.07±10.77 (-31.61±13.69)	>100
	Dry young male bud 95%EtOH	DMy95	-	-	-	9.812±3.619 (-45.183)±3.366	34.13±1.05 (-54.41)±0.51	>100
	Residue dry young male bud 95%EtOH	DMyr95	-	-	-	-1.971±1.586 (-16.113±13.338)	-4.81±1.26 (-23.47±6.94)	>100

Table 4.9 IC_{50} of NO inhibitory effect of prednisolone (µg/ml, n=3)

Positive control	%inhibition (µg/ml), (%survival of RAW264.7 cells)				IC_{50}±SEM (µg/ml)
	0.01	0.1	1	10	
Prednisolone	14.53±3.71 (-1.81±0.98)	37.26±0.54 (10.33±1.88)	55.75±2.20 (10.96±3.57)	61.49±3.45 (8.22±1.77)	0.16±0.01

Table 4.10 Summary of % yield, anti-oxidant activity, total phenolic contents and anti-inflammatory activities of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') extracts (n=3)

Part for used	Extraction	%yield		Anti-oxidant activity EC_{50}±SEM (µg/ml)		Total Phenolic contents (mg GAE/g, n=3)		Anti-inflammatory activity (IC_{50}±SEM, µg/ml, n=3)	
		Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
Male flower	Decoction	3.91	23.30	5.78±0.12	10.60±0.98	269.31±0.72	146.85±0.66	>100	5.98±0.19
	50%EtOH	1.88	23.28	>100	>100	8.54±0.57	11.67±0.37	>100	>100
	Residue 50%EtOH	0.48	38.20	>100	18.04±0.14	15.15±0.19	91.13±1.45	>100	31.17±1.37
	95%EtOH	3.51	8.97	33.76±0.50	>100	55.33±0.76	3.60±1.16	>100	>100
	Residue 95%EtOH	0.82	23.43	>100	12.85±0.36	13.60±0.26	123.74±0.76	>100	43.46±0.72
Bract	Decoction	2.35	34.83	41.35±0.93	23.32±0.58	42.96±0.74	25.50±0.42	>100	>100
	50%EtOH	1.50	23.71	>100	>100	3.27 ± 0.92	2.73±1.28	>100	>100
	Residue 50%EtOH	0.27	6.22	>100	56.30±0.36	8.90±0.34	23.69±0.56	>100	>100
	95%EtOH	1.55	12.13	>100	>100	9.67 ± 0.63	23.33±1.20	>100	>100
	Residue 95%EtOH	0.69	16.78	>100	44.85±0.18	45.96±0.96	46.13±0.65	>100	>100
Young male bud	Decoction	3.02	18.07	6.28±0.09	59.16±0.04	201.16±1.36	36.25±0.56	>100	20.91±0.23
	50%EtOH	0.75	16.09	>100	>100	2.31±1.35	27.90±1.40	>100	>100
	Residue 50%EtOH	0.34	12.85	>100	54.55±1.18	13.27±0.36	51.27±0.43	>100	>100
	95%EtOH	1.62	4.92	36.89±1.37	39.70±1.26	44.75±0.60	58.79±0.75	>100	>100
	Residue 95%EtOH	0.80	25.97	26.76±1.25	>100	18.96±0.77	75.58±0.67	>100	>100
	soaked in water	1.35	-	>100	-	19.72±0.78	-	>100	-

4.6 The stability tests of dry male flower decoction extract (DMfd)

Stability test of dry male flower decoction extract (DMfd) of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') was tested anti-oxidant activity by DPPH radical scavenging assay, total phenolic content and anti-inflammatory activity by NO inhibition from RAW264.7 cells that shows in Table 4.11. Dry male flower decoction (DMfd) was showed DPPH radical scavenging assay, total phenolic content of property and anti-inflammatory activity by NO inhibition which after Day 30 showed less anti-oxidant and anti-inflammatory activity and also less total phenolic content at accelerated condition (40±2ºC, 75±5% RH for 6 months) significantly different when compared with day 0. Thus, product from male flower should be kept in cool which should be continuously studies for temperature which keep product from male flower.

Table 4.11 Anti-oxidant activity, total phenolic contents and anti-inflammatory of the dry male flower decoction extract (DMfd) under accelerated condition (40±2ºC with 75±5%RH) for 6 months (n=3)

Day	Anti-oxidant activity EC_{50}±SEM µg/ml	Total phenolic contents (mg GAE/g) ±SEM	Anti-inflammatory activity IC_{50}±SEM µg/ml
0	9.55±0.45	147.15±5.77	8.89±0.31
15	10.89±1.20	155.02±3.35	19.54±1.36
30	15.63±1.13	137.29±2.63	96.21±0.34
60	24.14±0.52	32.36±0.36	>100
90	19.33±0.72	65.52±5.74	>100
120	20.21±0.73	60.36±1.02	>100
150	40.31±0.68	32.29±0.76	>100
180	54.90±0.73	55.44±4.31	>100

4.7 Bioassay-guided fractionation

Dry male flower decoction (DMfd) of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') shows high anti-oxidant and good anti-inflammatory which selected for isolated. 40 g of dry male flower decoction (DMfd) of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') was isolated using Diaion® HP-20 and using solvents ingredient from high-polarity to low polarity as mobile phase; 100% water, 25% methanol, 50% methanol, 75% methanol, 100% methanol and ethyl acetate (Figure 4.9). Each fraction (DF) was dried by rotary evaporator.

Table 4.12 IC_{50} values of anti-inflammatory property of column chromatography of the dry male flower decoction extract (DMfd) (n=3)

Sample	Anti-inflammatory activity IC_{50}±SEM, µg/ml	Anti-oxidant activity EC_{50}±SEM µg/ml
DMfd	146.85±0.66	10.60±0.98
100% Water	>100	-
25% MeOH	45.64±0.92	-
50% MeOH	>100	-
75% MeOH	>100	-
100% MeOH	27.75±1.76	-
EtOAc (Ethylacetate)	>100	-
BN1	>100	>100
BHT	-	14.12±0.81
Prednisolone	0.16±0.01	-

4.7.1 Column chromatography of active anti-inflammatory fraction

Weights of DA-DF were obtained in Figure 4.13. DA was obtained highest of weight (20.78 g) and DB (9.34 g), respectively. Six fractions were tested to determine their anti-inflammatory activity by NO inhibitory effect test. DE fraction performed is the highest of anti-inflammatory activity and DB fraction performed is

good of anti-inflammatory activity with IC_{50} values 27.75±1.76 and 45.64±0.92µg/ml, respectively (Table 4.12). DE fraction performed is not pure compound. Therefore, DB is high of weight and good anti-inflammatory activity which selected for isolation compound by column chromatography.

The fraction DB (5.02 g) (Figure 4.10) shows the highest % yield and good anti-inflammatory activity was selected to separate by column chromatography which % yield of DB is 9.34 g. The fraction of chromatography over a silica gel column were used as stationary phase and using of gradient solvent system from low polarity to high polarity: $CHCl_3$:MeOH:H_2O (9:3:0.5), $CHCl_3$:MeOH:Water (1:1:0.5), $CHCl_3$:MeOH:Water (3:7:0.5), MeOH:Water (100:0.5). After that, were collected $CHCl_3$:MeOH:H_2O (9:3:0.5) from solvent system which elution of solvent and fraction combine, followed by TLC examination and detection with acidic anisaldehyde spray and dried. This fraction gave four sub-fractions and fraction DBA were collected and separate on new column chromatography.

The fraction DBA was purified by column chromatography on silica gel using a gradient solvent system of $CHCl_3$:MeOH:H_2O, 9:3:0.5 and $CHCl_3$:MeOH, 1:1 and increasing stepwise polarity to 100% MeOH. This fraction gave three sub-fractions and fraction BA1 (Figure 4.13) was isolated as a pale yellow solid. Then, the pale yellow solid was recrystallized by one milliliter of methanol, chloroform and water. BN1 was tested by TLC examination which detection of acidic anisaldehyde spray (Figure 4.11). The structure was explained by HMBC and NOESY correlation (Figure4.15). BN1 showed not active on NO inhibitory activity and DPPH radical scavenging assay (Table 4.13).

Table 4.13 EC_{50} values of pure compound on Anti-oxidant activity and IC_{50} values of anti-inflammatory activity of the dry male flower decoction extract (DMfd) (n=3)

Sample	Anti-oxidant activity EC_{50}±SEM, µg/ml	Anti-inflammatory activity IC_{50}±SEM, µg/ml
BN1	>100	>100

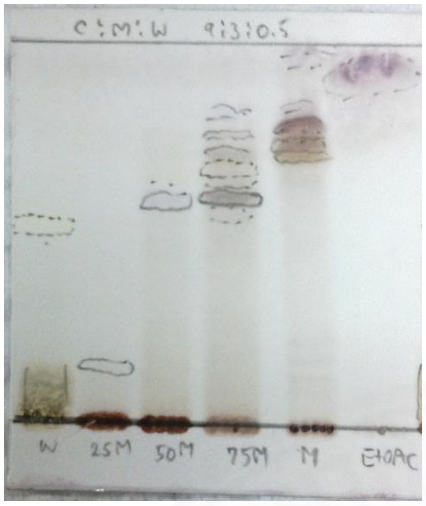


Figure 4.9 TLC of DA-DF
(Original picture)

Figure 4.10 TLC of DB
(Original picture)

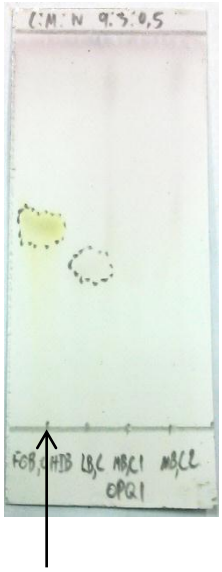
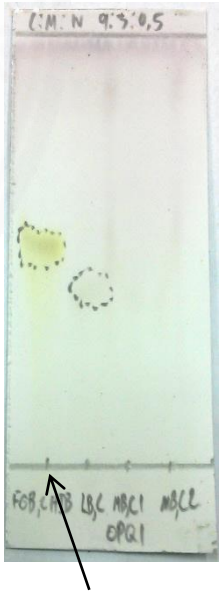


Figure 4.11 TLC of BN1
(Original picture)

(A)

(B)



(C)

Figure 4.12
After run of solvent system (A), (B) and (C) of TLC in BN1,
detected by UV light at 254 nm (Original picture)

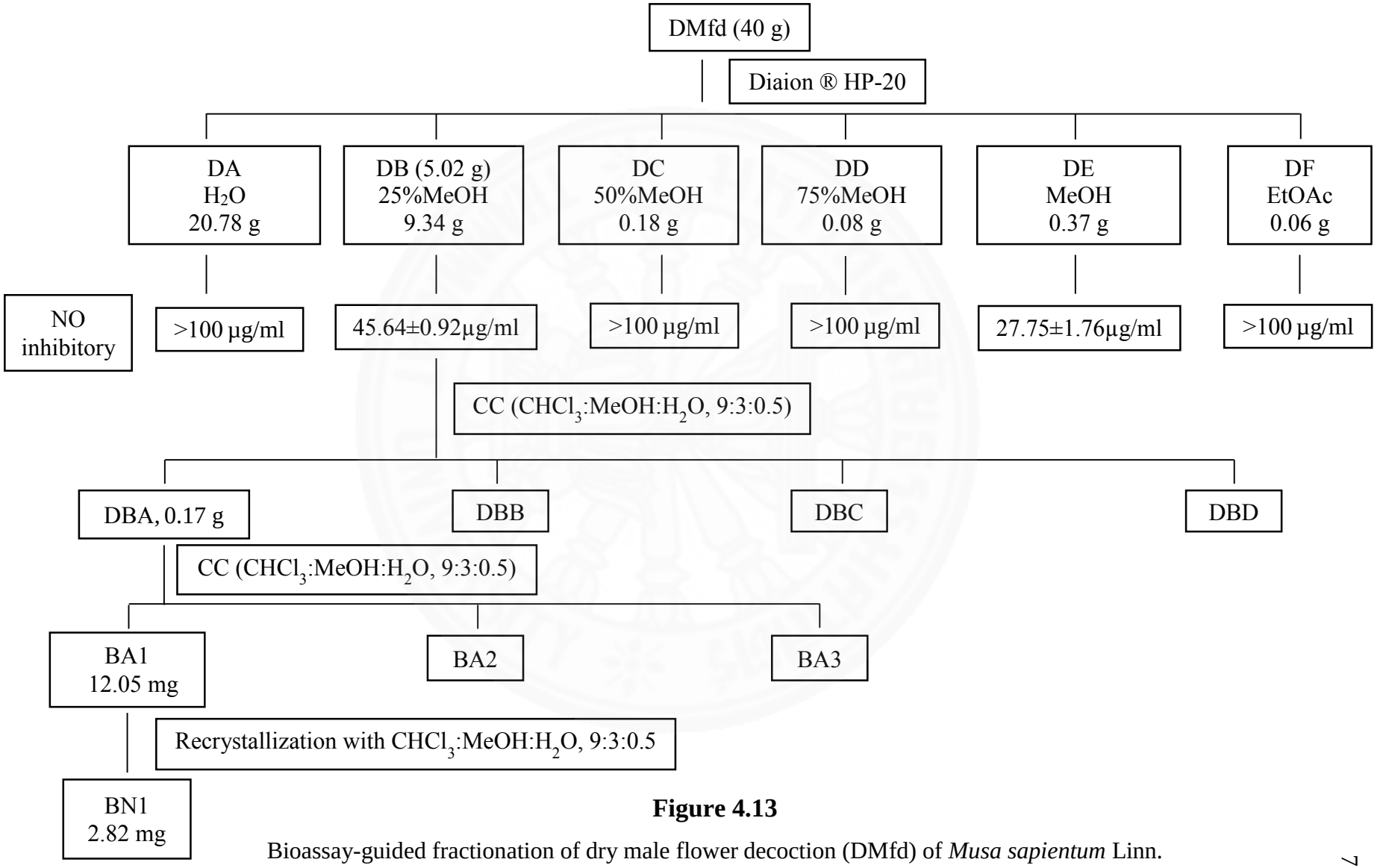


Figure 4.13

Bioassay-guided fractionation of dry male flower decoction (DMfd) of *Musa sapientum* Linn.

(*Musa* ABB GROUP CV. 'NAM WA')

4.8 Structure elucidation of BN1 from water extract of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA')

BN1 was obtained as a pale yellow solid. Its HR-ESI-TOF-MS $[M+Na]^+$ m/z at 290.09, corresponding to molecular formula $C_{10}H_{13}N_5O_4Na$. The UV absorption maxima at 260 nm.

^{13}C NMR, DEPT-135° and DEPT-90° experiment showed nine signals for ten carbons and ^1H NMR spectral data are shown Table 4.14. In the HMBC experiment, the aromatic methine proton at δ 8.18 (s, H-2) showed correlations with C-4 (δ 150.0) and C-6 (δ 157.6). The aromatic methine proton at δ 8.31 (s, H-8) showed correlations with C-4 (δ 150.0) and C-5 (δ 121.0). The anomeric ribonucleoside at δ 5.97 (d, J = 6.4, H-8) showed correlations with C-4 (δ 150.0), C-8 (δ 142.0) and C-2' (δ 75.5). In the NOESY experiment, H-1' also showed correlation with H-8 and H-4' (Figure 4.15). By comparison of the previously reported ^1H and ^{13}C NMR data with those of adenosine (2, Figure 4.16), the structure of BN1 was similar to adenosine (2) but different in that hydroxyl group appear at C-6 of BN1 while the amine group was in adenosine (2) (Ciuffreda *et al*., 2007). From the analysis of 1D and 2D spectral data, BN1 was assigned as (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol (Figure 4.14).

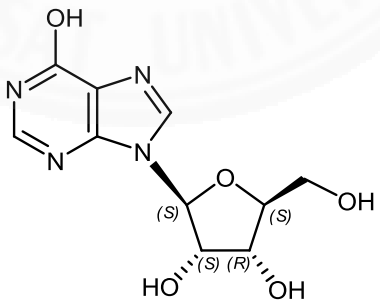


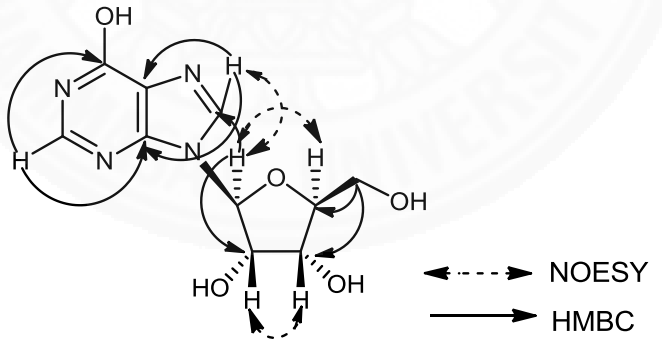
Figure 4.14

Isolated BN1 from water extract of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3, 4-diol

Table 4.14 ^{1}H (500 MHz), ^{13}C (125 MHz) NMR, DEPT and HMBC spectral data of BN1 in methanol-*d*$_4$

position	^{13}C	DEPT	^{1}H	HMBC
2	153.5	CH	8.18 (s)	C-4, C-6
4	150.0	C		
5	121.0[a]	C		
6	157.6	C		
8	142.0	CH	8.31 (s)	C-4, C-5
1'	91.2	CH	5.97 (d, *J* = 6.4)	C-4, C-8, C-2'
2'	75.5	CH	4.74 (t, *J* = 5.7)	C-1'
3'	72.7	CH	4.33 (q, *J* = 2.0)	
4'	88.2	CH	4.17 (brd, *J* = 2.3)	
5'	63.5	CH_2	3.89 (dd, *J* = 13.0, 2.5),	C-3', C-4'
			3.75 (dd, *J* = 13.0, 2.5)	

[a] Assigned based on HMBC experiment.



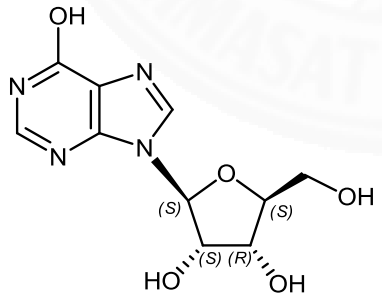
BN1

Figure 4.15

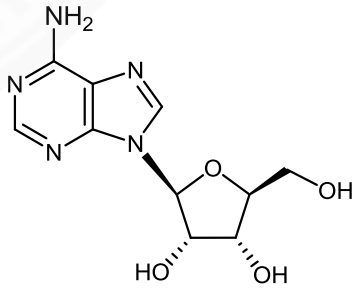
Selected HMBC and NOESY correlation of BN1

Table4.15 Comparison of ^{1}H and ^{13}C NMR spectral data of BN1 (in methanol-d_4) and adenosine (2, in DMSO-d_6, Ciuffreda *et al.*, 2007)

position	^{13}C		^{1}H	
	BN1	**2**	**BN1**	**2**
2	153.5	152.4	8.18 (s)	8.13 (s)
4	150.0	149.0		
5	121.0	119.3		
6	157.6	156.2		
8	142.0	139.9	8.31 (s)	8.34 (s)
NH_2				7.33 (brs)
1'α	91.2	87.9	5.97 (d, *J* = 6.4)	5.87 (d, *J* = 6.2)
2'β	75.5	73.4	4.74 (t, *J* = 5.7)	4.61 (ddd, *J* = 5.1, 6.2, 6.3)
3'	72.7	70.6	4.33 (q, *J* = 2.0)	4.14 (ddd, *J* = 3.0, 3.6, 3.7)
4'	88.2	85.9	4.17 (brd, *J* = 2.3)	3.96 (ddd, *J* = 3.0, 3.6, 3.7)
5'	63.5	61.6	3.89 (dd, *J* = 13.0, 2.5),	3.67 (ddd, *J* = 3.6, 4.4, 12.1),
			3.75 (dd, *J* = 13.0, 2.5)	3.55 (ddd, *J* = 3.7, 7.2, 12.1)



BN1



2

Figure 4.16

Chemical structure of isolated BN1 from water extract of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') compared to reference compound 2 (adenosine) from Ciuffreda *et al.*,2007

Adenosine is a purine nucleoside composed of a molecule of adenine attached to ribose sugar molecule (ribofuranose) moiety via a β-N9-glycosidic bond (Morton and Judith, 2012.; Buckingham, 1987.; Index, 2000). Adenosine is widely found in nature having an important role in biochemical processes such as energy transfers as adenosine triphosphate (ATP), adenosine diphosphate (ADP) and signal transduction of cyclic adenosine monophosphate (cAMP). It is a neuromodulator that believed to play a role in promoting sleep and suppressing of arousal. Role of adenosine is regulation of blood flow to several organs through vasodilation (Sato, 2005.; Costa and Biaggioni, 1998.; Morgan *et al*., 1991). Moreover, adenosine in endogenous forms are used as a medication as anti-arrhythmic agent (Morton and Judith, 2012.; Buckingham, 1987.; Index, 2000) for treating a number of forms of supraventricular tachycardia that can not be improved with vagal maneuvers (Kleinman *et al*., 2010). Common side effects are chest pain, shortness of breath sideways to tingling of senses and feeling faint (Kleinman *et al*., 2010). Serious side effects are worsening in dysrhythmia, low blood pressure and present to be safe in pregnancy (Kleinman *et al*., 2010). Drug interactions with dopamine can precipitate toxicity in a person. Carbamazepine can increase heart block. The dipyridamole of potentiates the action of adenosine requiring the use of lower doses. Methylxanthines (caffeine found in coffee, theophylline in tea and theobromine found in chocolate) antagonize adenosine's effects increased doses of adenosine may be required. The Nature of caffeine's purine structure can bind to some of the same receptors as adenosine (Sharron, 2001). Adenosine was first described more than 60 years ago. Adenosine receptors are shown on most cells and organs. A decade ago, the potent anti-inflammatory effect of adenosine was first described of, acting on specific A_2 receptors, inhibiting some but not at all of neutrophil functions. Adenosine can inhibit phagocytosis, generation of toxic oxygen metabolites and adhesion but does not inhibit degranulation or chemotaxis. The adenosine A_2 receptors modulates leukocyte function are linked to heterotrimetric GS signaling proteins, stimulation of adenylate cyclase, adenosine 3′, 5′-cyclic monophosphate does not act of the second messenger for the inhibition of the leukocyte function. The mechanisms still remain obscure on the occupancy of adenosine A_2 receptor on neutrophils that uncouples chemo attractant receptors from their stimulus of transduction proteins. The concentrations of

adenosine inhibiting the function of inflammatory cells of function are similarly observed *in vivo* and suggest a role for adenosine in modulation of inflammation *in vivo* (Cronstein, 1994).

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

The investigation of *Musa sapientum* Linn (male bud) was based on its use in Thai traditional medicine and Thai health food for preparation as part of postpartum breastfeeding care. Thirty-one samples of *Musa sapientum* Linn. (*Musa* ABB GROUP CV. 'NAM WA') of male buds (blossom) were separated into two parts to use as dried and fresh. The dried part was dried in a hot air oven at 50 °C before use and the fresh was used as a fresh sample. The male buds (blossom) were divided into three parts. First, the outside of bracts from the male bud. Second, the flowers inside the bract with anthers and ovary in the flower. Last, the young male bud inside dark violet and red of male bud. There were extraction by decoction, maceration in 50% ethanol, residue 50% ethanol, maceration in 95% ethanol, residue of 95% ethanol and blending with water of fresh young male buds only. The water extract of male bud by residue dry male flower maceration 50%EtOH (DMfr50) method showed the highest yield, followed by dry bract decoction (DMbd) and residue dry young male bud maceration 95%EtOH (DMyr95), respectively. Among ethanol extract of male bud by dry bract maceration 50%EtOH (DMb50) method showed the highest yield, followed by dry male flower maceration 50%EtOH (DMf50) and dry young male bud maceration 50%EtOH (DMy50), respectively.

Phytochemicals of *Musa sapientum* Linn. in male flowers bract and young male bud found as constituents include saponins, flavonoids, anthocyanin, alkaloids, deoxy sugar and tannins.

Anti-oxidant activity of all extracts of male bud were tested by DPPH radical scavenging assay. Fresh male flower decoction (FMfd) showed the highest antioxidant activity. Moreover, fresh young male bud decoction (FMyd), dry male flower decoction (DMfd) and residue dry male flower maceration 95%EtOH (DMfr95) also showed strong anti-oxidant activity (<15 µg/ml). These four extracts indicated stronger activity than BHT, as a positive control.

The total phenolic contents of male bud extracts was measured using Folin-Ciocalteu's method. Fresh male flower decoction (FMfd) also showed the

highest total phenolic contents. In addition, fresh young male bud decoction (FMyd), dry male flower decoction (DMfd) and residue dry male flower maceration 95%EtOH (DMfr95) also showed high total phenolic contents. These four extracts showed high content of total phenolic compounds with values more than 100 mg GAE/g.

Anti-inflammatory activity of male bud extracts were tested by determining their effects on inhibition of LPS-induced NO production in macrophages RAW 264.7 cells. The dry male flower decoction (DMfd) exhibited the most potent NO inhibitory activity follow by dry young male bud decoction (DMyd), residue dry male flower maceration 50%EtOH (DMfr50) and residue dry male flower maceration 95%EtOH (DMfr95).

The extract showing the highest of all the results with the anti-oxidant activity using DPPH assay, total phenolic contents using Folin-Ciocalteu's reagent, and anti-inflammatory by their effects on inhibition of LPS-induced NO production in macrophages RAW 264.7 cells on antigen-induced was tested for stability. Dry male flower decoction (DMfd) was showed poor results on total phenolic content, antioxidant and anti-inflammatory activities after Day 30 after it was keep in under the accelerated conditions (40±2 ºC, 75±5% RH for 6 months). All values at day 30 showed significantly different when compared with day 0. This result concluded that. The dry male flower decoction is not stable and activity reduces with in 2 months because this extract had no change phenolic contents and all activity within 15 days.

BN1 were isolated from dry male flower decoction (DMfd) using column chromatography. BN1 was assigned as (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol. BN1 has no anti-oxidant activity and anti-inflammatory activity.

In summary, dry male flower decoction (DMfd) was showed high antioxidant activity, high total phenolic contents values and high anti-inflammatory activity. Hence, this result shows the decoction method most suitable and support using this Thai medicinal plant for preparing food for postpartum care especially breast feeding, preventive in common family cuisine, convenient and appropriate for preparing dried male flower of postpartum care.

Further of studies should be continued to estrogenic activities for outstanding on anti-estrogen or no anti-estrogen of estrogen hormones from *Musa sapientum* Linn. extracts. Moreover, residue dry male flower maceration 50%EtOH (DMfr50), residue dry male flower maceration 95%EtOH (DMfr95) and dry young male bud decoction (DMyd) were also interesting because they showed high percent yield as well as anti-oxidant and anti-inflammatory. Therefore, they should be continued of study the stability and isolate active compounds from these active extract. Finally, *Musa sapientum* Linn. male bud extracts should be continued of study for anti-oxidant activity such as NBT assay and anti-inflammatory such as COX-1, COX-2 and TNF-α because there is no report on two these assay.

REFERENCES

Books and Book Articles

Cadenas, E., Packer, L. (2002). Handbook of antioxidants (2nd edition). California, Marcel Dekker.

Chandrasoma, P., Taylor, C.R. (1998). *Part A.General Pathology, Section II. The Host Response to Injury. Concise Pathology* (3rd ed.). New York, N.Y.: McGraw-Hill.

Crozied, A., Jaganath, I., Clifford, M.N., Ashihara, H. (2006). Phenols, polyphenols and tannins: an overview. *Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet,* Blackwell Publishing, Oxford, pp. 1-24.

Fernández, S. A.M. (2009). Antioxidants and diseases: Obesity. In: Morales González JA, Fernández Sánchez A M, Bautista Ávila M, Vargas Mendoza N, Madrigal Santil-lán E O. (eds.) Antioxidants and Chronic Degenerative Diseases. México: Ciencia al Día; 2009. P. 411-25.

Gallin, John, I., Snyderman, Ralph. (1999), Overvies. In D. T. Fearon, B. F. Haynes, C. Nathan (Eds.), *Inflammation: basic principles and clinical correlates* (3rd ed.). Philadelphia,USA:Lippincott Williams & Wilkins.

Gross, G.Gross. (1992). Enzymes in the biosynthesis of hydrolysable tannins. In: *Plant Polyphenols,* R.W. Heminway, P.E. Laks and S.J. Branham (eds.). Plenum Press, New York, pp. 43-60.

Kumar, V., Abbas, A. K., Fausto, N. (2005). *Robbin and Cotran pathologic basis of disease* (7th ed.). Philadelphia: Elsevier Saunders.

Ministry of Public Health (2000). *Thai Herbal Pharmacopoeia* (*THP*), Standardization of Thai Herbal Medicines. Volume 2, (pp. 134-138, 141), Department of Medical Science, Ministry of Public Health, Thailand.

Sabchalern, P. (2005). *The self-reliant health care with herbal medicine in basic health care.* Bangkok, Thailand: Samcharernpanit.

The National Archives of Thailand (1999). Patsartsongkroh: The Medical knowledge and the literary heritage of the nation. Bangkok, Thailand: Khurusapha Ladprao.

World Health Organization (2007). WHO guidlines for assessing quality of herbal medicines with reference to contaminants and residues. (p.24), WHO Library Cataloguing-in-Publication Data.

Articles

Agarwal, PK., Singh, A., Gaurav, K., Goel, S., Khanna, HD., Goel, RK. (2009). Evaluation of wound healing activity of extracts of plantain banana *Musa sapientum var. paradisiacal* in rats. *Indian Journal of Experimental Biology, 47*(1), 32-40.

Aktan, F. (2004). iNOS-mediated nitric oxide production and its regulation. *Life Sciences, 75,* 639-653.

Alexandra Pazmiño-Duran, E., Mónica Giusti, M., Ronald, E., Wrolstad, M., Beatriz, A., Glória. (2000), Anthocyanins from banana bracts (*Musa X paradisiaca*) as potential food colorants. *Journal of Food Chemistry*, *73*(2001), 327-332.

Balentine, D. A., Wiseman, S. A., Bouwens, L. C. (1997). The chemistry of tea flavonoids. *Critical review in Food science and Nutrition*, *37*(8), 693-704.

Beecher, G. R. (2003). Overview of dietary flavonoids: nomenclature, occurrence and intake. *Journal of Nutrition*, *133*(10), 3248S-3254S.

Bolisetty, S., & Jaimes, E. A. (2013). Mitochondria and reactive oxygen species: physiology and pathophysiology. *International Journal of Molecular Sciences*, *14*(3), 6306-6344.

Bor, J. Y., Chen, H. Y., Yen, G. C. (2006). Evaluation of antioxidant activity and inhibitory effect on nitric oxide production of some common vegetables. *Journal of Agricultural and Food Chemistry, 54,* 1680-6.

Bucci, M., Roviezzo, F., Posadas, I., Yu, J., Parente, L., Sessa, W. C., *et al*. (2005). Endothelial nitric oxide synthase activation is critical for vascular leakage during acute inflammation *in vivo. Proceedings of the National Academy of Sciences, 102*(3), 904-908.

Buckingham, J. (1987). Dictionary of Organic Compounds. *CRC Press.* pp. 75. ISBN 978-0-412-54090-5.

Chen, S., Kao, Y. C., Laughton, C. A. (1997). Binding characteristics of aromatase inhibitors and phytoestrogens to human aromatase. *Journal of Steroid Biochemistry and Molecular Biology, 61*, 107-15.

Ciuffreda, P., Casati, S., Manzocchi, A. (2007). Complete ^{1}H and ^{13}C NMR spectral assignment of α- and β-adenosine, 2′-deoxyadenosine and their acetate derivatives. *Magnetic Resonance in Chemistry*, *45*(9), 781-784.

Conner, E. M., & Grisham, M. B. (1996). Inflammation, free radicals, and antioxidants. *Nutrition*, *12*(4), 274-277.

Cooke, M. S., Evans, M. D., Dizdaroglu, M., Lunec, J. (2003). Oxidative DNA damage: mechanisms, mutation, and disease. *Faseb Journal*, *17*(10), 1195-1214.

Costa, F., Biaggioni, I. (1998). Role of nitric oxide in adenosine-induced vasodilation in humans. *Hypertension. 31*(5), 1061–1064. PMID 9576114. doi:10.1161/01.HYP.31.5.1061.

Cronstein, B.N. (1994). Adenosine, an endogenous anti-inflammatory agent. *Journal of Applied Physiology Published*, *76*(1), 5-13.

Darley-Usmar, V., Wiseman, H., Halliwell, B. (1995). Nitric oxide and oxygen radicals: a question of balance. *Federation of European Biochemical Societies*, *369*, 131-5.

Davis, K. L., Martin, E., Turko, I. V., Murad, F. (2001). Novel effects of nitric oxide. *Annual Review o fPharmacology and Toxicology, 41*, 203-236.

Dutra, R. C., Leite, M. N., Barbosa, N. R. (2008). Quantification of phenolic constituents and antioxidant activity of Pterodon emarginatus vogel seeds. *International Journal of Molecular Sciences*, *9*(4), 606-614.

Fang, Y. Z., Yang, S., Wu, G. (2002). Free radicals, antioxidants, and nutrition. *Nutrition*, *18*(10), 872-879.

Farnsworth, N. R. (1966). Biological and phytochemical screening of plants. *Journal of Pharmaceutical Sciences, 55,* 225-76.

Ferrero-Miliani, L., Nielsen, O. H., Andersen, P.S., Girardin, S. E. (2006). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. *Clinical and Experimental Immunology, 147,* 227-235.

Flora, S. J. (2009). Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxidative medicine and Cellular longevity*, *2*(4), 191-206.

Frie, B., Stocker, R., Ames, B.N. (1988). Antioxidant defences and lipid peroxidation in human blood plasma. *Proceedings of the National Academy of Sciences, 37,* 569-71.

Gatti, L. (2008). Maternal perceptions of insufficient milk supply in breastfeeding. *Journal of Nursing Scholarship, 40,* 355-63.

Goel, RK., Sairam, K., Rao, CV. (2001). Role of gastric antioxidant and anti-*Helicobactor pylori* activities in antiulcerogenic activity of plantain banana (*Musa sapientum* var. *Paradisiacal*). *Indian Journal of Experi Biology*, *39*(7), 719-22.

Guetens, G., De Boeck, G., Highley, M., Van Oosterom, A. T., De Bruijn, E. A. (2002). Oxidative DNA damage: biological significance and methods of analysis. *Critical Reviews in Clinical Baboratory Sciences*, *39*(4-5), 331-457.

Hermann, K. (1976). Flavonols and flavons in food plants: a review. *Journal of Food Technology*, 11, 433-448.

Hetrick, E. M., Schoenfisch, M. H. (2009). Analytical chemistry of nitric oxide. *Annual Revies of Analytical Chemistry, 2,* 409-433.

Hiroshi, A., Claudio, S., Natalia, L., Trevor, A. T. (2000). Purine metabolism during white spruce somatic embryo development: salvage of adenine, adenosine and inosine. *Journal of Plant Science, 160,* 647-657.

Hobbs, A. J., Higgs, A., Moncada, S. (1999). Inhibition of nitric oxide synthase as a potential therapeutic target. *Annual Review of Pharmacology and Toxicology. 39,* 191-220.

Index Nominum (2000). International Drug Directory. *Taylor & Francis. pp. 18-*. ISBN 978-3-88763-075-1.

Kannes, K., Tisserat, B., Berhow, M., Vandercook, C. (1993). Phenolic composition of various tissues of rutaceae species. *Phytochemistry*, *32*, 967-974.

Kim, B. H., Reddy, A. M., Lee, K. H., Chung, E. Y., Cho, S. M., Lee, H., *et al.* (2004). Inhibitory mechanism of chroman compound on LPS-induced nitric oxide production and nuclear factor-κB activation. *Biochemical and Biophysical Research Communications, 325,* 223-228.

Kim, N. Y., Kang, T. H., Song, E. K., Pae, H. O., Chung, H, T., Kim, Y. C. (2000). Inhibitory effects of butanol fraction of the aqueous extract of *Forsythia koreana* on the nitric oxide production by murine macrophage-like RAW 264.7 cell. *Journal of Ethnopharmacology, 73,* 323-327.

Kleinman, ME., Chameides, L., Schexnayder, SM., *et al*. (2010). Part 14: pediatric advanced life support: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation*. 122(18 Suppl 3), S876-908.

Kohen, R., Nyska, A. (2002). Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic Pathology*, *30*(6), 620-650.

Kurzer, M. S. (2002). Hormonal effects of soy in premenopausal women and men. *Journal of Nutrition*, *132*, 570S-573S.

Lewis, DA., Fields, WN., Shaw, GP. (1999). A natural flavonoid present in unripe plantain banana pulp (*Musa sapientum* L. *var. paradisiacal*) protects the gastric mucosa from aspirin-induced erosions. *Journal of Ethnopharmacology, 65*(3) 283-288.

Lobo, V., Patil, A., Phatak, A., Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, *4*(8), 118-126.

MacMicking, J., Xie, Q. W., Nathan, C. (1997). Nitric oxide and macrophage function. *Annual Review of Immunology, 15,* 325-350.

Mahmood, A., Ngah, N., Omar, MN. (2011). Phytochemicals constituent and antioxidant activities in *Musa x Paradisiaca* flower. *European Journal of Science Research, 66,* 311-318.

Mahmood, A., Omar, M. N., Ngah, N. (2012). Galactagogue effects of *Musa* x paradisiaca flower extract on lactating rats. *Asian Pacific Journal of Tropical Medicine*, *5*, 882-6.

Manach, C., Scalbert, A., Morand, C., Remesy, C., Jimenez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition*, *79*(5), 727-747.

Mateos, R., Bravo, L. (2007). Chromatographic and electrophoretic methods for the analysis of biomarkers of oxidative damage to macromolecules (DNA, lipids, and proteins). *Journal of Separation Science*, *30*(2), 175-191.

Matsuo, T., Itoo, S. (1981). Comparative studies of condensed tannins from several young fruits. *Engei Gakkai Zasshi, Journal of Japanese for Horticultural Science, 50*(2), 262-9.

Miliauskas, G., Venskutonis, P. R., Van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Journal of Food Chemistry*, *85*, 231-237.

Morgan, JM., McCormack, DG., Griffiths, MJ., Morgan, CJ., Barnes, PJ., Evans, TW. (1991). Adenosine as a vasodilator in primary pulmonary hypertension. *Circulation.84* (3), 1145–1149. PMID 1884445. doi:10.1161/01.CIR.84.3. 1145.

Morton, I.K., Judith, M., Hall. (2012). Concise Dictionary of Pharmacological Agents: Properties and Synonyms. *Springer Science & Business Media.* pp. 106. ISBN 978-94-011-4439-1.

Pannangpetch, P., Vuttivirojana, A., Kularbkaew, C., Tesana, S., Kongyingyoes, B., Kukongviriyapan, V. (2001). The antiulcerative effect of Thai *Musa* species in rat. *Journal of Phytotherapy Research, 15*(5) 407-410.

Prathan, Luecha., Kaoru, Umehara., Toshio, Miyase., Hiroshi, Noguchi. (2009). Antiestrogenic Constituents of the Thai Medicinal Plants Capparis flavicans and Vitex glabrata. *Journal of Natural Products, 72*, 1954-1959.

Reynolds, JEF. (1989). The extra pharmacopocia. London. *The Pharmaceutical Press,* 779.

Riggins, R. B., Bouton, A. H., Liu, M. C., Clarke, R. (2005). Antiestrogens, aromatase inhibitors and apoptosis in breast cancer. *Journal of Vitamins and Hormones*, *71*, 201-237.

Sabri, Fatima., Zohra, B. M., Sabri, Samira., Alsayadi Muneer M.S. (2012). Phytochemical Screening and identification of some compounds from Mallow. *Journal of Natural Product and Plant Resources, 2,* 512-516.

Saha, K., Lajis, N. H., Israf, D. A., Hamzah, A. S., Khozirah, S., Khamis, S., *et al.* (2004). Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. *Journal of Ethnopharmacology, 92,* 263-267.

Sato, A. (2005). Mechanism of vasodilation to adenosine in coronary arterioles from patients with heart disease. *American Journal of Physiology. Heart and Circulatory Physiology*. *288* (4), H1633–1640. *PMID 15772334*. *doi*:*10.1152/ajpheart.00575.2004.*

Scalbert, A., Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition, 130*(8S Suppl), 2073S-2085S.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental physiology, 82,* 297-295.

Sluyser, M., RijKers, AW., De Goeij, CC., Parker, M. and Hilkens, J. (1988) Assignment of estradiol receptor gene to mouse chromosome 10. *Journal of Steroi Biochem, 31,*757-761.

Sobti, J., Mathur, G. P., Guta, A., WHO (2002). WHO's proposed global strategy for infant and young child feeding: a viewpoint. *Journal of the Indian Medical Association , 100,* 502-4, 506.

Tezuka, Y., Irikawa, S., Kaneko, T., Banskota, A. H., Nagaoka, T., Xiong, Q., *et al*. (2001). Screening of Chinese herbal drug extracts for inhibitory activity on nitric oxide production and identification of an active compound of *Zanthoxylum bungeanum. Journal of Ethnopharmacology, 77,* 209-217.

Thiantanawat, A., Long, B. J. & Brodie, A. M. (2003). Signaling pathways of apoptosis activated by aromatase inhibitors and antiestrogens. *Journal of Cancer Research, 63,* 8037-50.

Tewtrakul, S., Itharat, A. (2007). Nitric oxide inhibitory substances from the rhizomes of *Dioscorea membranacea*. *Journal of Ethnopharmacology*, *109*, 412-6.

Tewtrakul, S., Subhadhirasakul, S. (2008). Effects of compounds from *Kaempferia parviflora* on nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha production in RAW264.7 macrophage cells. *Journal of Ethnopharmacology*, *120*, 81-4.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The Internationa Journal of Biochemistry and Cell Biology*, *39*(1), 44-84.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico Biological Interactions*, *160*(1), 1-40.

Vitrac, X., Bornet, A., Vanderlinde, R., Valls, J., Richard, T., Delaunay, J. C., Mérillon, J.M., Teissedre, P. L. (2005). Determination of stilbenes (delta-viniferin, trans-astringin, trans-piceid, cis- and trans-resveratrol, epsilon-viniferin) in Brazilian wines. *Journal of Agricultural and Food Chemistry*, *53*(14), 5664-5669.

WHO/UNICEF (1990). Protecting, promoting and supporting breastfeeding: the special role of maternity services. A joint WHO/UNICEF statement. *International Journal of Gynecology & Obstetrics*, 31 Suppl 1, 171-83.

Wickens, A. P. (2001). Ageing and the free radical theory. *Respiratory Physiology*, *128*(3), 379-391.

Yamazaki, K, H. A., Kokusenya, Y., Miyamoto, T., Sato, T. (1994). Electrochemical method for estimating the antioxidative effect of methanol extracts of crude drugs. *Journal of the Chemical and Pharmaceutical Bulletin*, *42*, 1663-1665.

Electronic Media

Thai Breastfeeding Center Foundation (2016). First breastfeeding. Available from: http://www.thaibreastfeeding.org/page.php?id=61.

National Statistical Office, Ministry Of Information And Communication Technology (2006). Report Of 2006 Reproductive Health Survey. Available from: http://web.nso.go.th/index.htm.

Sharron, W. Smith. (2001). Coffee: How Much Is Too Much?. *Consumer Reports,* 64–65. Available from:http://www.chemistryexplained.com/BoCe/Caffeine .html.

World Health Organization (WHO) (2016). Stability Testing of Pharmaceutical Products in a Global Environment. Retrieved May 1, 2016 from http://www. google.co.th/url?sa=t&rct=j&q=&esrc=s&source=web&cd=6&cad=rja&uact= 8&ved=0ahUKEwi6pbmb4PbMAhWIpo8KHdGVApkAFghKMAU&url=http %3A%2F%2Fwww.who.int%2Fmedicines%2Fareas%2Fquality_safety%2Fqu ality_assurance%2FRAJ2006WHOStability.pdf&usg=AFQjCNHvaSGBhVd_ 0R1SkeqNLuoJ0Pm2rw&bv.122852650,d.c2I.

Thesis

Itharat, A. (2003). Studies on bioactivity and compounds of five Thai medicinal plants called 'Hua-Khao-Yen'. Doctoral Thesis (Ph.D.), University of London.

Itharat, A., Sakpakdeejaroen, I. (2010). Development of banana extract for drug and health food. Faculty of Medicine, Thammasat University.

Panyoyai, Panu. (2008). *Development of Banana Fiber for Textile.* Unpublished master's thesis dissertation, Chiang Mai University, Faculty of Engineering.

Phuaklee, P. (2012). Anti-allergic, Anti-inflammatory Activities and Development of Cream Product for Skin Allergies from *Musa sapientum* Extracts. Faculty of Medicine, Thammasat University.

Srikwan, K. (2013). Biological activities of *Smilax glabra* extracts. Faculty of Medicine, Thammasat University.

Makchuchit, S. (2010). Anti-inflammatory and Anti-allergic Activities of Thai Traditional Medicine Preparation Called Prasaprohyai. Faculty of Medicine, Thammasat University.

Tesorn, R. (2009). Antimicrobial activity of Thai traditional medicinal plants extract incorporated alginate-tapioca starch based edible films against food related bacteria including foodborne pathogens. Unpublished doctoral dissertation, University of Hohenheim, Faculty of Agricultural sciences, Institute for environmental and animal hygiene and veterinary medicine (with animal clinic).

Wibuloutai, J. (2006). Inhibitory effect of nitric oxide production on inflammation and apoptosis in macrophage RAW 264.7 by extract from seed coat of *Tamarindus indica* L. Unpublished doctor of philosophy's thesis, Suranaree University of Technology, Faculty of Sciences.

Yuenyongsawat, S., & Kongthong, S. (1994). *Study on the Properties of Polysaccharide Extracts from Musa peels.* Prince of Songkla University, Faculty of Pharmaceutical sciences.

APPENDICES

APPENDIX A

Chemical Reagents

Reagent for DPPH radical scavenging assay

Preparation of stock $6x10^{-5}$ M DPPH in absolute ethanol

DPPH	2.4 mg
Absolute ethanol	100 ml

Keep it in the volumetric flask protected from light with wrapped foil and stored at 4ºC

Reagent for total phenolic content

0.2M of Folin-Ciocalteu's reagent

Stock of 2M Folin-Ciocalteu's reagent was diluted 10 fold with distilled water. (Prepare fresh daily)

7.5% w/w Sodium carbonate (Na_2CO_3)

Na_2CO_3	7.5 g
Distilled water	100 ml

Reagent for NO inhibitory effect using the Griess reagent

Griess reagent

Sulfanilamide	1 g
N-(1-naphthyl)ethylenediamine dihydrochloride	0.1 g
Phosphoric acid (H_3PO_4)	2.5 g
Distilled water	100 ml

The reagent was protected from light with wrapped foil and stored at 4ºC

MTT solution (5 mg/ml)

3-(4,5-Dimethyl-2-thiazolyl)-2,5-dipheyl-2*H*-tetrazolium bromide or Thiazolyl blue tetrazolium bromide	200 g
PBS	40 ml

The reagent was protected from light with wrapped foil and stored at 4ºC

0.04 M HCl in Isopropanol

HCl	0.83 ml
Isopropanol	250 ml

(Stored at room temperature)

Reagent for cell culture

RPMI medium 1640

10.4 g of RPMI medium 1640 powder is dissolved with sterile water in 500 ml. 2.0 g of sodium bicarbonate is diluted with sterile water in 1000 ml. 10% NaOH or 10% HCl adjust pH 7.2-7.4 and filter though 0.2 micron membrane filter. Keep in sterile bottle.

400 ml of the complete RPMI medium 1640 is mixed with 40 ml fetal bovine serum and 4 ml penicillin-streptomycin and stored at 4ºC.

10% Hydrochloric acid (HCl)

Conc. HCl (37%)	27 ml
Distilled of water	100 ml

10% Sodium hydroxide (NaOH)

NaOH	10 g
Distilled water	100 ml

Fetal bovine serum (FBS)

Slowly thaw the frozen FBS (inactivate), heat 56ºC for 30 min

(Aliquot, stored at -20 ºC)

Phosphate buffer saline (PBS)

PBS	1 tablet
Distilled water	100 ml

(Sterilize by autoclave 121ºC for 15 min, stored at 4 ºC)

Penicillin-streptomycin (P/S)

Slowly thaw the frozen P/S in water bath at 37 ºC until complete thaw

(Aliquot, stored at -20 ºC)

Trypsin-EDTA

Slowly thaw the frozen 0.5% trypsin-EDTA in water bath at 37 ºC until complete thaw (Aliquot, stored at -20 ºC)

APPENDIX B

Figures

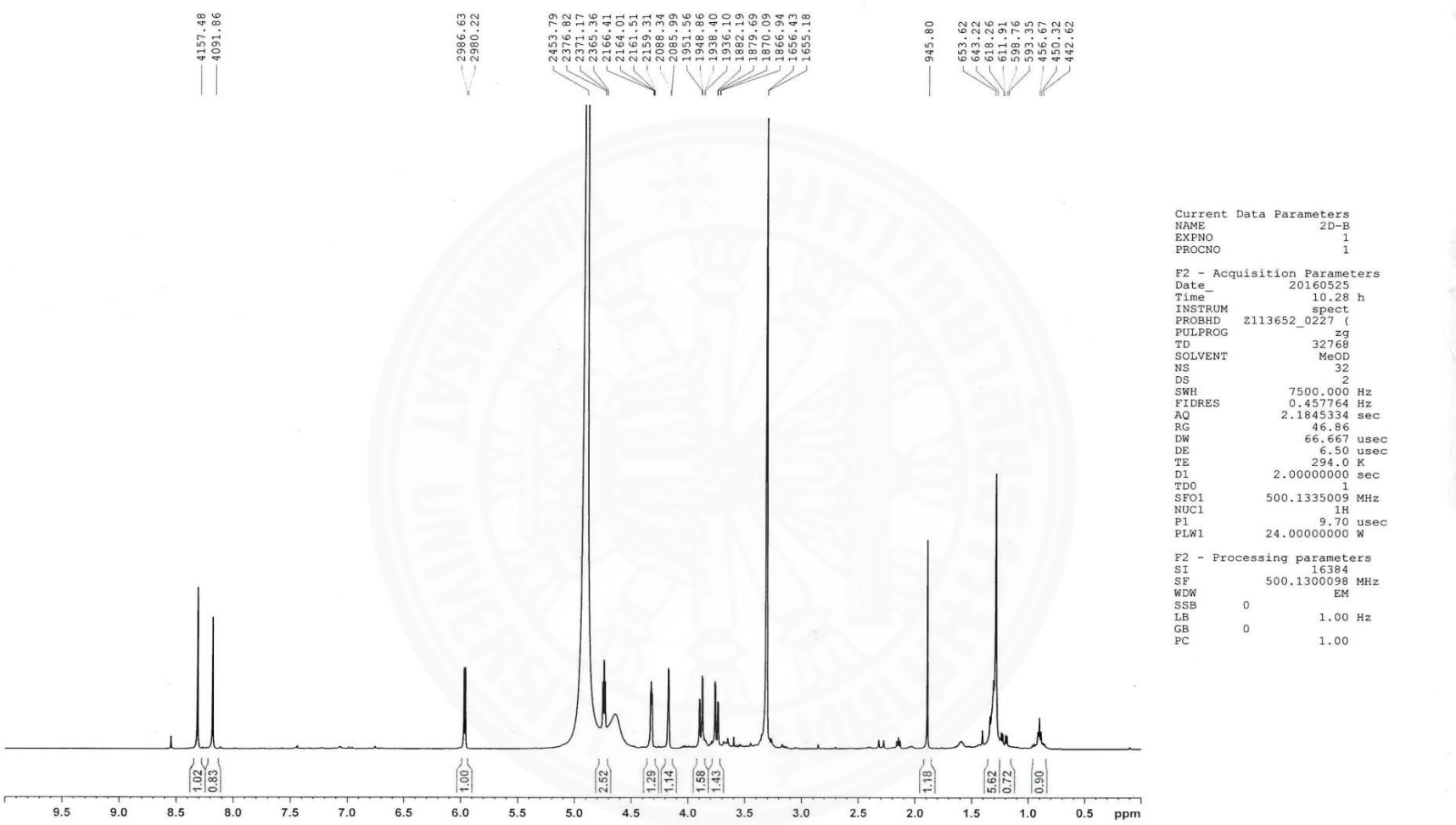


Figure 1

^{1}H-NMR Spectrum of (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol in MeOH, $CHCl_3$ and H_2O

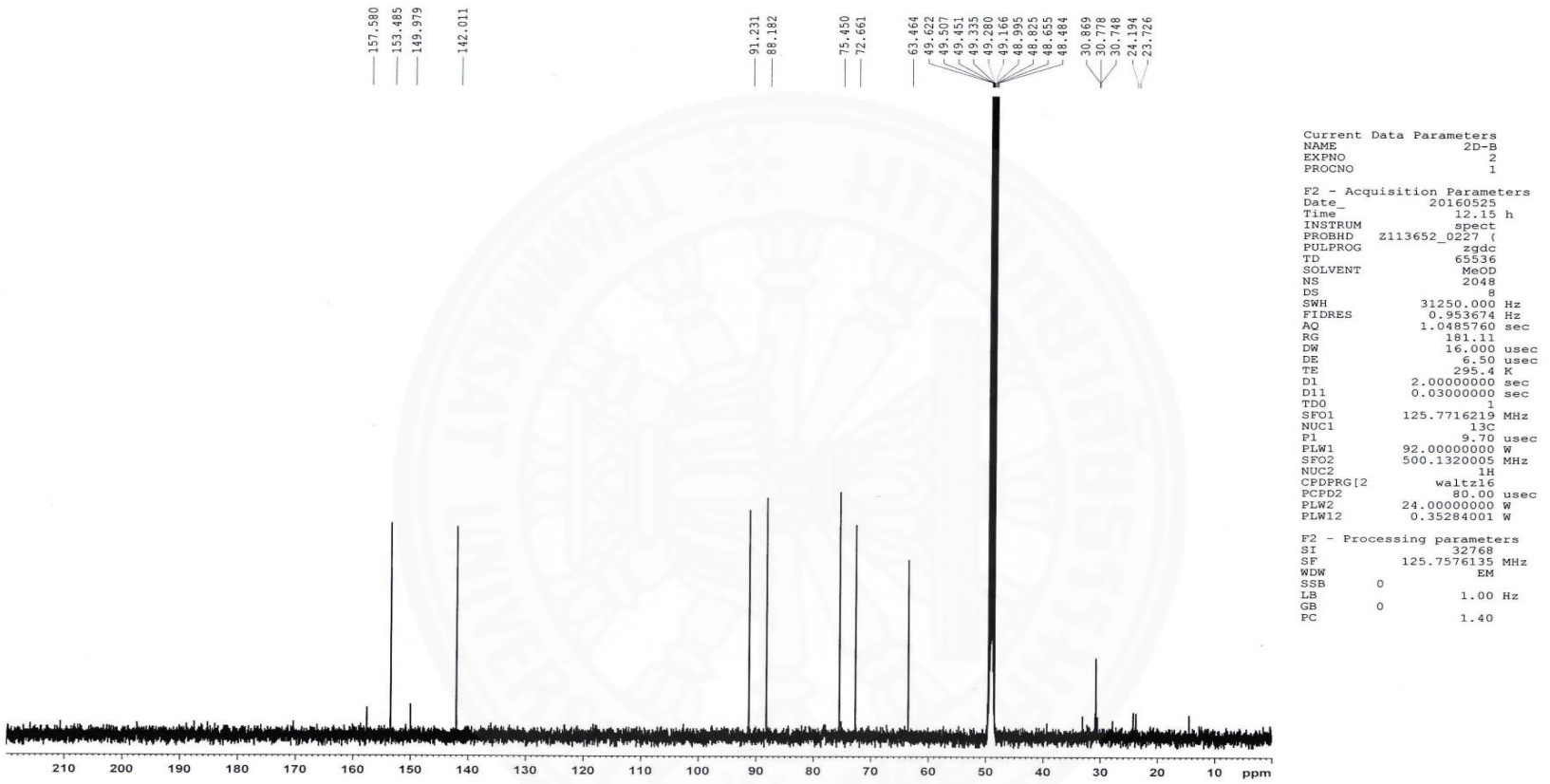


Figure 2

^{13}C-NMR Spectrum of (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol in MeOH, $CHCl_3$ and H_2O

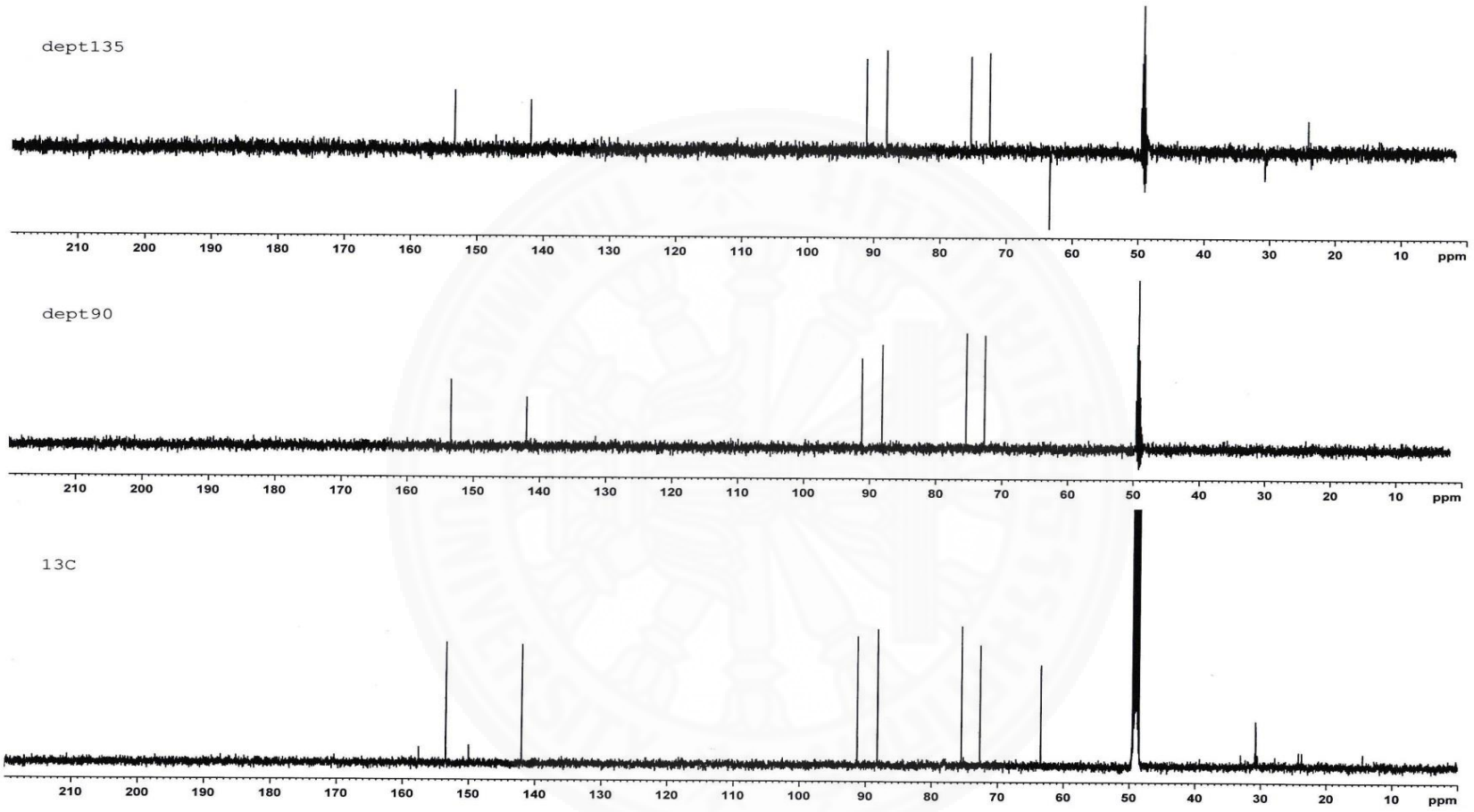


Figure 3

NMR Spectrum of (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol in MeOH, $CHCl_3$ and H_2O

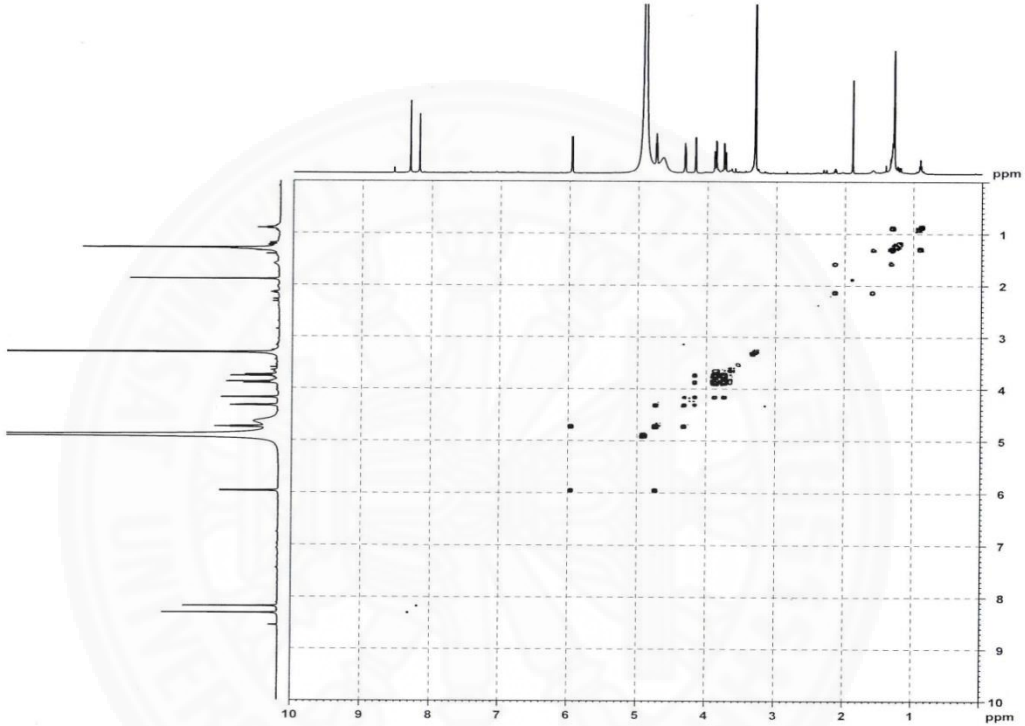


Figure 4

COSY of (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol in MeOH, $CHCl_3$ and H_2O

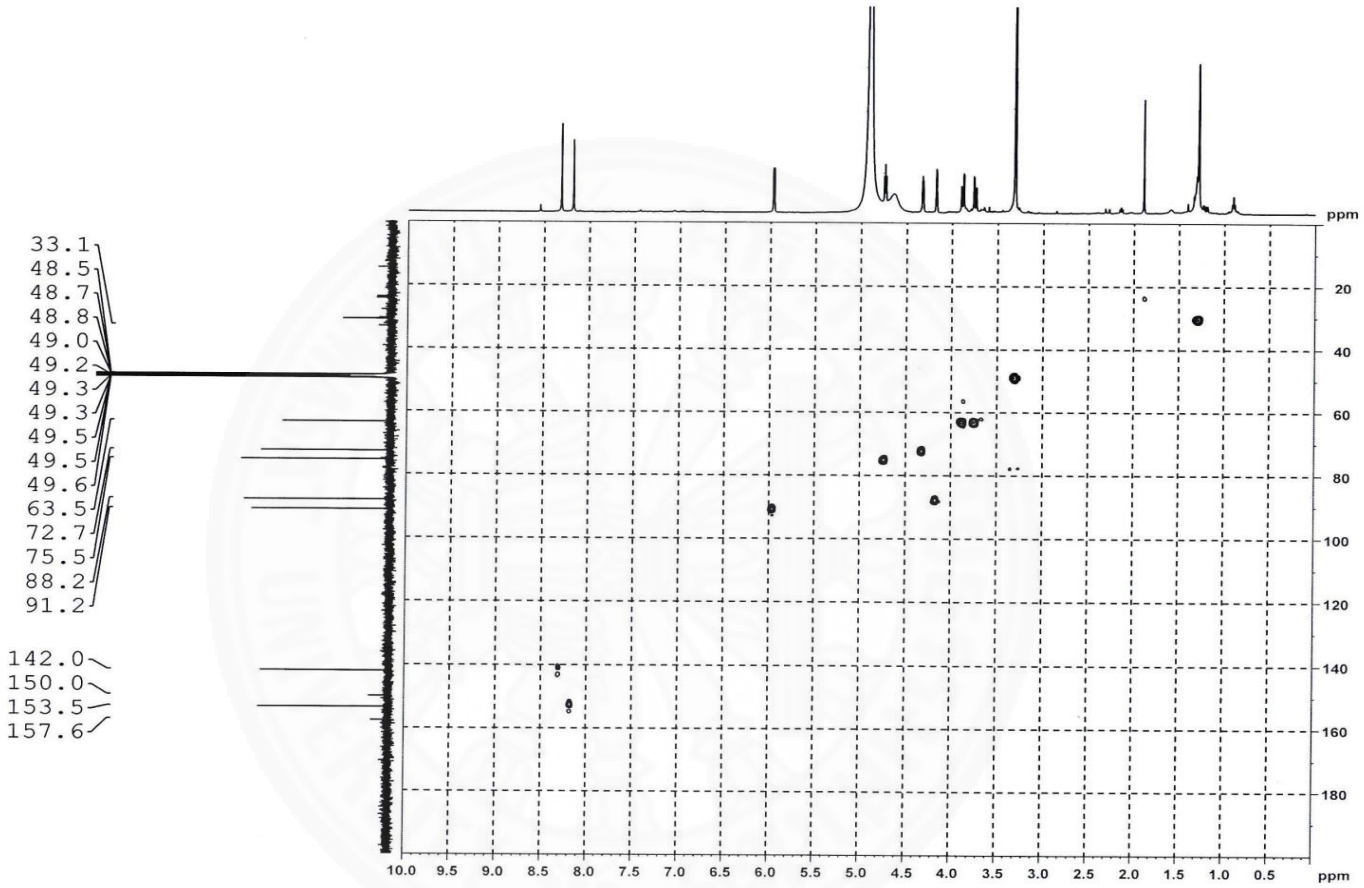


Figure 5

HSQC of (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol in MeOH, $CHCl_3$ and H_2O

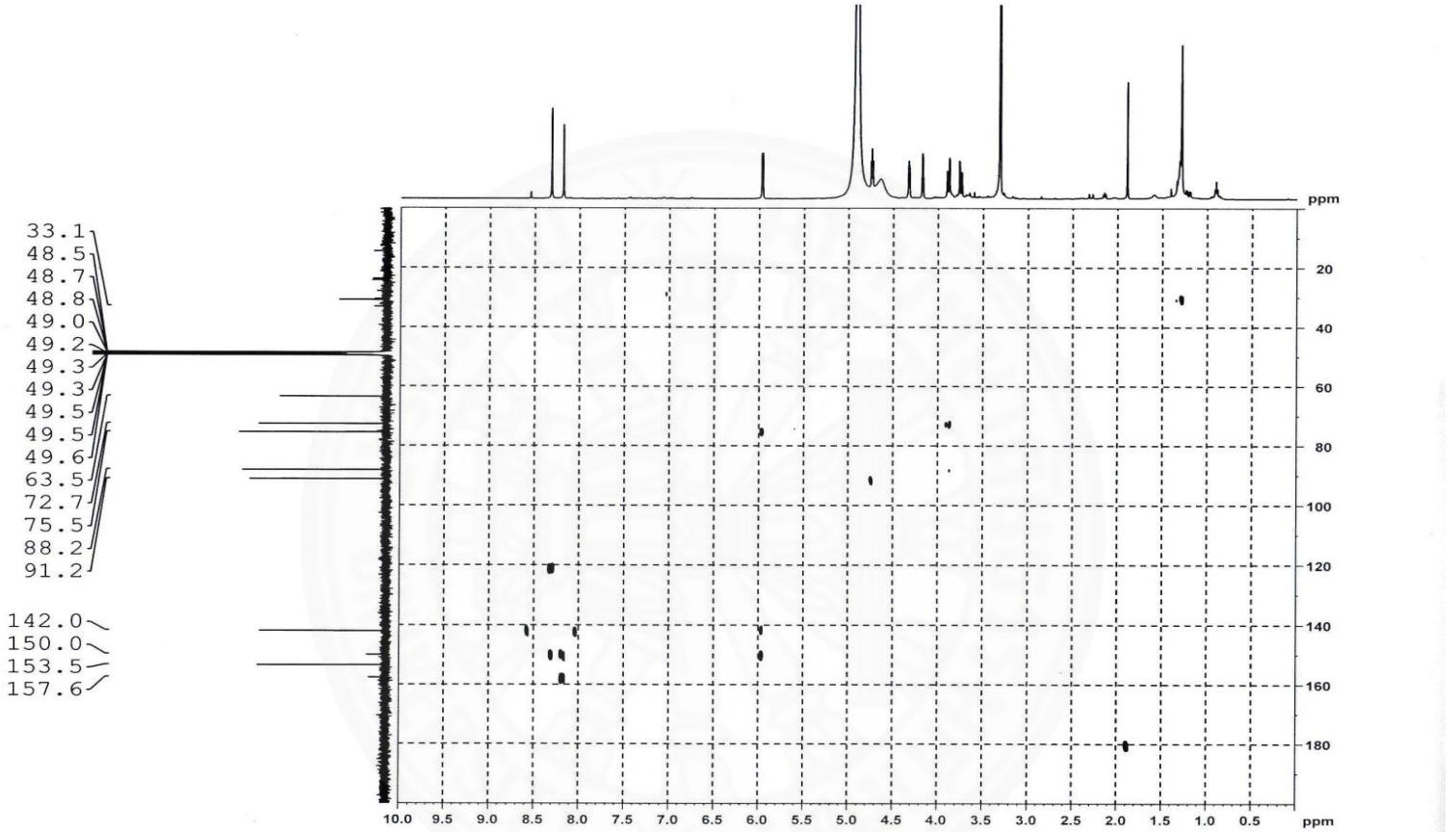


Figure 6

HMBC of (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol in MeOH, $CHCl_3$ and H_2O

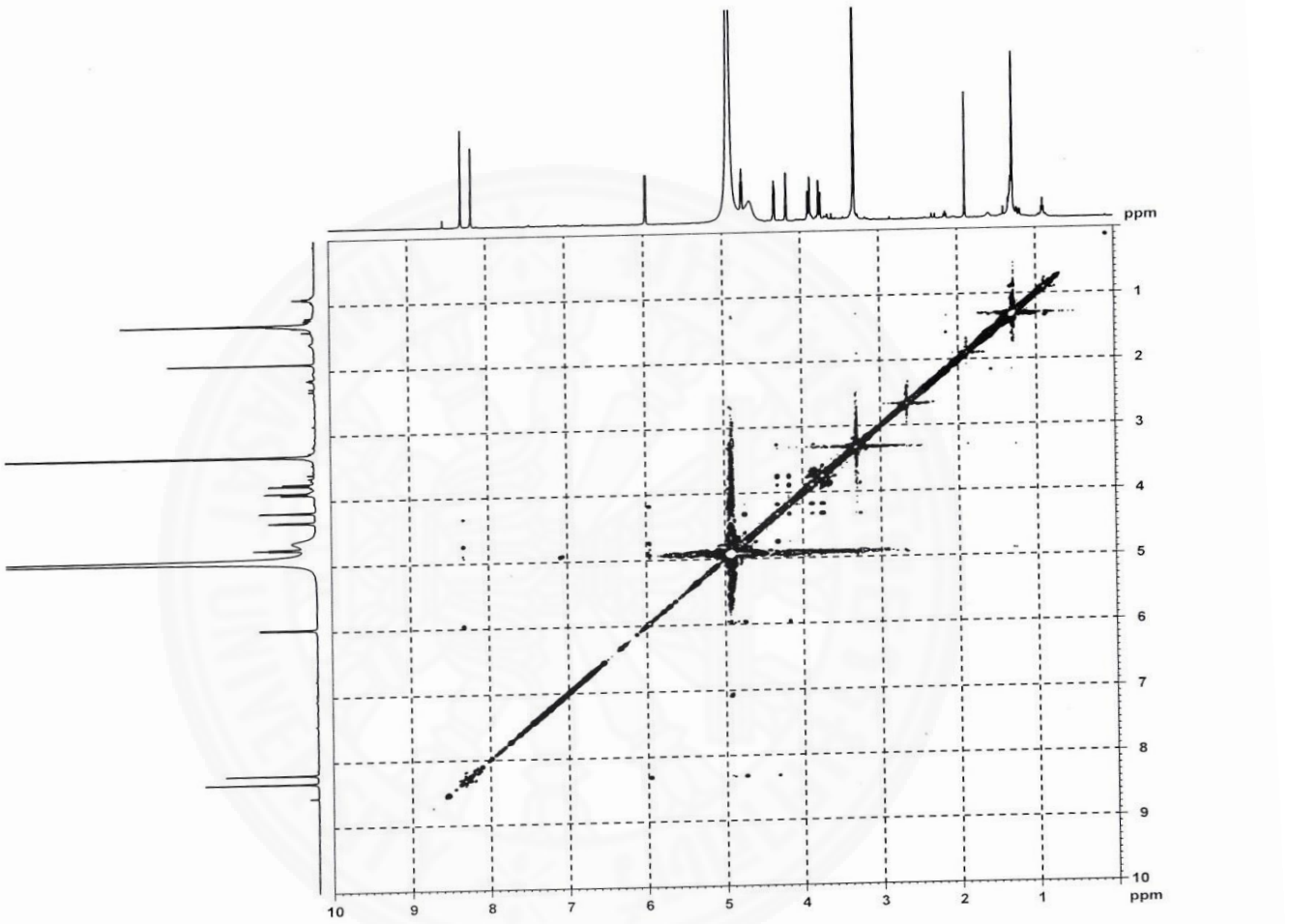


Figure 7

NOESY of (2S,3S,4R,5S)-2-(6-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol in MeOH, $CHCl_3$ and H_2O

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Publication

Khongkhon S., Ruangnoo S., Itharat A. Inhibition of LPS-Induced Nitric Oxide Production in RAW 264.7 Cell Lines, DPPH Radical Scavenging and Total Phenolic Content of Banana (*Musa sapientum*) Blossom Extracts. *Journal of the Medical Association of Thailand* 2017 (in press).

Conferences and presentations

Khongkhon S., Itharat A. Anti-oxidant and Total phenolic content of *Musa sapientum* Young Male Bud Extracts. 18th World Congress on Clinical Nutrition (WCCN) "Agriculture, Food and Nutrition for Health and Wellness", December 1-3, 2014, Ubon Ratchathani University, Ubon Ratchathani, Thailand (Poster Presentation).

Khongkhon S., Ruangnoo S., Itharat A. Anti-oxidant, Anti-inflammatory activities and Total Phenolic Content of *Musa sapientum* male flower Extracts. *MED TU Forum* 2016, June 20-22, 2016, Faculty of Medicine, Thammasat University, Pathumthani, Thailand (Oral presentation).

Award

The compliment award for oral presentation of Anti-oxidant, Anti-inflammatory activities and Total Phenolic Content of *Musa sapientum* male flower Extracts in *MED TU Forum* 2016, June 20-22, 2016, Faculty of Medicine, Thammasat University, Pathumthani, Thailand.

Work Experiences 2011-2013: Applied Thai Traditional Medicine, Chomphu Tambon Health Promoting Hospital, Phitsanulok, Thailand