MACROSCOPIC MICROSCOPIC MOLECULAR EVALUATIONS MANGIFERIN CONTENT AND BIOACTIVE POTENTIALS OF *MANGIFERA INDICA* LEAVES IN THAILAND

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การประเมินลักษณะทางมหทรรศน์ จุลทรรศน์ อณูโมเลกุล ปริมาณสารแมงจิเฟอริน และ ฤทธิ์ทางชีวภาพ ของใบมะม่วงในประเทศไทย

นางสาวอัญญาชุลี กนกพิชญ์ไกร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Thesis Title	MACROSCOPIC MICROSCOPIC MOLECULAR EVALUATIONS MANGIFERIN CONTENT AND BIOACTIVE POTENTIALS OF *MANGIFERA INDICA* LEAVES IN THAILAND
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อัญญาชุลี กนกพิชญไกร : การประเมินลักษณะทางมหทรรศน์ จุลทรรศน์ อณูโมเลกุล ปริมาณสารแมงจิเฟอริน และ ฤทธิ์ทางชีวภาพของใบมะม่วงในประเทศไทย (MACROSCOPIC MICROSCOPIC MOLECULAR EVALUATIONS MANGIFERIN CONTENT AND BIOACTIVE POTENTIALS OF *MANGIFERA INDICA* LEAVES IN THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. ชนิดา พลานุเวช, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ภก. ดร. นิจศิริ เรืองรังษี, ผศ. ดร. กาญจนา รังษีหิรัญรัตน์, 192 หน้า.

มะม่วง (*Mangifera indica*) เป็นหนึ่งในไม้ผลที่เก่าแก่และมีคุณค่าที่สุด และถูกพิจารณาให้เป็น “ราชาแห่งผลไม้ในทางอายุรเวท” นอกจากนี้ยังเป็นแหล่งสำคัญของแมงจิเฟอรินซึ่งเป็นสารที่มีผลทางเภสัชวิทยาอย่างหลากหลาย สามารถตรวจพบได้ในทุกส่วนของต้นมะม่วง ในประเทศไทยต้นมะม่วงถูกปลูกขึ้นตั้งแต่สมัยก่อนประวัติศาสตร์ คนไทยใช้ใบมะม่วงรับประทานเป็นผักซึ่งมีฤทธิ์ช่วยแก้อาการบิดและท้องอืด ใบซึ่งเป็นวัสดุที่ได้จากการตัดแต่งกิ่งหลังการเก็บเกี่ยว เหมาะกับการนำมาเป็นแหล่งของแมงจิเฟอริน ในปัจจุบันมีสายพันธุ์มะม่วงไทย ถูกปลูกมากกว่า 174 สายพันธุ์ สายพันธุ์ต่างๆเหล่านั้นเผชิญกับความสับสนเนื่องมาจากการมีชื่อสามัญหลายชื่อ จึงเป็นเหตุจำเป็นที่ต้องได้รับการระบุสายพันธุ์ให้ถูกต้อง อย่างไรก็ตามก่อนหน้านี้ไม่มีการศึกษาลักษณะทางมหทรรศน์ จุลทรรศน์ หรืออณูโมเลกุลของมะม่วงรวมกันมาก่อน และยังคงมีข้อมูลน้อยเกี่ยวกับการตรวจหาปริมาณสารแมงจิเฟอรินในใบมะม่วงเช่นเดียวกับการออกฤทธิ์ทางชีวภาพ เช่นฤทธิ์ต้านเบาหวาน ต้านเชื้อจุลชีพ หรือต้านมะเร็ง การศึกษานี้จะศึกษาลักษณะ ทางมหทรรศน์ จุลทรรศน์ และ อณูโมเลกุลโดยใช้เครื่องหมาย ISSR ของมะม่วง 17 สายพันธุ์ที่นิยมปลูกในประเทศไทย นอกเหนือจากนี้ ยังมีการประเมินปริมาณสารแมงจิเฟอรินในใบมะม่วงอกร่อง และศึกษาการออกฤทธิ์ทางชีวภาพได้แก่ ฤทธิ์ต้านเบาหวาน ต้านเชื้อจุลชีพ หรือต้านมะเร็งของสารสกัดจากใบมะม่วงและสารแมงจิเฟอริน ประเมินลักษณะทางมหทรรศน์ จุลทรรศน์ และอณูโมเลกุลของมะม่วง 17 สายพันธุ์ โดยแต่ละสายพันธุ์เก็บจาก 3 แหล่งปลูกที่แตกต่างกัน และใช้มะม่วงเบา (*M. caloneura*) และมะปราง (*B. macrophylla*) เป็นพืชเปรียบเทียบนอกกลุ่ม ลักษณะทางมหทรรศน์ร่วมกับลักษณะทางอณูโมเลกุลมีประสิทธิภาพที่จะใช้ระบุสายพันธุ์ต่างๆของมะม่วงได้เช่นเดียวกับค่าคงที่ของใบทางจุลทรรศน์ใช้เป็นหลักฐานเพื่อสนับสนุนเมื่อรวมกับลักษณะทางมหทรรศน์และลักษณะทางอณูโมเลกุลแล้วจะช่วยให้การยืนยันสายพันธุ์ถูกต้องมากขึ้น ใบมะม่วงสายพันธุ์อกร่องถูกเก็บจากสิบห้าแหล่งที่แตกต่างกันทั่วประเทศไทยเพื่อวิเคราะห์หาปริมาณสารแมงจิเฟอริน ใบมะม่วงทั้งหมดจะถูกตรวจวัดด้วยวิธีทินเลเยอร์โครมาโทกราฟี-เด็นซิโทเมทรีและวิธีการวิเคราะห์ทางรูปภาพ-ทินเลเยอร์โครมาโทกราฟี วิธีวิเคราะห์มีความเที่ยงตรง ใช้ตัวทำละลายเอทิลอะซิเตท ต่อ เมทานอล และ กรดฟอร์มิก (3.9:6:0.1) แมงจิเฟอรินถูกตรวจวัดภายใต้แสงอัลตราไวโอเลตได้ชัดเจนที่ความยาวคลื่น 254 นาโนเมตร โดยวิธีทั้งสองพบปริมาณสารแมงจิเฟอริน 4.992±1.025 และ 4.311±0.987 กรัม/100 กรัมของน้ำหนักแห้ง ตามลำดับ ศึกษาฤทธิ์ต้านเบาหวานโดยวัดการยับยั้งเอ็นไซม์แอลฟากลูโคสิเดสจากเชื้อยีสต์แซคคาโรไมซีส ซีรีวิซิอี และ เอ็นไซม์แอลฟากลูโคสิเดสจากผงลำไส้เล็กของหนู โดยใช้ 1 มิลลิโมลาร์ ของ พารา-ไนโตรฟีนิล-แอลฟา-ดี-กลูโคไพราโนไซด์ทำหน้าที่เป็นสับสเตรท ในขณะที่ เอมไซม์แอลฟาอะไมเลสจากตับอ่อนหมู ใช้ 1 มิลลิโมลาร์ ของ 2-4-คลอโร-ไนโตรฟีนอล-แอลฟา-ดี-มอลโตไซด์ ทำหน้าที่เป็นสับสเตรท ตรวจวัดสารไนโตรฟีนอลที่เกิดขึ้นภายใต้แสงอัลตราไวโอเลตที่ความยาวคลื่น 405 นาโนเมตร ทั้งสารสกัดจากใบมะม่วงและสารแมงจิเฟอริน มีความสัมพันธ์ระหว่างปริมาณสารที่ใช้ทดสอบดังกล่าวกับการยับยั้งที่เกิดขึ้น โดยเฉพาะอย่างยิ่ง เอ็นไซม์แอลฟากลูโคสิเดสจากเชื้อยีสต์ (สารสกัดจากใบมะม่วง; IC_{50}=0.050 มิลลิกรัม/มิลลิลิตร) เอ็นไซม์แอลฟากลูโคสิเดสจากหนู (สารละลายแมงจิเฟอริน; IC_{50}=0.433 มิลลิกรัม/มิลลิลิตร) เมื่อเปรียบเทียบกับสารอะคาร์โบส (IC_{50}=11.929 และ 0.449 มิลลิกรัม/มิลลิลิตร ตามลำดับ) สำหรับการทดสอบฤทธิ์ต้านเชื้อจุลชีพ เชื้อซึ่งเป็นตัวแทนจากกลุ่มแบคทีเรียแกรมบวก แบคทีเรียแกรมลบ และ เชื้อรา ถูกนำมาทดสอบเพื่อแสดงขอบเขตการยับยั้งต่อเชื้อ ค่าต่ำสุดในการยับยั้งต่อเชื้อ ค่าต่ำสุดในการฆ่าเชื้อแบคทีเรีย และเชื้อรา สำหรับการทดสอบเพื่อแสดงขอบเขตการยับยั้งต่อเชื้อ สารสกัดจากใบมะม่วงแสดงขอบเขตการยับยั้งต่อเชื้อแบคทีเรียแกรมบวกบางชนิด ในขณะที่ สารแมงจิเฟอรินแสดงขอบเขตการยับยั้งต่อเชื้อแบคทีเรียทั้งแกรมบวกและแกรมลบบางชนิด ทั้งสารสกัดจากใบมะม่วงและสารแมงจิเฟอรินมีประสิทธิภาพสูงสุดต่อการยับยั้งต่อเชื้อ โคคูเรีย ไรโซฟิลา ค่าระดับความเข้มข้นต่ำสุดในการยับยั้งต่อเชื้ออยู่ที่ 15.63 และ 62.5 ไมโครกรัม/มิลลิลิตร และ ค่าระดับความเข้มข้นต่ำสุดในการฆ่าเชื้ออยู่ที่ 2,000 และมากกว่า 2,000 ไมโครกรัม/มิลลิลิตรตามลำดับ การทดสอบความเป็นพิษต่อเซลล์มะเร็งห้าชนิดที่แยกมาจากเซลล์มะเร็งของมนุษย์เปรียบเทียบกับเซลล์ที่แยกมาจากเซลล์ปกติของมนุษย์ พบว่าสารสกัดจากใบมะม่วง (≥200 ไมโครกรัม/มิลลิลิตร) แสดงการยับยั้งเซลล์ที่แยกมาจากเซลล์มะเร็งของมนุษย์ที่ทดสอบ ทั้งสารสกัดจากใบมะม่วงและสารแมงจิเฟอริน มีประสิทธิภาพในการเพิ่มอัตราการรอดชีวิตของเซลล์ปกติจากผิวหนังมนุษย์

สาขาวิชา วิทยาศาสตร์สาธารณสุข ลายมือชื่อนิสิต .....

ปีการศึกษา 2559 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KEYWORDS: MANGIFERA INDICA CULTIVARS / MACROSCOPIC / MICROSCOPIC / STOMATAL NUMBER / VEINLET TERMINATION NUMBER / PALISADE RATIO / MOLECULAR / ISSR FINGERPRINT / MANGIFERIN CONTENT / TLC-DENSITOMETRY / TLC-IMAGE ANALYSIS / ANTIDIABETIC / YEAST ALPHA-GLUCOSIDASE / RAT ALPHA-GLUCOSIDASE / PANCREATIC ALPHA-AMYLASE / ANTIMICROBIAL / ZONE OF INHIBITION / MIC / MBC / MFC / ANTICANCER / MTT / ACTIVITY

AUNYACHULEE GANOGPICHAYAGRAI: MACROSCOPIC MICROSCOPIC MOLECULAR EVALUATIONS MANGIFERIN CONTENT AND BIOACTIVE POTENTIALS OF *MANGIFERA INDICA* LEAVES IN THAILAND. ADVISOR: ASST. PROF. CHANIDA PALANUVEJ, Ph.D., CO-ADVISOR: ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., ASST. PROF. KANCHANA RUNGSIHIRUNRAT, Ph.D., 192 pp.

Mango (*Mangifera indica*) has been noted that it is one of the most ancient and valuable fruit crop and has been considered to be 'Ayurveda king of fruit'. It is also found to be the major sources of mangiferin, which has many pharmacological effects, that can be detected in all parts of mango. Mango trees have long been cultivated since the early history of Thailand. Thai people ate mango leaves as vegetables with anti-dysentery and anti-flatulence properties. The leaves, a waste material gained from timming of post-harvest could be used as the good reasonable source of mangiferin. Currently, Thai mangoes have over 174 cultivars have been cultivated. They have confronted with confusions about numerous synonym nomenclatures and needed to be correctly identified. However, neither of previous studies provided any macroscopic, microscopic nor molecular descriptive evidences in combination. There still has little information about mangiferin content in Thai mango leaves as well as their biological activities such as antidiabetic, antimicrobial or anticancer. This study investigated selected seventeen Thai mango cultivars popularly cultivated in Thailand, on macroscopic, microscopic leaf characteristics and their genetic relationships using ISSR markers; in addition, it also evaluated mangiferin content in selected mango leaves and some biological activities such as antidiabetic, antimicrobial and anticancer of mango leaf extract and mangiferin. For selected Thai mango identifications, seventeen Thai mango cultivars, *M. caloneura* and *B. macrophylla* were collected throughout Thailand (each of them from three different locations). Macroscopic characters together with their genetic characters had a potential to identify among seventeen Thai mango cultivars as well as microscopic leaf constant number, as a supporting evidence, in combination with macroscopic and molecular characteristics was able to use as a helpful tool for more accurate identification. Fifteen *Mangifera indica* 'Okrong' leaf samples were collected from different locations in Thailand for evaluated mangiferin content. They were determined by TLC-densitometry and TLC-image analysis. TLC quantitation was validated. The TLC plate was developed with a saturated mobile phase; ethyl acetate: methanol: formic acid (3.9 : 6 : 0.1). Mangiferin spots were clearly detected under UV 254 nm. Mangiferin contents were 4.992 ± 1.025 and 4.311 ± 0.987 g / 100 g of dried crude drug, respectively. For antidiabetic activities, yeast α-glucosidase activity (from *Saccharomyces cerevisiae*) and rat α-glucosidase activity (from intestinal acetone powders from rat) were determined by using 1 mM of p-nitrophenyl-α-D-glucopyranoside (PNPG) as the substrate; while, pancreatic α-amylase activity (from porcine pancreas) using 1 mM of 2-chloro-4 nitrophenol-α-D-maltotroside (CNPG-3) as substrate. The absorbance was measured at 405 nm. Both mango leaf extract and mangiferin possessed a dose response relationship with a great inhibitions, especially yeast α-glucosidase (mango leaf extract; IC_{50}=0.050 mg/ml), rat α-glucosidase activity (mangiferin; IC_{50} = 0.433 mg/ml) when compared to acarbose (IC_{50} = 11.926 and 0.449 mg/ml, respectively). For antimicrobial activities, thirteen representatives gram-positive bacteria, gram-negative bacteria and fungi were used to demonstrate zone of inhibitions and MIC, MBC and MFC. For disk diffusion, mango leaf extract showed inhibition zones against some of tested gram-positive bacteria; whereas, mangiferin showed inhibition zones against some of tested both gram-positive and gram-negative bacteria. For broth microdilution, mango leaf extract and mangiferin showed the most potent inhibition against *Kocuria rhizophila* with MIC values of 15.63 and 62.5 µg/ml and MBC values of 2000 and ≥ 2000 µg/ml, respectively. Anticancer activity was evaluated against five human cancer cell lines compared to two human normal cell lines using MTT assay. For cytotoxicity, mango leaf extract, ≥ 200 µg/ml, showed cytotoxicity against tested cancer cell lines. Both mango leaf extract and mangiferin increased % survival of skin fibroblast.

Field of Study: Public Health Sciences
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Student's Signature .....
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LIST OF ABBREVIATIONS

°C	=	degree Celsius
µg	=	microgram
µg/ µl	=	microgram per microliter
µg/ spot	=	microgram per spot
µl	=	microliter
µm	=	micrometer
µM	=	micromolarity
ATCC	=	American type culture collection
bp	=	base pair
CFU	=	colony forming unit
cm	=	centimeter
DNA	=	deoxyribonucleic acid
DMSO	=	dimethyl sulfoxide
dNTPs	=	deoxyribonucleotide triphosphate
EDTA	=	ethylenediaminetetraacetic acid
g	=	gram
hr	=	hour
ICH	=	International Conference on Harmonisation
ISSR	=	inter simple sequence repeat
LOD	=	limit of detection
LOQ	=	limit of quantification
M	=	molarity
MBC	=	minimum bactericidal concentration
MFC	=	minimum fungicidal concentration
mg	=	milligram
mg/g	=	milligram per gram
mg/ml	=	milligram per milliliter
$MgCl_2$	=	magnesium chloride

MHA	=	Mueller hinton agar
MHB	=	Mueller hinton broth
min	=	minute
ml	=	milliliter
ml/min	=	milliliter per minute
mm	=	millimeter
mM	=	millimolarity
mm^2	=	square millimeter
nm	=	nanometer
PCR	=	polymerase chain reaction
RNA	=	ribonucleic acid
rpm	=	round per minute
RSD	=	relative standard deviation
SD	=	standard deviation
SDA	=	Sabouraud dextrose agar
SDB	=	Sabouraud dextrose broth
sec	=	second
SSR	=	single sequence repeat
Taq	=	*Taq* DNA polymerase
TBE buffer	=	Tris Boric EDTA buffer
TIF	=	tagged image file
TLC	=	thin layer chromatography
Tm	=	temperature for annealing
UV	=	ultra violet
V	=	volt

CHAPTER I
INTRODUCTION

Background and rationale

Mango (*Mangifera indica* L.) is one of the most ancient and important tropical fruit in the world, especially in Asia. It has been cultivated since at least 4,000 years ago and has been often referred as 'Ayurveda King of fruits' in the tropical world [1]. All mangoes belong to the Anacardiaceae family consisting of various kinds of species with over 1,000 cultivars [2]. It possesses pharmacological effects i.e. antidiabetic, antioxidant, antimicrobial, anticancer, anti-inflammatory properties [3]. In Thailand, mangoes have been cultivated since the early history of the Kingdom; as many as 174 cultivars have been recorded, mainly for domestic consumption, slightly for export. Mangoes are now widely grown throughout the Kingdom. Among mango cultivars currently cultivated in Thailand, 'Okrong' is an admired commercial cultivar that is typically offered as fresh fruit [4, 5]. Their leaves are consumes as vegetables with anti-dysentery and anti-flatulence properties [6].

Macroscopic and microscopic examinations should be the first step to identify the plants, they are primary importance that should be carried out before any tests will be undertaken [7]. These judgments may vary in size or shape from time to time because of the environmental conditions [8]; however, their characters are very much considerable as far as taxonomy and pharmacognostical value concerned [9]. Both macroscopic and microscopic evaluations are useful for identification, standardization and quality assurance purposes [7, 8, 10]. Recently, there have been growing interests in mango characteristics. Many researchers observed a variation in that macroscopic characters which could be benefited in differentiation among mango cultivars [11-13]. On the other hand, only a few studies have been investigated leaf microscopic characteristics based on their constant values [14-16]; besides, those cultivars have not been cultivated in Thailand. There have still never been the studies on microscopic leaf constant values (stomatal number, veinlet termination number and palisade ratio) among Thai mango cultivars.

Currently, molecular markers based on polymerase chain reaction (PCR) have been extensively used because they are a powerful tools to evaluate genetic diversity and provide a genetic relationships of the plant [17, 18]. Molecular markers are less affected by age, physiological and environmental conditions [19]. Among the various molecular marker techniques, inter simple sequence repeat (ISSR) is valuable due to not only no required prior genetic information but also its rapidity, reproducibility, simplicity and cost effectiveness. ISSR can be performed to judge the genetic diversity and identify closely related cultivars in many species [20-22]. It also provides typically highly polymorphism. ISSR marker is based on a repeat sequence and amplifies the sequence between two microsatellites, which appear in both nuclear and organelle genomes [23-25]. Many molecular markers have been used for mango cultivars identification for example AFLP [26, 27], RAPD [28], ISSR [29-31] and SSR [32-34]. However, there have been few studies on molecular characteristics among Thai mango cultivars [26, 33] especially no studies by ISSR marker.

Mango is a plentiful source of various polyphenolic compounds, especially mangiferin, which is the major component that can be detected in all parts of mango [35]. This compound is a xanthone, commonly called C-glucosyl xanthone that referred as a super antioxidant [36]. It also has been found pharmacological effects including radioprotective, antiallergic, antidiabetic, anticancer, antimicrobial, immunomodulatory , anti-inflammatory activities [37, 38]. Due to their high mangiferin content, the leaves, which are waste material gained from timming of post-harvest, could be used as the good reasonable source of mangiferin. For quantitative analysis, TLC-densitometry as well as TLC-image analysis were developed.

Diabetes mellitus is a chronic metabolic disorder characterized by uncontrolled increase in blood glucose level. An infectious disease is a health problems caused by pathogenic microorganisms, such as virus, bacteria and fungi. Cancer is a group of diseases differentiated by the uncontrolled growth and spread of abnormal cells. All of them are main global public health problems, which affect several million people worldwide, especially in developing countries. Currently, chemical agents like acarbose, amikacin, ampicillin and doxorubicin are available for treatment of diabetes,

infection and cancer. However, these treatments are related with undesirable side effects as well as drug resistance occurred frequently [39-44] leading to increasing interest in the complementary and alternative use of medicinal plants because of their safer and less destructive to the body.

Objectives of the study

1. To investigate selected seventeen Thai mango cultivars that popularly cultivated in Thailand, on macroscopic and microscopic characteristics as well as the genetic diversity and genetic relationships using ISSR marker system.
2. To evaluate the mangiferin content of *Mangifera indica* 'Okrong' leaves via TLC combined with image analysis using image J software compared to TLC-densitometry.
3. To evaluate biological activities consisted of antidiabetic, antimicrobial and anticancer properties of *Mangifera indica* 'Okrong' leaf extract and mangiferin compound.

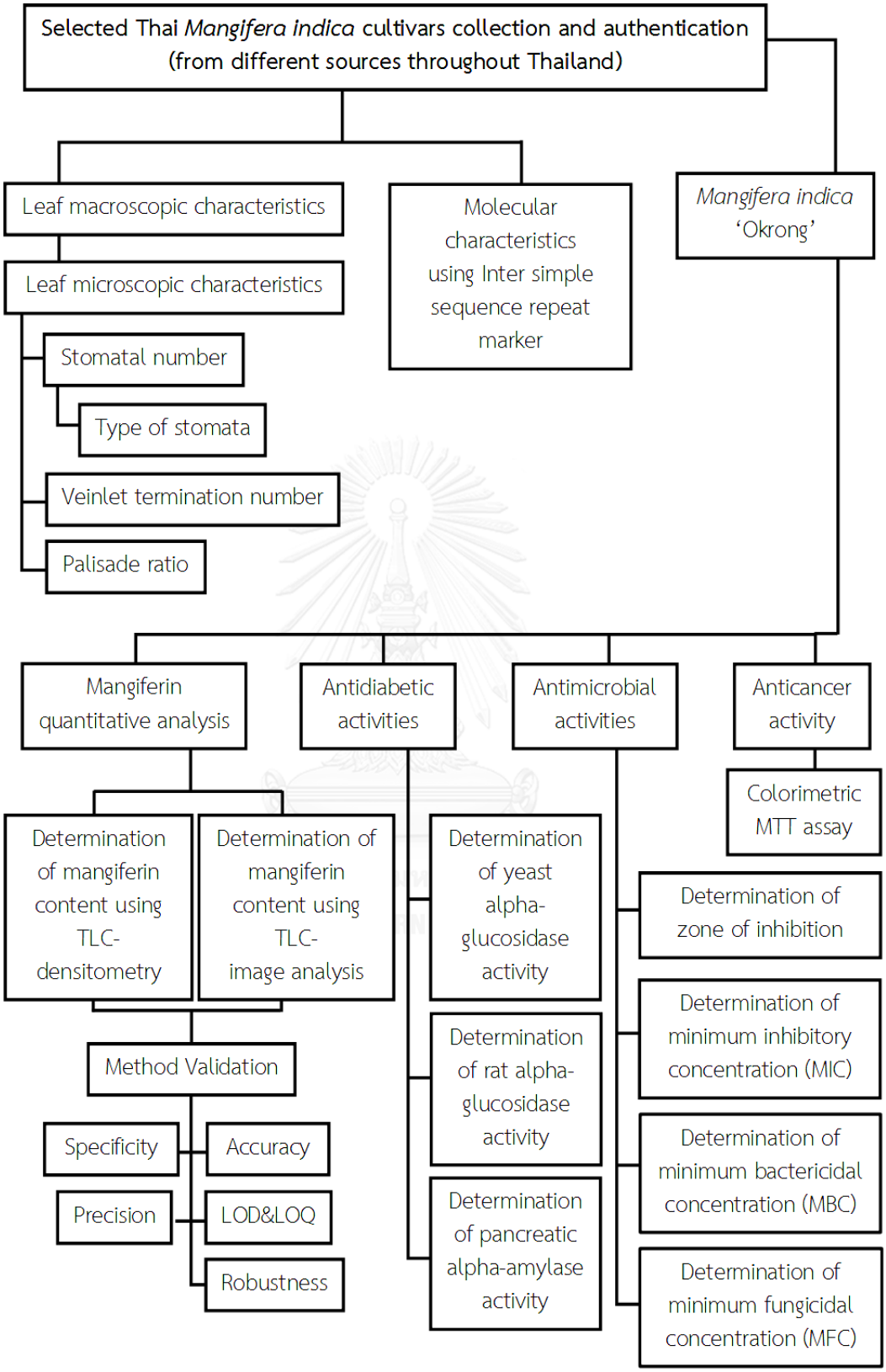


Figure 1 The conceptual framework

CHAPTER II
LITERATURE REVIEWS

Botanical description

Anacardiaceae

Anacardiaceae, the cashew or sumac family, include about 82 genera in over 800 known species [45]. This flowering plants family is cultivated throughout the world, mainly in tropical, subtropical, and temperate areas. Examples of economically prominent crops of Anacardiaceae comprise mango (*Mangifera indica*), cashew (*Anacardium occidentale*) and marian plum (*Bouea macrophylla*) [45-47]. The resinous sap 'urushiol' also found in this family, poisonously cause a contact dermatitis, especially in *Toxicodendron* genus [45, 46, 48-50].

"Trees or shrubs, also woody climbers or perennial herbs, resiniferous secretory ducts in bark and foliage, plants turpentine-smelling, blackening when wounded, hermaphroditic, polygamo-dioecious or dioecious. Leaves often clustered distally, alternate, exstipulate, simple, trifoliolate or imparipinnate [46]*. The flowers can be either unisexual or bisexual, with 5 (sometimes 3) sepals united at the base and 5 (sometimes 3 or 0) petals. There are 5 or 10 stamens. The ovary is positioned superior and consists of 3 united carpels forming a single* chamber [48]*. Fruits drupes or samaras (rarely syncarps, utricles, nut-like, or baccates), fleshy or dry, occasionally subtended by a fleshy hypocarp or an accrescent, chartaceous or fleshy calyx; mesocarp sometimes with prominent black resin canals* [51]*"*

Mangifera

Mangifera genus is one of 82 genera that belong to Anacardiaceae family. It consists of 69 species mostly occur in south and south-east Asia. It has been divided into two subgenera based on morphological characters, namely *Limus* and *Mangifera* species. Subgenus *Limus* has been divided into two sections, section *Deciduae* (deciduous trees) and section *Perennes* (non-deciduous tree). Subgenus *Mangifera* has been divided into four sections, section *Marchandora* Pierre, *Euantherae* Pierre, *Rawa*

Kosterm and *Mangifera* Ding Hou. *Mangifera caloneura*, which is closely related and can be mistaken for Mangifera *indica*, belongs section *Euantherae* Pierre; while, *Mangifera indica* belongs section *Mangifera* Ding Hou.

Section *Mangifera* Ding Hou, the largest section of subgenus *Mangifera*, had more than 30 species. It has been divided into three groups based on floral structure and organ number variation; pentamerous flowers, tetramerous flowers and intermediate (having both pentamerous and tetramerous flowers) group of species. Common mango (*Mangifera indica*) belongs the intermediate group [49, 51].

Mangifera indica

Mangifera indica is the most popular economically important fruit tree with over 1,000 cultivars. Mangoes are grown from seeds and are known as "seedlings". They are long-live, some mango trees being known to be 300 years old and still fruiting [52-54].

Scientific classification [55-57]

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Rosidae

Order: Sapindales

Family: Anacardiaceae – Sumac family

Genus: *Mangifera* L. – mango

Species: *Mangifera indica* L. – mango

"*Trees, 10-20 m tall; branchlets brown, glabrous. Petiole 2-6 cm, grooved apically, inflated basally; leaf blade oblong to oblong-lanceolate, 12-30 × 3.5-6.5 cm, leathery, deep green adaxially, light green abaxially, glabrous on both sides, base cuneate to obtuse, margin entire, undulate, apex acute to long acuminate, lateral veins 20-25 pairs, midrib prominent on both sides, reticulate venation obscure. Inflorescence paniculate, terminal, 20-35 cm, glabrous to tomentose-pilose; bracts ca. 1.5 mm,*

lanceolate pubescent. Pedicels 1.5-3 mm, articulate. Sepals ovate-lanceolate, 2.5-3 × ca. 1.5 mm, glabrous to pubescent, acuminate. Petals light yellow with prominent

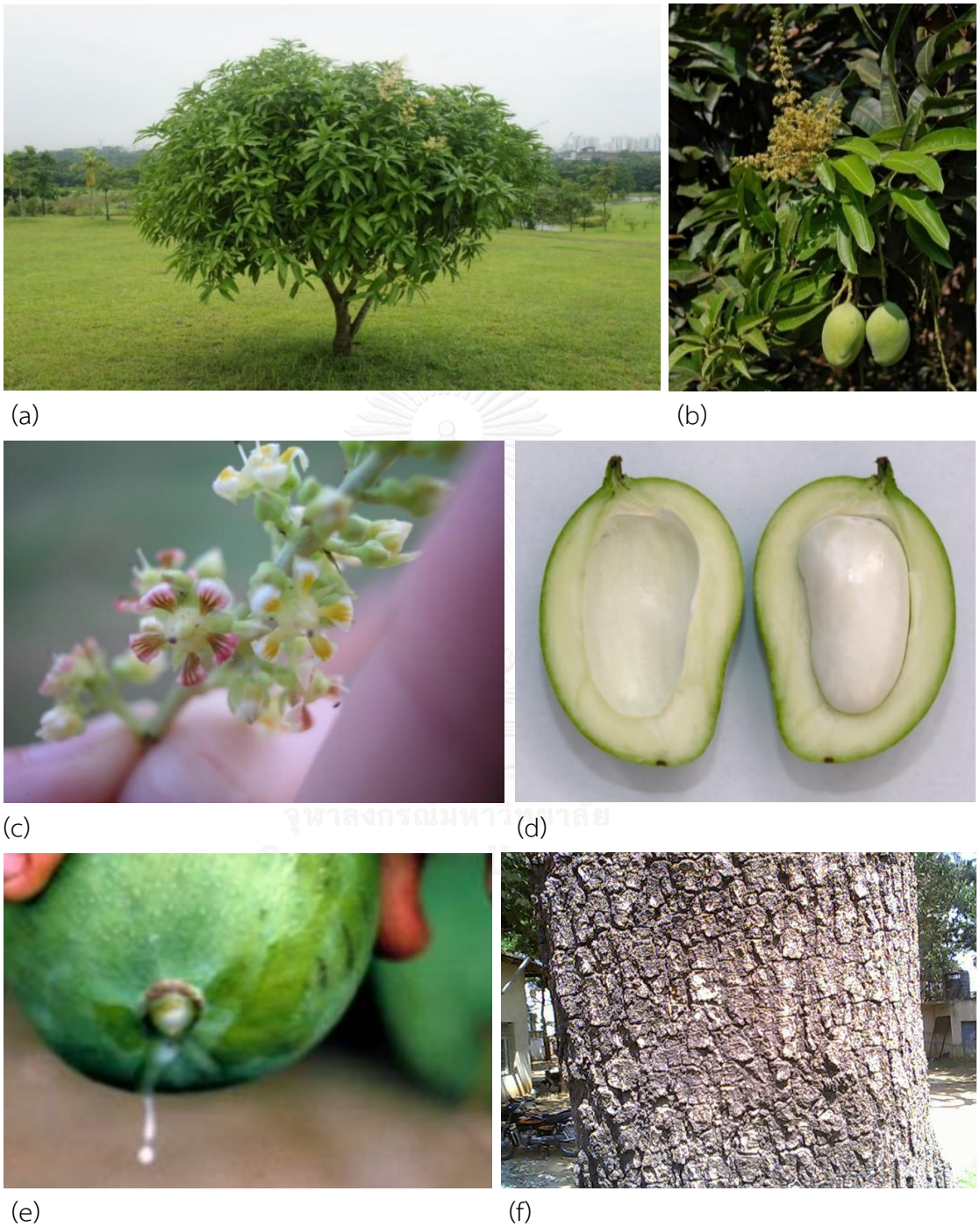


Figure 2 *Mangifera indica* L.; (a) Mango tree, (b) Mango leaves, (c) Mango flowers, (d) Mango cross section, (e) Mango sap and (f) Mango bark [58-62]

red tree-shaped pattern adaxially, oblong or oblong-lanceolate, 3.5-4 × ca. 1.5 mm, glabrous, recurved at anthesis. Fertile stamen 1, ca. 2.5 mm, with ovate anther; staminodes 4, 0.7-1 mm. Disk inflated, fleshy, 5-lobed. Ovary oblique, ovate, ca. 1.5 mm in diam. at anthesis; style ca. 2.5 mm, eccentric. Drupe oblong to subreniform, greenish yellow to red, 5-10 × 3-4.5 cm; fleshy mesocarp bright yellow; endocarp ± compressed." [55]

Table 1 List of *Mangifera indica* cultivars located in Thailand [63]

Mangifera indica 'Keao'	*Mangifera indica* 'Khunthip'
Mangifera indica 'Kratae Luemrang'	*Mangifera indica* 'Khiaokhaika'
Mangifera indica 'Krasuay'	*Mangifera indica* 'Khiaopuket'
Mangifera indica 'Kluay'	*Mangifera indica* 'Khiaosawoey'
Mangifera indica 'Kalonthong'	*Mangifera indica* 'Khiaosawoey Rotchana'
Mangifera indica 'Karaket'	*Mangifera indica* 'Khaituek'
Mangifera indica 'Kalamae'	*Mangifera indica* 'Khlay khiaosawoey'
Mangifera indica 'Kampan'	*Mangifera indica* 'Khonokkaeo'
Mangifera indica 'Kaemdaeng'	*Mangifera indica* 'Khangkao Luemrang'
Mangifera indica 'Kaeo Khao'	*Mangifera indica* 'Kham'
Mangifera indica 'Kaeo Khieo'	*Mangifera indica* 'Ku'
Mangifera indica 'Kaeo Tawai'	*Mangifera indica* 'Nga Khaomonyao'
Mangifera indica 'Kaeo Luemkon'	*Mangifera indica* 'Nga Khiao'
Mangifera indica 'Kaeo Luemrang'	*Mangifera indica* 'Nga Chang'
Mangifera indica 'Kaeo Sampi'	*Mangifera indica* 'Nga Dap'
Mangifera indica 'Kaeo Hom'	*Mangifera indica* 'Nga Daeng'
Mangifera indica 'Khochang'	*Mangifera indica* 'Nga Thongruae'
Mangifera indica 'Khaituek'	*Mangifera indica* 'Nga Mon'
Mangifera indica 'Khitai'	*Mangifera indica* 'Chanchaokha'
Mangifera indica 'Khithup'	*Mangifera indica* 'Ngo'
Mangifera indica 'Champa'	*Mangifera indica* 'Thongdam Klaipan'
Mangifera indica 'Chaokhunthip'	*Mangifera indica* 'Thongdam Mirong'
Mangifera indica 'Chaopraya'	*Mangifera indica* 'Thongdaeng'

Table 1 (cont.) List of *Mangifera indica* cultivars located in Thailand [63]

Mangifera indica 'Chaosawoey'	*Mangifera indica* 'Thongthawai'
Mangifera indica 'Changtoktuek'	*Mangifera indica* 'Thongprakaisat'
Mangifera indica 'Chok Sopon'	*Mangifera indica* 'Thongplaikhean'
Mangifera indica 'Chok Anan'	*Mangifera indica* 'Thongmairuwai'
Mangifera indica 'Chok Anan Kanchompu'	*Mangifera indica* 'Thurian'
Mangifera indica 'Talapnak'	*Mangifera indica* 'Thunthawai'
Mangifera indica 'Tapianthong'	*Mangifera indica* 'Thepnimit'
Mangifera indica 'Tuppet'	*Mangifera indica* 'Thepparot'
Mangifera indica 'Ta Te-Lan'	*Mangifera indica* 'Nuanchan'
Mangifera indica 'Thaeng Kwao'	*Mangifera indica* 'Nuanthaeng'
Mangifera indica 'Thawai Dueankao'	*Mangifera indica* 'Nathap'
Mangifera indica 'Thongkhao'	*Mangifera indica* 'Namdokmai'
Mangifera indica 'Thongkhaoklom'	*Mangifera indica* 'Namdokmai Thawai'
Mangifera indica 'Thongkhaoyao'	*Mangifera indica* 'Namdokmai No.4'
Mangifera indica 'Thongchaopat'	*Mangifera indica* 'Namdokmai No.5'
Mangifera indica 'Thongdam'	*Mangifera indica* 'Namdokmai Phrapradaeng'
Mangifera indica 'Namdokmai Sithong'	*Mangifera indica* 'Phimsen Preow'
Mangifera indica 'Namdokmai Suphan'	*Mangifera indica* 'Phimsen Man'
Mangifera indica 'Namtan Chin'	*Mangifera indica* 'Phetbanlat'
Mangifera indica 'Namtan Tao'	*Mangifera indica* 'Falan'
Mangifera indica 'Namtan Pakkrabok'	*Mangifera indica* 'Fa-apple'
Mangifera indica 'Namtansainak'	*Mangifera indica* 'Faep'
Mangifera indica 'Nampueng'	*Mangifera indica* 'Maprang'
Mangifera indica 'Banyen'	*Mangifera indica* 'Malila'
Mangifera indica 'Bunbandan'	*Mangifera indica* 'Manbangkhunsi'
Mangifera indica 'Pakhirio Hothong'	*Mangifera indica* 'Mankom'
Mangifera indica 'Payaluemfao'	*Mangifera indica* 'Manthawai'
Mangifera indica 'Payasawoey'	*Mangifera indica* 'Manthawai Nakrop'
Mangifera indica 'Phruankho'	*Mangifera indica* 'Manthong Aek'

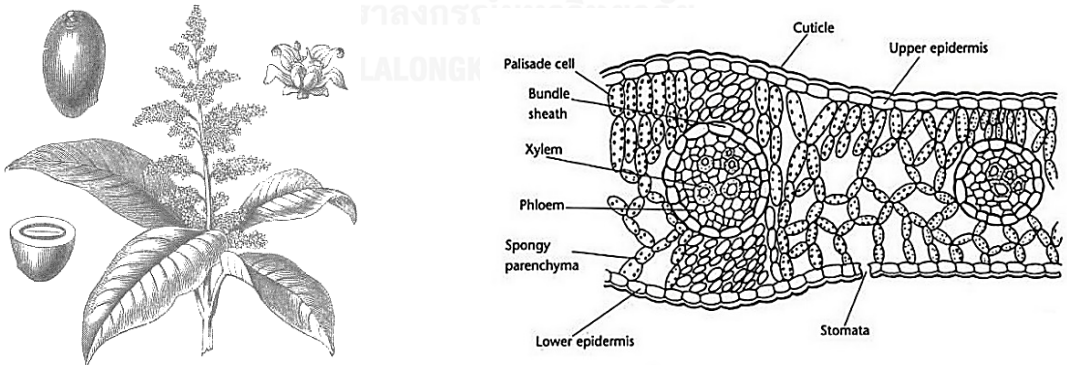
Table 1 (cont.) List of *Mangifera indica* cultivars located in Thailand [63]

Mangifera indica 'Phram Konkho'
Mangifera indica 'Phram Nueadaeng'
Mangifera indica 'Phram Nuealueang'
Mangifera indica 'Phatnampueng'
Mangifera indica 'Phimsen Klaipan'
Mangifera indica 'Phimsen Daeng'
Mangifera indica 'Manmu'
Mangifera indica 'Manyot'
Mangifera indica 'Manwan'
Mangifera indica 'Manhaeo'
Mangifera indica 'Man Ayuthaya'
Mangifera indica 'Maletnim'
Mangifera indica 'Maelukdok'
Mangifera indica 'Maeosao'
Mangifera indica 'Yaiglam'
Mangifera indica 'Rotchana'
Mangifera indica 'Radenkhao'
Mangifera indica 'Radenkhiao'
Mangifera indica 'Raet'
Mangifera indica 'La'
Mangifera indica 'Lin Nguhao'
Mangifera indica 'Lukklom'
Mangifera indica 'Lukdaeng'
Mangifera indica 'Lukyon Phra-in'
Mangifera indica 'Lepmuenang'
Mangifera indica 'Salaya'
Mangifera indica 'Haeo'
Mangifera indica 'Haeo Luanging'
Mangifera indica 'Okrong'
Mangifera indica 'Manthalufa'
Mangifera indica 'Manbanlat'
Mangifera indica 'Mahacharnok'
Mangifera indica 'Manpiset'
Mangifera indica 'Mansadet'
Mangifera indica 'Mansaifa'
Mangifera indica 'Sangkhaya'
Mangifera indica 'Sampi'
Mangifera indica 'Samruedu'
Mangifera indica 'Saithip'
Mangifera indica 'Sainamkang'
Mangifera indica 'Saifon'
Mangifera indica 'Saonoi Kratuepho'
Mangifera indica 'Sampan'
Mangifera indica 'Sisom'
Mangifera indica 'Saengthong'
Mangifera indica 'Hongthong'
Mangifera indica 'Hongsa'
Mangifera indica 'Hongsawadi'
Mangifera indica 'Nongsaeng'
Mangifera indica 'Nangklangwan'
Mangifera indica 'Monthong'
Mangifera indica 'Wannampueng'
Mangifera indica 'Hoikrang'
Mangifera indica 'Horakang'
Mangifera indica 'Hinthong'
Mangifera indica 'Okrong Saiyok'
Mangifera indica 'Okrong Phikunthong'
Mangifera indica 'Okrong Phonthip'

Table 1 (cont.) List of *Mangifera indica* cultivars located in Thailand [63]

Mangifera indica 'Okrong Kati'	*Mangifera indica* 'Okrong Man'
Mangifera indica 'Okrong Khao'	*Mangifera indica* 'Okrong Homthong'
Mangifera indica 'Okrong Khiao'	*Mangifera indica* 'Onman'
Mangifera indica 'Okrong Thong'	*Mangifera indica* 'Inthorachit'
Mangifera indica 'Okrong Thongdamklaiphan'	*Mangifera indica* 'Ai- Huap'

Mango cultivars can generally be categorized into two groups, Indian and IndoChinese, based on peel pigments and sensory characteristics of the pulp [64]. All selected seventeen mango cultivars in this study categorized into IndoChinese group. Mango fruits had two stages of maturity. Green fruit is used to eat as Thai salad 'Yam' or eat with sweet-spicy sauce 'Nam Pla Wan'; whereas, ripe fruits is used to make Thai dessert 'Mango with glutinous rice' [4, 64]. Due to their high polyphenolic content, mango has been found pharmacological effects including as antioxidant, antidiabetic, antimicrobial, anticancer, antispasmodic, antipyretic, anti-inflammatory activities and immunomodulatory [3, 64-68].



(a) (b)

Figure 3 *Mangifera indica* L.; (a) Herbarium and (b) transverse section of mango leaf [69, 70]

Cultivars Nga Khao [63, 71]

Species *Mangifera indica*

Genus *Mangifera*

Family Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	elliptical	**Leaf apex**	acute
	Leaf base	acute	**Leaf margin**	entire

Agricultural descriptor

Flower	**Flowering**	intermediate		
zzFruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	110-120 days	**Yield/10 years**	300 mangoes
	Size (HXLXW)	19 X 7.43 X 6.61 cm	**Fruit weight**	500-600 g
	Flesh thickness	2.47 cm	**Skin thickness**	0.16 cm
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	yellow-green	**Green fruit colour**	green
	Ripe fruit taste	sweet	**Green fruit taste**	sour
	Brix	18 °Bx	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	16.48 x 3.67 x 1.8 cm
	Weight	38 g		

Cultivars Nangklangwan [63, 72]

Species *Mangifera indica*

Genus *Mangifera*

Family Anacardiaceae

Characteristics

Canopy	**Canopy**	large		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	elliptical	**Leaf apex**	acute
	Leaf base	acute	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	abundant		
Fruit	**Fruit setting**	abundant	**Fruiting season**	out of season
	Harvesting index	100-120 days	**Yield/10 years**	300 mangoes
	Size (HXLXW)	16.5 X 7.26 X 6.42 cm	**Fruit weight**	300-600 g
	Flesh thickness	2.46 cm	**Skin thickness**	0.22 cm
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	yellow-green	**Green fruit colour**	yellow-green
	Ripe fruit taste	-	**Green fruit taste**	sour
	Brix	-	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	15.22 x 3.48 x 1.63 cm
	Weight	30 g		

Cultivars	Khiaoyai [63, 73]
Species	*Mangifera indica*
Genus	*Mangifera*
Family	Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	lanceolate	**Leaf apex**	acute
	Leaf base	obtuse	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	100-110 days	**Yield/10 years**	300-400 mangoes
	Size (HX**L**X**W)**	NA	**Fruit weight**	300-600 g
	Flesh thickness	NA	**Skin thickness**	NA
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	yellow-green	**Green fruit colour**	green
	Ripe fruit taste	sweet	**Green fruit taste**	sweet-sour
	Brix	NA	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (H**X**L**X**W)**	NA
	Weight	NA		

Cultivars	Mankhunsi [63, 74]
Species	*Mangifera indica*
Genus	*Mangifera*
Family	Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	cracked		
	Climbling of branch	no		
Leaf	**Leaf shape**	oblong	**Leaf apex**	acute
	Leaf base	acute	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	100-110 days	**Yield/10 years**	300 mangoes
	Size (HXLXW)	13.65 X 5.49 X 4.91 cm	**Fruit weight**	230 g
	Flesh thickness	1.78 cm	**Skin thickness**	0.10 cm
	Fruit juiciness	intermediate	**Fiber**	present
	Ripe fruit colour	orange-green	**Green fruit colour**	green
	Ripe fruit taste	-	**Green fruit taste**	sour
	Brix	-	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	15.73 x 3.54 x 1.99 cm
	Weight	40 g		

Cultivars	Namdokmai [63, 72]
Species	*Mangifera indica*
Genus	*Mangifera*
Family	Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	oblong	**Leaf apex**	acuminate
	Leaf base	obtuse	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	100 days	**Yield/10 years**	300 mangoes
	Size (HXLXW)	15.25 X 7.27 X 6.59 cm	**Fruit weight**	300 g
	Flesh thickness	2.45 cm	**Skin thickness**	0.14 cm
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	yellow-green	**Green fruit colour**	yellow-green
	Ripe fruit taste	sweet	**Green fruit taste**	sour
	Brix	22 °Bx	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	10.27 x 4.03 x 1.10 cm
	Weight	20 g		

Cultivars	Mahacharnok [63, 74]
Species	*Mangifera indica*
Genus	*Mangifera*
Family	Anacardiaceae

Characteristics

Canopy	**Canopy**	sparse		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	linear-oblong	**Leaf apex**	acuminate
	Leaf base	obtuse	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	NA	**Yield/10 years**	NA
	Size (HXLXW)	NA	**Fruit weight**	280-380 g
	Flesh thickness	NA	**Skin thickness**	0.14 cm
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	orange-green	**Green fruit colour**	green
	Ripe fruit taste	sweet-sour	**Green fruit taste**	-
	Brix	18 °Bx	**Flesh aroma**	strong
Stone	**Shape**	NA	**Size (HXLXW)**	NA
	Weight	NA		

Cultivars Kaemdaeng [63, 75]

Species *Mangifera indica*

Genus *Mangifera*

Family Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	cracked		
	Climbling of branch	no		
Leaf	**Leaf shape**	lanceolate	**Leaf apex**	acute
	Leaf base	obtuse	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	100 days	**Yield/10 years**	300 mangoes
	Size (HX**L**X**W)**	13.01 X 7.38 X 6.26 cm	**Fruit weight**	325 g
	Flesh thickness	1.72 cm	**Skin thickness**	0.13 cm
	Fruit juiciness	intermediate	**Fiber**	present
	Ripe fruit colour	yellow-orange-green	**Green fruit colour**	green
	Ripe fruit taste	sour-sweet	**Green fruit taste**	sour
	Brix	19 °Bx	**Flesh aroma**	strong
Stone	**Shape**	oblong	**Size (H**X**L**X**W)**	10.88 x 4.20 x 1.38 cm
	Weight	50 g		

Cultivars	Okrong [63, 71]
Species	*Mangifera indica*
Genus	*Mangifera*
Family	Anacardiaceae

Characteristics

Canopy	**Canopy**	large		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	oblong	**Leaf apex**	acuminate
	Leaf base	acute	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	100 days	**Yield/10 years**	500 mangoes
	Size (HXLXW)	11.11 X 6.25 X 5.46 cm	**Fruit weight**	230 g
	Flesh thickness	1.57 cm	**Skin thickness**	0.01 cm
	Fruit juiciness	abundant	**Fiber**	present
	Ripe fruit colour	yellow-orange-green	**Green fruit colour**	green, yellow-green
	Ripe fruit taste	sweet	**Green fruit taste**	sour
	Brix	20 °Bx	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	9.91 x 3.61 x 1.68 cm
	Weight	30 g		

Cultivars Chok Anan [63, 74]

Species *Mangifera indica*

Genus *Mangifera*

Family Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	elliptical	**Leaf apex**	attenuate
	Leaf base	acute	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	abundant		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	out of season
	Harvesting index	110-120 days	**Yield/10 years**	400 mangoes
	Size (HXLXW)	11.12 X 6.25 X 5.39 cm	**Fruit weight**	209 g
	Flesh thickness	2.95 cm	**Skin thickness**	0.01 cm
	Fruit juiciness	abundant	**Fiber**	absent
	Ripe fruit colour	yellow-orange	**Green fruit colour**	yellow-green
	Ripe fruit taste	sweet	**Green fruit taste**	sour
	Brix	20 °Bx	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	8.94 x 3.35 x 1.93 cm
	Weight	29 g		

Cultivars	Raet [63, 76]
Species	*Mangifera indica*
Genus	*Mangifera*
Family	Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	oblong-lanceolate	**Leaf apex**	attenuate
	Leaf base	obtuse	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	abundant	**Fruiting season**	season
	Harvesting index	100 days	**Yield/10 years**	400 mangoes
	Size (HXLXW)	12.44 X 7.42 X 6.12 cm	**Fruit weight**	300 g
	Flesh thickness	2.13 cm	**Skin thickness**	0.1 cm
	Fruit juiciness	intermediate	**Fiber**	present
	Ripe fruit colour	yellow-orange	**Green fruit colour**	yellow-green
	Ripe fruit taste	sour-sweet	**Green fruit taste**	sweet-sour
	Brix	20 °Bx	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	10.82 x 3.6 x 1.8 cm
	Weight	15 g		

Cultivars Talapnak [63, 77]

Species *Mangifera indica*

Genus *Mangifera*

Family Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	cracked		
	Climbling of branch	no		
Leaf	**Leaf shape**	oblong	**Leaf apex**	acute
	Leaf base	acute	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	100 days	**Yield/10 years**	200-300 mangoes
	Size (HX**L**X**W)**	9.23 X 8.91 X 7.88 cm	**Fruit weight**	400 g
	Flesh thickness	2.55 cm	**Skin thickness**	0.12 cm
	Fruit juiciness	abundant	**Fiber**	present
	Ripe fruit colour	yellow-orange	**Green fruit colour**	green
	Ripe fruit taste	sweet	**Green fruit taste**	sour
	Brix	14 °Bx	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (H**X**L**X**W)**	6.77 x 5.04 x 2.46 cm
	Weight	45 g		

Cultivars Kaeo [63, 72]

Species *Mangifera indica*

Genus *Mangifera*

Family Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	elliptical	**Leaf apex**	acuminate
	Leaf base	acute	**Leaf margin**	entire

Agricultural descriptor

Flower	**Flowering**	abundant		
Fruit	**Fruit setting**	abundant	**Fruiting season**	season
	Harvesting index	100 days	**Yield/10 years**	400-500 mangoes
	Size (HX**L**X**W)**	10.44 X 6.86 X 5.94 cm	**Fruit weight**	250 g
	Flesh thickness	1.81 cm	**Skin thickness**	0.07 cm
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	yellow-orange	**Green fruit colour**	green
	Ripe fruit taste	sweet	**Green fruit taste**	sour
	Brix	23 °Bx	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (H**X**L**X**W)**	8.17 x 3.65 x 1.86 cm
	Weight	30 g		

Cultivars	Tongdam [63, 78]
Species	*Mangifera indica*
Genus	*Mangifera*
Family	Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	elliptical	**Leaf apex**	acute
	Leaf base	acute	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	100 days	**Yield/10 years**	300 mangoes
	Size (HX**L**X**W)**	NA	**Fruit weight**	350 g
	Flesh thickness	NA	**Skin thickness**	NA
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	orange-green	**Green fruit colour**	green
	Ripe fruit taste	sweet	**Green fruit taste**	sour
	Brix	NA	**Flesh aroma**	strong
Stone	**Shape**	oblong	**Size (H**X**L**X**W)**	NA
	Weight	NA		

Cultivars Khiaosawoey [63, 79]

Species *Mangifera indica*

Genus *Mangifera*

Family Anacardiaceae

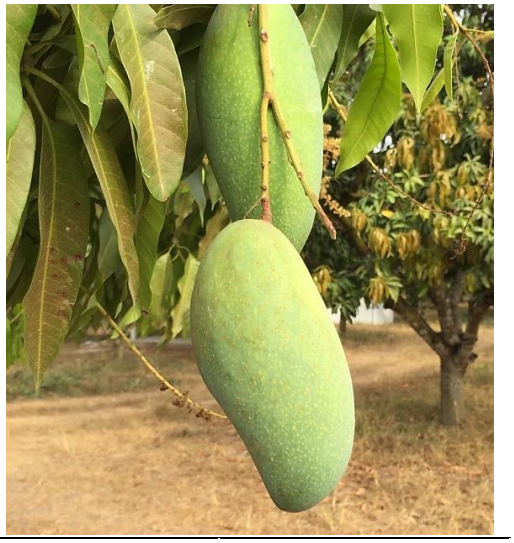
Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	oblong	**Leaf apex**	attenuate - acuminate
	Leaf base	attenuate	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	100-110 days	**Yield/10 years**	200 mangoes
	Size (HXLXW)	15.83 X 7.21 X 6.83 cm	**Fruit weight**	400 g
	Flesh thickness	2.35 cm	**Skin thickness**	0.15 cm
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	yellow-orange	**Green fruit colour**	green
	Ripe fruit taste	sweet	**Green fruit taste**	sweet-sour
	Brix	18.5 °Bx	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	NA
	Weight	NA		

Cultivars	Falan [63, 80]
Species	*Mangifera indica*
Genus	*Mangifera*
Family	Anacardiaceae



Characteristics

Canopy	**Canopy**	small		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	linear-oblong	**Leaf apex**	acute
	Leaf base	acute	**Leaf margin**	entire

Agricultural descriptor

Flower	**Flowering**	abundant		
Fruit	**Fruit setting**	abundant	**Fruiting season**	out of season
	Harvesting index	95 days	**Yield/10 years**	400-500 mangoes
	Size (HXLXW)	16.73 X 7.45 X 6.9 cm	**Fruit weight**	400 g
	Flesh thickness	1.96 cm	**Skin thickness**	0.11 cm
	Fruit juiciness	intermediate	**Fiber**	present
	Ripe fruit colour	yellow-green	**Green fruit colour**	yellow-green
	Ripe fruit taste	-	**Green fruit taste**	slightly sweet
	Brix	-	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	13.6 x 3.71 x 1.11 cm
	Weight	30 g		

Cultivars Phetbanlat [63, 81]

Species *Mangifera indica*

Genus *Mangifera*

Family Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	oblong-lanceolate	**Leaf apex**	acuminate
	Leaf base	acute	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	100 days	**Yield/10 years**	300 mangoes
	Size (HXLXW)	10.14 X 6.71 X 5.71 cm	**Fruit weight**	250 g
	Flesh thickness	1.88 cm	**Skin thickness**	0.10 cm
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	yellow-green	**Green fruit colour**	green
	Ripe fruit taste	sweet	**Green fruit taste**	sour
	Brix	19.6 °Bx	**Flesh aroma**	strong
Stone	**Shape**	oblong	**Size (HXLXW)**	8.31 x 3.43 x 1.87 cm
	Weight	30 g		

Cultivars Nongsaeng [63, 82]

Species *Mangifera indica*

Genus *Mangifera*

Family Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	oblong-lanceolate	**Leaf apex**	acuminate
	Leaf base	acute	**Leaf margin**	entire

Agricultural descriptor

Flower	**Flowering**	abundant		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	90-100 days	**Yield/10 years**	200-300 mangoes
	Size (HXLXW)	11.2 X 6.96 X 6.04 cm	**Fruit weight**	300 g
	Flesh thickness	1.88 cm	**Skin thickness**	0.10 cm
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	yellow-orange	**Green fruit colour**	green
	Ripe fruit taste	sweet	**Green fruit taste**	-
	Brix	25 °Bx	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	NA
	Weight	NA		

Cultivars	Bao [63, 83]
Species	*Mangifera caloneura*
Genus	*Mangifera*
Family	Anacardiaceae

Characteristics

Canopy	**Canopy**	medium		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	oblong	**Leaf apex**	acute
	Leaf base	acute	**Leaf margin**	undulate

Agricultural descriptor

Flower	**Flowering**	abundant		
Fruit	**Fruit setting**	abundant	**Fruiting season**	out of season
	Harvesting index	100 days	**Yield/10 years**	500 mangoes
	Size (HX**L**X**W)**	5.5 X 4.48 X 3.87 cm	**Fruit weight**	56.5 g
	Flesh thickness	0.77 cm	**Skin thickness**	0.10 cm
	Fruit juiciness	intermediate	**Fiber**	present
	Ripe fruit colour	yellow-orange	**Green fruit colour**	yellow-green
	Ripe fruit taste	sweet	**Green fruit taste**	sour
	Brix	10 °Bx	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (H**X**L**X**W)**	4.35 X 2.57 X1.63 cm
	Weight	10 g		

Cultivars	-
Species	*Bouea macrophylla* [84, 85]
Genus	*Bouea*
Family	Anacardiaceae

Characteristics

Canopy	**Canopy**	dense		
	Bark texture	smooth		
	Climbling of branch	no		
Leaf	**Leaf shape**	ovate-oblong	**Leaf apex**	acute-acuminate
	Leaf base	acute-acuneate	**Leaf margin**	entire

Agricultural descriptor

Flower	**Flowering**	intermediate		
Fruit	**Fruit setting**	intermediate	**Fruiting season**	season
	Harvesting index	NA	**Yield/10 years**	NA
	Size (HXLXW)	NA	**Fruit weight**	NA
	Flesh thickness	NA	**Skin thickness**	NA
	Fruit juiciness	intermediate	**Fiber**	absent
	Ripe fruit colour	yellow-orange	**Green fruit colour**	green
	Ripe fruit taste	sweet	**Green fruit taste**	slightly sweet
	Brix	NA	**Flesh aroma**	mild
Stone	**Shape**	oblong	**Size (HXLXW)**	NA
	Weight	NA		

Macroscopic and microscopic characteristics

Macroscopic characteristics play a great role on the classification of the plants. Natural variations in size and shape are common due to the environment factors. Leaf macroscopic characteristics such as leaf shape, leaf apex, leaf base and leaf margin need to be investigated. Leaf microscopic evaluation is based on the cellular structure observation using a microscope. Microscopic leaf constant values are possibly used to distinguish between some closely related both species and cultivars of which cannot clearly characterized by general microscopy [10]. Both macroscopic and microscopic evaluations should be the first step to identify the plants.

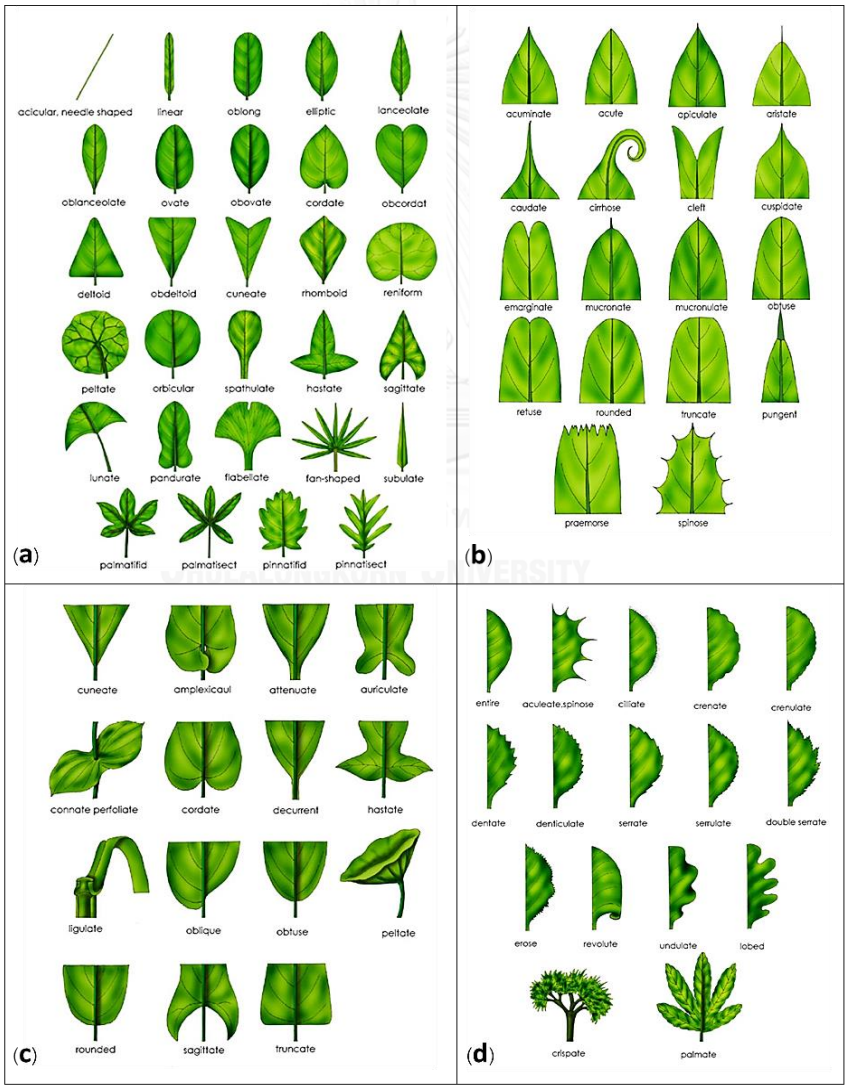


Figure 4 Leaf macroscopic patterning; (a) leaves shape, (b) leaves apex, (c) leaves base and (d) leaves margin [86]

Stomatal number

Stoma is a pore, found in the epidermis of leaf, stem or other organs which can be used to control gas exchange. The pore is surrounded by a pair of guard cells (specialized parenchyma cells) that are responsible for controlling the size of the opening. In leaves, the stomatal patterning distribution is highly variable among species, but is controlled by a mechanism that sustains a minimum of one cell spacing between stomata [87]. Stomatal density is commonly highest on the lower epidermis surface, which probably helps to prevent water loss since that surface is less exposed to heating [88]. Four considerably different stoma types are distinguished by their form and their arrangement of the surrounding cells, particularly the subsidiary cells, as follows

• The anomocytic or ranunculaceous (irregular-celled) type: the stoma is bordered by a varying number of cells, normally not different from the epidermis.

• The anisocytic or cruciferous (unequal-celled) type: the stoma is typically bordered by three or four subsidiary cells, one of which is clearly smaller than the others.

• The diacytic or caryophyllaceous (cross-celled) type: the stoma is complemented by two subsidiary cells, the common wall of which is at right angles to the stoma.

• The paracytic or rubiaceous (parallel, celled) type: the stoma has two subsidiary cells, that the long axes are parallel to the axis of the stoma. [7]

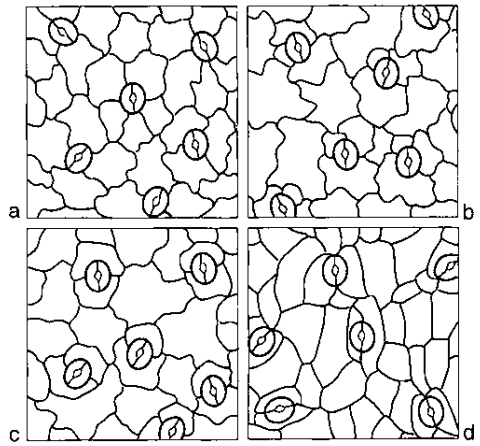


Figure 5 Leaf stomatal patterning; (a) anomocytic type, (b) anisocytic type, (c) diacytic type and (d) paracytic type [7]

The stomatal number is a very specific criteria for identification and characterization of leaves. Stomatal number is the average number of stomata per mm^2 of epidermis and the number on each surface of a leaf. However, this number varies depending on the environment condition and geographical sources where plants were grown [8].

The environment also has considerable effects on stomatal development. Stomatal density seemingly increases or decreases in response to altering conditions, such as light intensity, water availability, temperature, and carbon dioxide concentration. They have been revealed to influence the frequency that stomatal develop on leaves [89, 90].

Air including carbon dioxide and oxygen move in plant through the stomata and are used in photosynthesis in mesophyll cells and respiration, respectively. Oxygen produced as a by-product of photosynthesis diffused out to the atmosphere through these stomata. Furthermore, water vapor is released into the atmosphere throughout the stomata in a process called transpiration. Moreover, to opening and to closing the stomata (stomata behavior), plants possibly apply control over their gas exchange rates by varying stomata density in new leaf when it is produced. The more stomata per unit area; the more carbon dioxide can be taken up, and the more water can be possible released. Consequently, higher stomata number may significantly clarify the potential for behavioral control over water loss rate and carbon dioxide uptake [91].

Veinlet termination number

Veinlet, a vascular tissue, which consisted of xylem and phloem cells surrounded in parenchyma, sometimes sclerenchyma, and is bounded by bundle sheath cells [92]. Veins provide support and protect for the leaf and transfer both water and minerals (using vein xylem) and sugars (using phloem) through the leaf and on to the rest of the plant [93].

Vascular tissue systems are vary greatly across major plant lineages. Normally, there are three orders of lower-order veins, known as ‘major veins’, often ribbed with sclerenchyma. One or more first-order veins run from the petiole to the leaf apex, with

second-order veins branching at intervals, and third-order veins branching between [94].

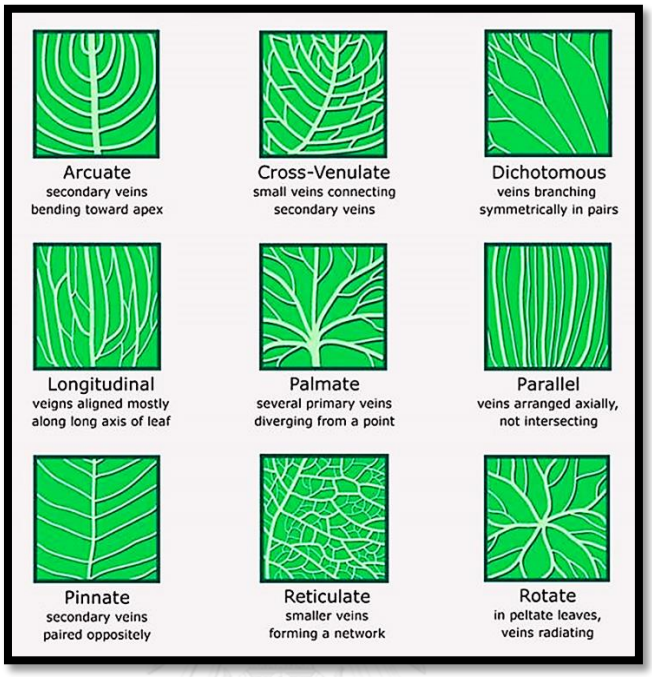


Figure 6 Leaf vein patterning [95]

Leaves are vary greatly in the occurrence and number of veinlet termination. Their number correlates with the total leaf vein length. In most species, veinlet terminations have little or no phloem inside.

Veinlet termination is an ultimate free end of a veinlet, and the number of the veinlet termination per mm^2 of leaf surface is termed as veinlet termination number. It may be used as a distinguishing character for the leaves, both species and cultivars [8, 10]. However, there have never been researches about veinlet termination number of Thai mango cultivars.

Palisade ratio

Palisade cells are plant cells also found inside the mesophyll in leaves. They comprised of elongated usually chlorenchyma cells (parenchyma cells containing chloroplasts) which occur bordering to the epidermis or hiding more deeply in the cortex or mesophyll of plant stems and leaves [96].

Light can possibly have a direct effect in the palisade cells; increased light intensity shows two effects i.e. photosynthesis increasing and later influencing in revealing itself to modify the starch-sugar ratio, and so results in a high concentration of sugar and therefore a high osmotic value in the cells.

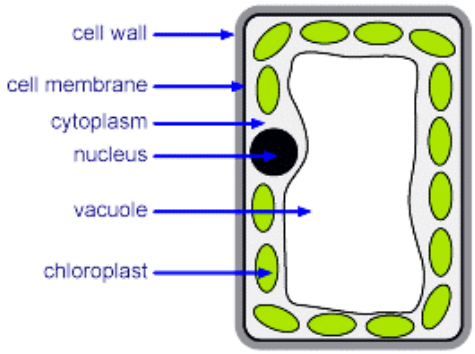


Figure 7 The palisade cell structure [97]

Palisade cells comprise of the largest number of chloroplasts that make them the primary position of photosynthesis in the leaf, changing the energy in light to the chemical energy of carbohydrates. Below the palisade mesophyll cells are the spongy mesophyll cells that perform photosynthesis. They are irregularly-shaped cells, which have many intercellular spaces that allow the passage of gases essential for photosynthesis.

Figure 8 Counting the palisade cells [98]

The average number of palisade cells, which present below each upper epidermal cells, can be used to identify and to evaluate the leaf. The finding may be used as a distinguishing character among the species. This value does not vary based on geographical variation. For that reason, palisade ratio is a very useful diagnostic feature for characterization and identification of different plant species.

The palisade cells are below the epidermal cells; the numbers of total palisade cells under 4 epidermal cells are divided by 4, which provide the average number of palisade cell under each epidermal cell.

Molecular characteristics

Deoxyribonucleic acid (DNA) is a molecule, which encodes the genetic information of all known living organisms including viruses. Eukaryotic organisms keep most DNA inside nucleus and some DNA in organelles (chloroplast genome, mitochondria genome); whereas, prokaryotes keep their DNA only in the cytoplasm [99].

Plant genomes are all the genetic material in plant cell consisted of nuclear genome and organelle genome. The nuclear genome consists of inherited information; it is crowded with nongenic DNA. The organelle genome can be divided into two parts: The mitochondrial genome which is lack of inherited information, and the chloroplast genome which is crowded with genes [100].

Relying on their genomic organization, repetitive DNA elements can be classified as either interspersed or tandemly repeated. Interspersed repeats are present at multiple sites throughout the genome. Tandem repeats are restricted to fewer loci and are composed of arrays of two to several thousand-sequence units arranged in a head-to-tail fashion [101].

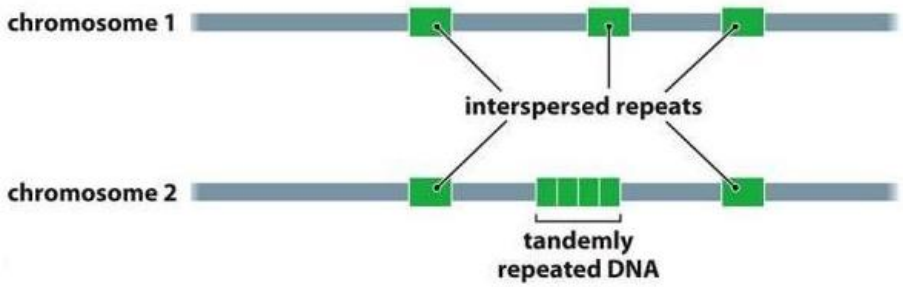


Figure 9 Interspersed and tandemly repeats DNA

Tandem-repetitive DNA can be possibly classified according to the length and copy number of the basic repeat units as well as its genomic localization; (1) Satellite DNA, contains very high numbers of repetitions, usually 1000 to 100,000 copies of a sequence motif. Monomer sizes are possibly range from two to several thousand base

pairs, but 100 to 300 base pairs are most common. They are often located only in subtelomeric or centromeric regions; (2) Minisatellite DNA is compose of approximately 1000 copies of a sequence motif. Monomer sizes are seemingly in the range from 10 to 60 base pairs. They are mostly located in subtelomeric or centromeric regions; (3) Microsatellite DNA, simple repetitive sequences (SRS), simple sequence repeats (SSRs), or simple tandem repeats (STRs), consists of approximately 10 to 60 copies of a sequence motif with very short monomer sizes are in the range from 1 to 6 base pairs. Microsatellites are found throughout the genome [102].

Mononucleotide repeats:	...AAAAAAAAAAAAAAAAAAAAAAA...
Dinucleotide repeats:	...CACACACACACACACACACACACA...
Trinucleotide repeats:	...CGTCGTCGTCGTCGTCGTCGTCGT...
Tetranucleotide repeats:	...CAGACAGACAGACAGACAGACAGA...
Pentanucleotide repeats:	...AAATTAAATTAAATTAAATTAAATT...
Hexanucleotide repeats:	...CTTTAACTTTAACTTTAACTTTAA...

Figure 10 Examples of perfect microsatellite repeats

Alternative way to classify microsatellites relates to the degree of perfectness of the arrays, including (1) perfect repeats that consist of a single, uninterrupted array of a particular motif; (2) imperfect repeats that are interrupted by one or several out-of-frame bases; (3) compound repeats that are combined perfect or imperfect arrays of several motifs.

Perfect repeats:	$\ldots(AG)_{32}\ldots$
	$\ldots(TAT)_{25}\ldots$
	$\ldots(CAA)_{7}\ldots$
Imperfect repeats:	$\ldots(TC)_{6}A(TC)_{13}\ldots$
	$\ldots(AG)_{12}GG(AG)_{3}\ldots$
Compound repeats:	$\ldots(AT)_{6}(GT)_{42}AT(GT)_{5}(GT)_{10}\ldots$
	$\ldots(AT)_{14}(AG)_{8}\ldots$
	$\ldots(GAA)_{21}\ldots(TA)_{23}\ldots$

Figure 11 Examples of perfect, imperfect and compound microsatellites

The polymerase chain reaction

The polymerase chain reaction (PCR) is an *in vitro* technique, which allows amplifying a specific DNA region to high copy numbers. To amplify a specific DNA sequence, two single-stranded complementary primers are designed. The primer sequences are selected to allow base-specific binding to the two template strands in reverse location, thermostable DNA polymerase in an appropriate buffer system and cyclic programming of denaturation steps, primer annealing and primer extension lead to the exponential amplification of the sequence between the primer-binding sites, as well as the primer sequences within a few hours [103].

In the first step of cycle, the template DNA is made single-stranded by raising the temperature to about 94°C (denaturing step). Then, lowering the temperature to about 35 to 65°C (depending on primer sequence) results in primers annealing to their target sequences on the template DNA (annealing step). For the last step, a temperature is chosen at which the activity of the thermostable polymerase is optimal; i.e., usually 65 to 72°C (elongation step). The polymerase now extends from the 3-ends of the DNA–primer hybrids toward the other primer binding site [103].

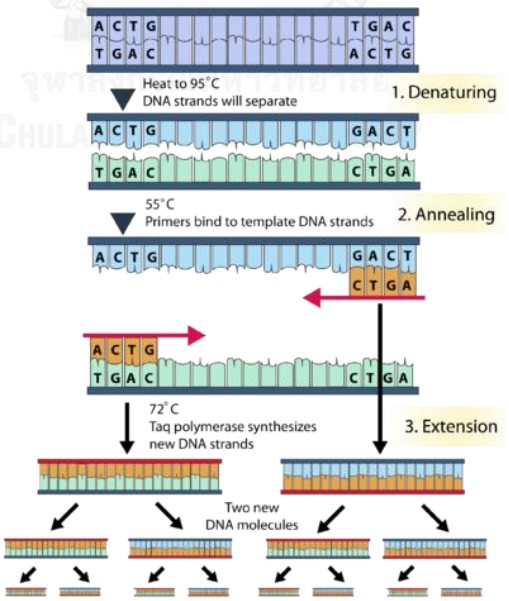


Figure 12 The polymerase chain reaction [104]

Various problems may occurred when template DNA, primers, Mg2+, dNTPs or *Taq* DNA polymerase quantities are not balanced. If quantity of template DNA is too high, this may increase nonspecific PCR products; whereas, if quantity of template DNA is too low, this may reduce the accuracy of the amplification. If quantity of primers is too high, this may increase mispriming and nonspecific PCR products; whereas, if quantity of primers is too low, this may reduce the accuracy of the amplification. If quantity of Mg^{2+} is too high, this may increase nonspecific PCR products; whereas, if quantity of Mg^{2+} is too low, this may reduce the yield of PCR products. If quantity of dNTPs is not balanced, the PCR products may severely increase. If quantity of *Taq* DNA polymerase is too high, this may increase nonspecific PCR products; whereas, if quantity of *Taq* DNA polymerase is too low, this may reduce the yield of PCR products. If set up at room temperature, this may increase nonspecific products [102, 105].

The molecular marker is used as a marker for genetic diversity evaluation and is based on polymorphisms in proteins or DNA. They are also less affected by age, sample physiological condition and environmental factors. The observing properties would commonly be desirable for a molecular marker such as moderately to highly polymorphic, co-dominant inheritance, frequent occurrence in the genome, distribution throughout the genome, high reproducibility and reasonable price for both marker development and assay [102].

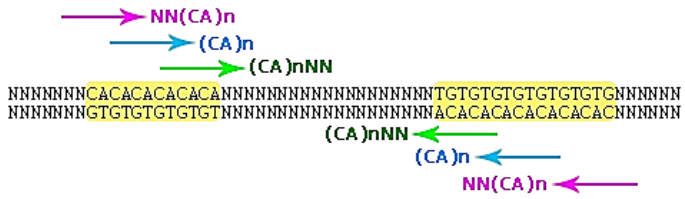


Figure 13 Inter simple sequence repeat amplification [106]

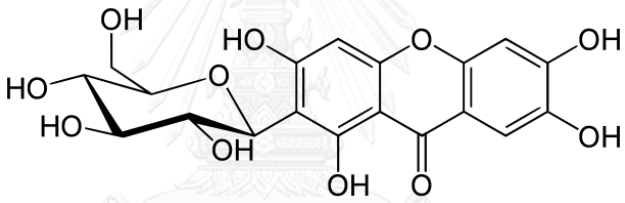
Inter simple sequence repeat amplification (ISSR) is a simple, quick and reliable technique used in various species and cultivars for detecting polymorphism and genetic mapping. ISSR is a general term for a genome region between microsatellite loci. The complementary sequences to two neighboring microsatellites are used as PCR primers; the variable regions between them get amplified. Sequences amplified can be used for DNA fingerprinting [107].

Mangiferin quantitative analysis

Mangiferin

Mangiferin is a xanthone, commonly called C-glucosyl xanthone, which is a natural polyphenolic antioxidant present in the bark, fruits, roots, and leaves of mango tree and a few other medicinal plants [108]. It is one of the most powerful antioxidants; it is thought to be more effective than both vitamin C or vitamin E. Sometimes it is referred to "super antioxidants" [35]. Mango is found to be the major source of mangiferin. It also has a medicinal benefit. Many studies of mangiferin and its extracts from mango have been reported as radioprotective, antiallergic, antidiabetic, anticancer, antimicrobial, immunomodulatory, anti-inflammatory activities. [37, 38]

Table 2 Chemical descriptions of mangiferin [109]



Chemical Name	Mangiferin	Molecular Formula	$C_{19}H_{18}O_{11}$
IUPAC Name	1,3,6,7-Tetrahydroxyxanthone C2-β-D-glucoside		
Synonym	Alpizarin, Chinonin, Xanthone-c-glucoside		
Molecular Weight	422.33962 g/mol	Melting point	261-264 °C
Appearance	Yellow to yellow-green		

Thin-layer chromatography (TLC) is a chromatographic technique that is a fast screening method to identify and to separate the compounds. TLC comprises of three steps - spotting, development, and visualization. This technique has some advantages, for instance easy to use, reasonable cost of instrumentation and short time for analysis. TLC can also be used for quantitative analysis [110, 111].

TLC-Densitometry

The compounds separated by TLC are quantified by *in situ* measurement of absorbed visible, UV light or emitted fluorescence upon excitation with UV light. Signal diminution (absorbance) or increase (fluorescence) between zone and blank area is measured upon that quantitative analysis. It can be converted into densitogram [111].

Scanning Densitometry

Figure 14 The CAMAG TLC scanner 4 [112]

Densitometer such as the CAMAG TLC scanner 4 contains a single wavelength, multiple wavelengths up to 31 selected wavelengths or a combination of measurements in absorption and fluorescence detection mode with winCATS software for evaluation after scanning. It consists of three light sources - deuterium, halogen-tungsten and high pressure mercury lamp. It can measure a reflection, either in absorbance or fluorescence mode. Spectrums are range from 190 to 900 nm. The deuterium lamp is used in the UV range of 190-450 nm, and the halogen-tungsten lamp in the visible region, i.e., 350-900 nm. A high-pressure mercury vapor lamp, provides high energy at 254 – 578 nm [111, 112].

Deuterium lamp, halogen-tungsten lamp or high pressure mercury lamp can be positioned in the light path by a motor drive (1). For scanning at wavelengths below 200 nm it is advisable to flush the monochromator with nitrogen. A monochromator bandwidth of 5 nm or 20 nm can be selected. Five nm bandwidth is used for spectra recording, multi-wavelength scanning, and when spectral selectivity is required. Twenty nm bandwidth offers higher light intensity (improves the signal to noise ratio and thus the reproducibility of the measurement) and enables measurement of several fractions with slightly different absorption maxima in one scan (2). The lens system with 190 –

900 nm transmission range features automatic positioning for micro and macro slit sizes. This ensures that the light energy available with small slits in the micro position is almost the same as that for the corresponding slit in the macro position, which is four times larger (3). The light beam strikes the object at right angle. The photomultiplier is aligned at an angle of 30° (5).The signal of the measuring photomultiplier is continuously offset against the signal of the reference photomultiplier (4 and 5) [112].

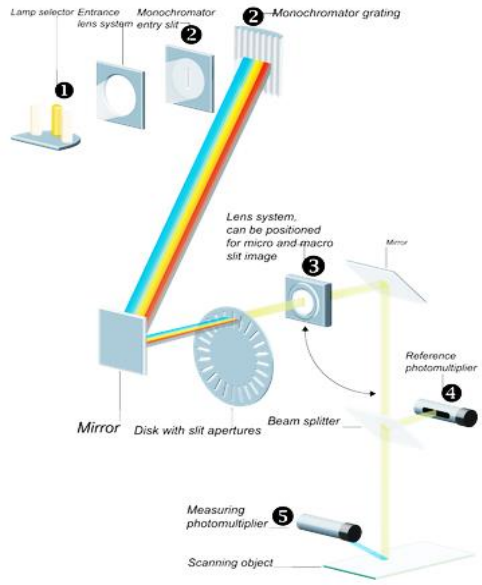


Figure 15 The densitometer optical system [112]

TLC-image analysis

Image J is one of the several image analysis softwares that extract quantifiable data of the image from digital camera. It is a public domain Java image processing program developed at the National Institutes of Health, USA. Image J supports standard image processing functions, for instance contrast manipulation, smoothing, sharpening, edge detection and median filtering. It can read many image file formats, including TIFF, PNG, GIF, JPEG, BMP, DICOM, and FITS. ImageJ can calculate area and pixel value statistics of user-defined selections and intensity thresholded objects. Users can develop and can fix this program. It is available for Microsoft Windows, Mac OS, OS X, Linux, and the Sharp Zaurus PDA. It can be free downloaded from http://rsbweb.nih.govtlyindex.html. The source code for ImageJ is freely available. [113, 114].

Antidiabetic activities

Diabetes mellitus is a group of metabolic diseases, characterized by people with chronic high blood sugar level (hyperglycemia), because of defects in insulin secretion, insulin action, or both. The symptoms includes polyuria, polydipsia, weight loss, polyphagia, and blurred vision [115, 116]. There are two main diabetes types:

Type 1 diabetes (Juvenile-onset diabetes/ Insulin-dependent diabetes mellitus)

- An absolute deficiency of insulin secretion due to a cellular-mediated autoimmune destruction of the ß -cells include islet cell of the pancreas
- Onset mostly in children (5-10 % of diabetes patient)
- Treated with insulin injections (usually given subcutaneously)

Type 2 diabetes (Adult-onset diabetes / Noninsulin-dependent diabetes mellitus)

- A combination of insulin resistance and/or an inadequate insulin secretory response
- Onset mostly in adults (90–95% of diabetes patient)
- Treated with lifestyle changes and medications with or without insulin

Fasting blood glucose level is normally maintained between 70 mg/dl and 110 mg/dl. Blood glucose levels below 70 mg/dl is hypoglycemia; whereas, a blood glucose above 180 mg/dl is hyperglycemia [115, 116].

Carbohydrates (polyhydroxylated aldehydes or ketones and their derivatives) are major biomolecules of organic compounds founded in all living organisms. Monosaccharide (saccharide = sugar) is the simplest and smallest unit of the carbohydrates, which are colorless, crystalline solids, freely soluble in water, insoluble in nonpolar solvents, for instance, glucose (dextrose), fructose (levulose) and galactose. Disaccharide consists of two monosaccharides joined by an O-glycosidic bond for example, maltose, a homosaccharide that α (1⟶4) glycosidic linkage joins two glucose units; sucrose, a heterosaccharides that anomeric carbon atoms joined a glucose unit and a fructose unit. Lactose, a heterosaccharides that ß (1⟶4) glycosidic linkage joined galactose unit and glucose unit. Oligosaccharide, a simple sugars, comprises a small number

(typically three to ten) of component sugars. Polysaccharides, which are very large macromolecules, insoluable in water and no sweet taste, consist of many monosaccharides joined together by glycosidic bonds [117, 118].

As the human food, carbohydrates are the main metabolic energy supply of which only monosaccharides can be absorbed at the small intestine. Key enzymes for hydrolysis of carbohydrates are α-amylase and α-glucosidase. α-Amylase, located in mouth (saliva) and small intestine, is participating in hydrolysis of polysaccharides and oligosaccharides through the cleavage of α-D-(1-4) glycosidic bonds. α-Glucosidase, located in the brush border of the small intestine, further hydrolyes di- and tri-saccharides to glucose and other monosaccharides through the cleavage of α-D-(1-4) and α-D-(1-6) glycosidic bonds [117, 118].

Acarbose, which is obtained from the fermentation processes of *Actinoplanes utahensis* [119], may be used to inhibit α-amylase and α-glucosidase activities. It decreases the glucose absorption rate from the gastrointestinal tract by delay the hydrolysis of polysaccharides and oligosaccharides in the small intestine.

α-amylase and α-glucosidase inhibition can be measured by hydrolysis of synthesis substrates 2-chloro-4 nitrophenol-α-D-maltotroside (CNPG-3) and p-nitrophenyl-α-D-glucopyranoside (PNPG), respectively. According to α-amylase or α-glucosidase activities, the yellow color of nitrophenol is seen, which implies that enzyme-induced hydrolysis of polysaccharides or oligosaccharides to monosaccharides. If the tested inhibitors possess α-amylase or α-glucosidase inhibitory activities, the intensity of yellow color will be less. Both activities are measured at initial rate of those substates utilization when no products are present.

Antimicrobial activities

Microbiology is the study of microorganisms. It includes bacteria, fungi (which are microscopic organisms that exist as single cells or cell clusters); it also comprises of viruses (which are not cellular).

Bacteria are prokaryotic microorganisms. Normally, their sizes are a few micrometers in length; spheres (cocci) or rods (bacilli) in shapes. They live in symbiotic and parasitic relationships with both plants and animals. A fungi are eukaryotic microorganisms (contain membrane-bound nuclei). They comprise of microorganisms such as yeasts and molds, as well as the more familiar mushrooms. Viruses are a minute infectious agent that replicates only inside the living cells. Viruses can infect all types of life forms, including animals, plants and microorganisms.

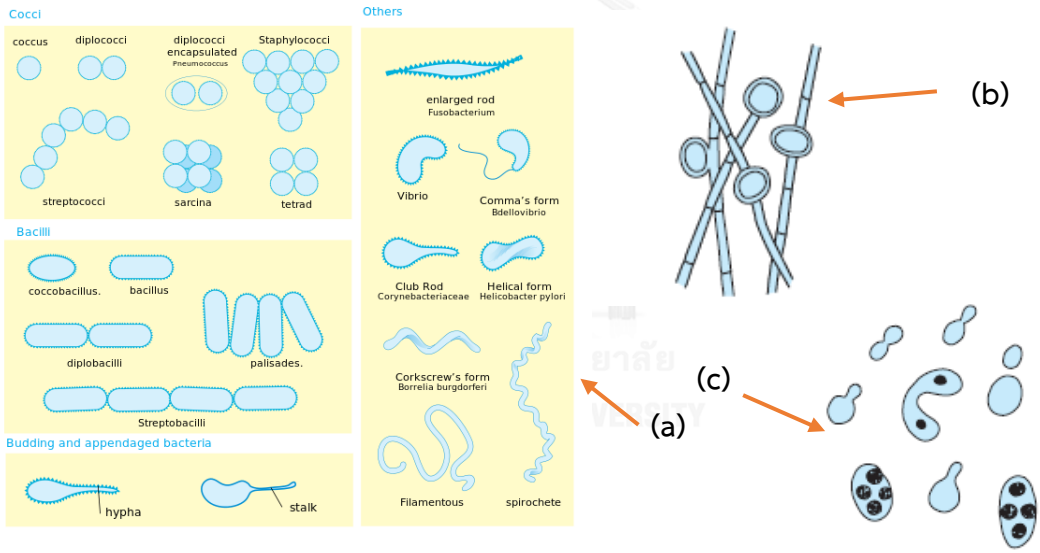


Figure 16 Microorganism morphology; (a) bacteria; (b) fungi in mold form and (c) yeast form [120]

Microorganisms that do not cause disease are nonpathogen. They are the part of the normal flora. Agents capable of causing disease only when the host is immunocompromised referred to opportunistic pathogen. A microorganism capable of causing disease is pathogen. The capability of an infectious agent to cause disease is pathogenicity [121-123].

Table 3 Characteristics and pathogenesis of Gram-positive bacteria, Gram-negative bacteria and fungi

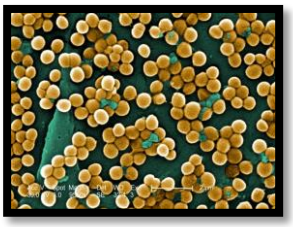
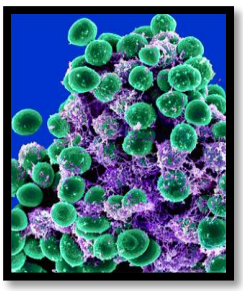
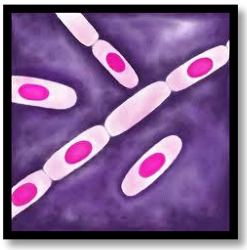
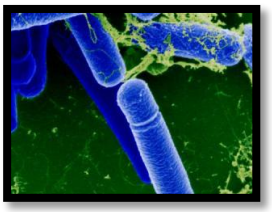
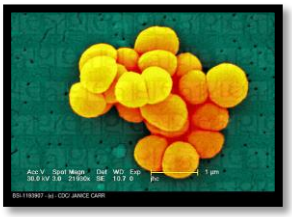
Gram-positive bacteria	Characteristics	Pathogenesis	References
Staphylococcus aureus 	■ Facultative anaerobe ■ Non spore forming ■ Cocci shaped, gray to deep golden yellow colonies, arranged in grape-like irregular clusters	■ Food poisoning ■ Minor skin infections ■ Skin infections ■ Bloodstream infections	[121, 124]
Staphylococcus epidermidis 	■ Facultative anaerobe ■ Non spore forming ■ Cocci shaped, gray to white colonies, arranged in grape-like irregular clusters ■ Normal human flora; the skin flora	■ Infections normally hospital-acquired or in immunocompromised patient	[121, 125]
Bacillus cereus 	■ Facultative anaerobe ■ Spore forming ■ Rod shaped, occurring in chains	■ Food poisoning 1. The emetic type, related with fried rice 2. The diarrheal type, related with meat dishes and sauces ■ Eye infections: severe keratitis, endophthalmitis, and panophthalmitis	[121, 126]

Table 3 (Cont.) Characteristics and pathogenesis of Gram-positive bacteria, Gram-negative bacteria and fungi

Gram-positive bacteria	Characteristics	Pathogenesis	References
Bacillus subtilis 	▪ Facultative anaerobe ▪ Spore forming ▪ Rod shaped, occurring in chains	▪ Food poisoning in immunocompromised patient	[121, 127]
Kocuria rhizophila 	▪ Obligate aerobe ▪ Non spore forming ▪ Cocci shaped, yellow colonies, arranged in grape-like irregular clusters ▪ Normal human flora; upper respiratory tract	▪ Infections in immunocompromised patient especially HIV patient	[121, 128]

Gram-negative bacteria	Characteristics	Pathogenesis	References
Escherichia coli 	▪ Facultative anaerobic ▪ Non spore forming ▪ Rods shaped ▪ Normal human flora; the gut flora	▪ Food poisoning ▪ Urinary tract infections ▪ Respiratory illness ▪ Pneumonia	[121, 129]

Table 3 (Cont.) Characteristics and pathogenesis of Gram-positive bacteria, Gram-negative bacteria and fungi

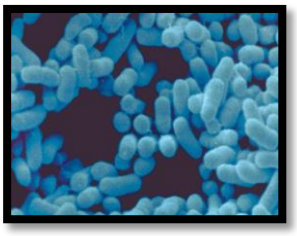
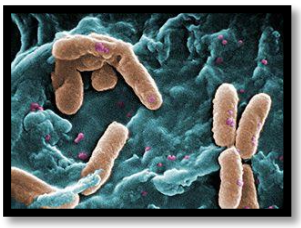
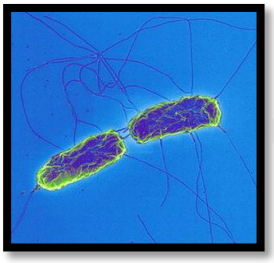
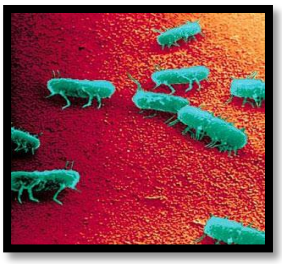
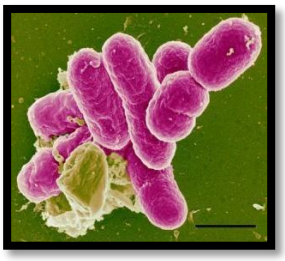
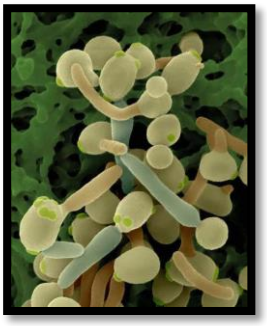
Gram-negative bacteria	Characteristics	Pathogenesis	References
Enterobacter aerogenes 	■ Facultative anaerobic ■ Non spore forming ■ Rods shaped	■ Urinary tract infections ■ Sepsis ■ Opportunistic infections in immunocompromised patient	[121, 130]
Pseudomonas aeruginosa 	■ Aerobic ■ Non spore forming ■ Producing the blue-green bacterial pigment ■ Coccobacillus shaped	■ Pneumonia ■ Septic shock ■ Urinary tract infection ■ Gastrointestinal infection ■ Skin and soft tissue infection	[121, 131]
Salmonella typhi 	■ Aerobic ■ Non spore forming ■ Rod shaped	■ Typhoid fever in human	[121, 132]
Salmonella typhimurium 	■ Aerobic ■ Non spore forming ■ Rod shaped	■ Typhoid fever in cattle, swine, sheep, horse, rodent ■ Infections in immunocompromised patient	[121, 132]

Table 3 (Cont.) Characteristics and pathogenesis of Gram-positive bacteria, Gram-negative bacteria and fungi

Gram-negative bacteria	Characteristics	Pathogenesis	References
***Shigella* spp.** 	■ Facultative anaerobic ■ Non spore forming ■ Rod shaped	■ Dysentery	[121, 131]

Fungi	Characteristics	Pathogenesis	References
Candida albicans 	■ Yeastlike fungi ■ Reproduced by budding	■ Genital infection in human ■ Oral candidiasis ■ Nail plate infection ■ Hospital-acquired infection ■ Opportunistic oral and genital infection in human	[133, 134]
Saccharomyces cerevisiae 	■ Yeast ■ Reproduced by budding ■ The most useful yeast; winemaking, baking, and brewing	■ Opportunistic oropharyngeal infection	[133, 135]

Disk diffusion assay [136, 137]

(Kirby–Bauer testing)

Disk diffusion assay, a quantitative screening assay, is commonly applied for screening the antimicrobial agents.

According to the Clinical Laboratory Standards Institute (CLSI) guideline, the inoculum density must be adjusted to the 0.5 McFarland standard in sterile saline, at the final concentration of 1 x 10^8 CFU/ml. These suspensions need to be used with in 15 minutes. The agar plates are usually 150 mm (less than 12 sample disks) or 90 mm (less than 5 sample disks) in size. Appropriate medium must be completely dried before all sample disks to be placed on, incubated only in a side up position in a standard times for tested microorganisms. Many other variations in that agar plate, for instance, depth can directly influence zone sizes. Larger zones are probably due to slow growing microorganisms; whereas, smaller zones are possibly owing to high molecular weight compounds.

For interpretation, the presence of an inhibition zone implied antimicrobial growth and no zone implied microbial growth.

Microbroth dilution assay [121, 136, 137]

Microbroth dilution assay, a quantitative estimate assay, is modified from the macrobroth dilution assay for determining minimal inhibitory concentration (MIC) of samples or antimicrobial agents to microorganisms.

From the CLSI guideline, microbroth dilution assay is the accepted assay of MIC determination. It is applied only small volumes of reagents, allowed a large number of microorganisms and tested relatively quickly. Standardization of the inoculum density is still at the final concentration of 1 x 10^8 CFU/ml (0.5 McFarland standard), and approximately 5 x10^5 CFU/ml in each well. The suspensions must be used within 15 minutes. The incubation times must be appropriated for selected microorganisms. This assay suggests two-fold dilutions of samples or antimicrobial agents into the broth media with microorganisms in each well of 96-microtiter plates, the lowest concentration that no visible growth is considered as the MIC. Because minimal

inhibitory concentration is the capability of inhibitory status, if that samples or antimicrobial agents are removed, the microorganisms possibly start to grow again.

For determining minimal bactericidal or fungicidal concentration (MBC or MFC), there can be examined by subculturing clear microbial suspended broth from microbroth dilution assay to new sterile agar plates. The lowest concentration of antibacterial agent that killing the majority (99.9%) of a bacterial inoculum is considered as the minimal bactericidal concentration (MBC) and the lowest concentration of an antifungal agent killing the majority (99.9%) of a fungal inoculum is considered as the minimal fungicidal concentration (MFC)

Müller-Hinton and Sabouraud dextrose mediums are recommended by CLSI as bacteria and fungi growth mediums, respectively that is generally used for these testing.

Tested antibiotics

Antibiotics are frequently refer to either bacteriostatic or bactericidal. Bacteriostatic defines antibacterial agents that temporarily inhibit the growth of bacteria; whereas, bactericidal defines antibacterial agents that causes bacteria death.

Ampicillin is a beta-lactam antibiotic used to treat bacterial infections. It is approximately equal to amoxicillin in terms of activity. Ampicillin performs as an inhibitor of bacterial cell walls synthesis, which finally causes cell lysis. It is active against both Gram-positive and Gram-negative bacteria except some bacteria such as *Pseudomonas aeruginosa*.

Amikacin is an aminoglycoside antibiotic also used to treat bacterial infections. Its functions are binding to the bacterial 30S ribosomal subunit, affecting misreading of mRNA and departing the bacteria unable to synthesize proteins essential to bacteria growth. Amikacin is frequently used to treat severe bacterial infections or hospital-acquired infections with multidrug-resistant Gram-negative bacteria for example *Pseudomonas aeruginosa* [137].

Anticancer activity

Proliferation and cytotoxicity assay [138-140]

(MTT cell proliferation/ MTT tetrazolium reduction assay**)**

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay, a quantitative colorimetric assay, is one of the most often used for screening the samples to reveal their cell proliferation or cytotoxic properties.

This method detects the number of viable eukarotic cells (surviving cells) in 96-well plates using MTT, which is measuring mitochondrial activity. Viable eukaryotic cells in culture maintain mitochondria redox reaction and capable to reduce MTT substrate to formazan (figure 17) which is directly proportional to that viable cell numbers present (death cells lose the ability to change the MTT substrate to formazan product). The formazan is an insoluble precipitate product, which needed to be solubilized agents before record a maximum absorbance at 570 nm. The common suitable solubilized agents are acidified isopropanol, DMSO, dimethylformamide, SDS and detergent and organic solvent in combinations.

A quantification of viable signal is related to several parameters comprising the MTT concentration (at a final concentration of 0.2-0.5 mg/ml), the length of the incubation time (1 to 4 hours), the number of viable cells (1,000-100,000 cell per well) and their metabolic activity.

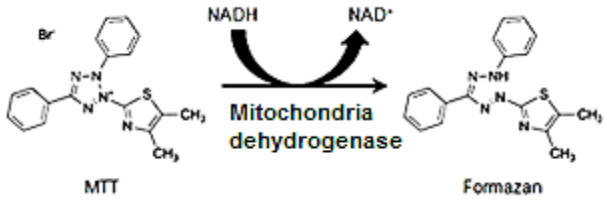


Figure 17 MTT structure and formazan product

Possible problems may occurred for instance, generated signal absorbance readings are too low or too high, blanks absorbance readings is too high or when the experiments are repeated, they give a different values. If quantitative generated signal is too low, this may be because the number of viable cells per well is too low or the

length of incubation time is too short. If quantitative generated signal is too high, this may be because the number of viable cells per well is too high or the number of viable cells is contaminated with bacteria/ yeast cultures. If a blank absorbance reading is too high, this may be because that medium is contaminated with cell cultures or reducing compounds such as ascorbic acid, glutathione and coenzyme A (decrease tetrazolium salts non-enzymatically then make possible to increase absorbance values). If the experiments are repeated then they give different values, this may be because inaccurate pipetting.

For interpretation, a lower absorbance rate than control cells implies a reduction rate of cell proliferation; on the contrary, a higher absorbance rate implies an increase in cell proliferation.

Doxorubicin is on the WHO's List of essential medicines used to treatment of cancer. It is an anthracycline chemotherapy drug that slows or stops the growth of cancer cells by blocking an topo isomerase 2 enzyme that cancer cells need to divide and grow. Doxorubicin may be used in combination with other chemotherapy [141].

CHAPTER III

MATERIALS AND METHODOLOGY

Chemicals

2-chloro-4 nitrophenol-α-D-maltotroside (CNPG-3)	Sigma-Aldrich, USA
Acarbose	Sigma-Aldrich, USA
Agaross	Vivantis Inc., USA
Alpha amylase from porcine pancreas	Sigma-Aldrich, USA
Alpha-glucosidase from *Saccharomyces cerevisiae*	Sigma-Aldrich, USA
Boric	Ajax Finechem Pty. Ltd., New Zealand
Chloral hydrate	Ajax Finechem Pty. Ltd., New Zealand
DNA marker	Thermo Fisher Scientific Inc., USA
DNeasy® plant mini kit	QIAGEN, USA
dNTPs	Thermo Fisher Scientific Inc., USA
EDTA	Ajax Finechem Pty. Ltd., New Zealand
Ethanol	RCI Labscan Limited, Thailand
Ethidium bromide	ACROS, USA
Ethyl acetate	Mallinckrodt® Inc., USA
Formic acid	RCI Labscan Limited, Thailand
GeneRuler 1 kb DNA ladder	Thermo Fisher Scientific Inc., USA
Haiter® solution (6% sodium hypochlorite)	Kao Corp., Japan
Hydrochloric acid	RCI Labscan Limited, Thailand
Intestinal acetone powders from rat	Sigma-Aldrich, USA
Loading dye	Thermo Fisher Scientific Inc., USA
Magnesium chloride	Thermo Fisher Scientific Inc., USA
Mangiferin	MIRA, China
Methanol	RCI Labscan Limited, Thailand
MTT (3-(4,5-dimethyl-thiazol-2-yl) 2, 5-diphenyl-tetrazolium bromide;)	Sigma-Aldrich, USA

Chemicals (Cont.)

Mueller Hinton agar	Merck, Germany
Mueller Hinton broth	Merck, Germany
Primer	Eurofins MWG Operon Inc., USA
P-nitrophenyl-α-D-glucopyranoside (PNPG)	Sigma-Aldrich, USA
Sabouraund Dextrose agar	Merck, Germany
Sabouraund Dextrose broth	Merck, Germany
Sodium carbonate	Sigma-Aldrich, USA
Taq DNA polymerase	Thermo Fisher Scientific Inc., USA

Other chemicals were analytical grade.

Water was ultrapure water.

Materials

Beaker	Pyrex, Germany
Filter paper No.4	Whatman™ paper, UK
Forceps	City-med, Thailand
Glass slide and coverglass	HDA, China
silica gel 60 F_{254}	Merck, Germany
Mortar and pestel	

Instruments and Equipments

-20°C Freezer	Sharp, Japan
AxioVision40 software (V 4.6.3.0)	Zeiss Inc., Germany
UV viewing cabinet (CC-80)	Spectronics Corp., USA
CAMAG TLC Chamber	CAMAG, Switzerland
CAMAG TLC Scanner 4	CAMAG, Switzerland
Centrifugation machine	Sigma, Germany
Digital camera (Canon PowerShot A640)	Canon Inc., Japan
Digital camera (Canon PowerShot A650 IS)	Canon Inc., Japan

Instruments and Equipments (Cont.)

GeneDirectory software	Syngene, UK
GeneTools software	Syngene, UK
Image J software	National Institutes of Health, USA
InGenius 3 with GeneSis software	Syngene, UK
Micropipette	Gibthai, Thailand
Microplate reader (Anthos Zenyth 200 RT)	Biochrom, England
Microscope (Axio imager A2)	Zeiss Inc., Germany
Proflex PCR system thermocycler	Thermo Fisher Scientific Inc., USA
Ultraviolet fluorescence analysis	Spectronic corp., USA
UV visualize gel documentation machine	Auto Chemi System, USA
Vortex mixer (K-550-GE)	Scientific Industries, Inc., USA
Water bath	Brinkmann, USA
Water purification systems	Heal Force Bio-meditech Holdings Ltd., China
winCATS software	CAMAG, Switzerland

Sample collection

Leaf samples of Thai *Mangifera indica* cultivars, *Mangifera caloneura* and *Bouea macrophylla* were collected during June to July in 2014. Each sample was collected from three different locations per cultivar listed in Table 4. They were authenticated by Assoc. Prof. Dr. Nijsiri Ruangrungsi. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand.

Table 4 *Mangifera indica* cultivars and two outgroups used in this study (n=57)

Scientific name	Source of collection
Mangifera indica 'Nga Khao'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Nangklangwan'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Khiaoyai'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Mankhunsi'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Namdokmai'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Mahacharnok'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Kaemdaeng'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Okrong'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Chok Anan'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Raet'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Talapnak'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Kaeo'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Tongdam'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Khiaosawoey'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Falan'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Phetbanlat'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera indica 'Nongsaeng'	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand
Mangifera caloneura	Nakhon Si Thammarat, Surat Thani, Songkhla provinces, Thailand
Bouea macrophylla	Chiang Mai, Nonthaburi and Uttaradit provinces, Thailand

Macroscopic characteristics

The observations on selected seventeen Thai *Mangifera indica* cultivars, *Mangifera caloneura* and *Bouea macrophylla* leaf samples using naked eyes on fruits (fruit shape) and leaves (leaf shape, leaf apex, leaf base and leaf margin) were recorded.

Microscopic characteristics

Microscopy

A microscope was used to observe stomatal number, veinlet termination and palisade ratio from each cast under the objective lens magnification of 20X, 5X and 40X, respectively and the eyepiece lens magnification of 10X. The microscope was attached to a digital camera interfaced with a personal computer using an AxioVision40 software for image labeling.

Determination of stomatal number, veinlet termination number and palisade ratio

All mature leaf samples were cleaned and the lamina were cut into small pieces approximately 10 x 5 mm^2 in size. Calcium oxalates were removed, and tissues were disintegrated by poaching leaf samples in 10% hydrochloric acid under low heat for 1 hour. They were bleached with Haiter® solution. When bleaching was complete, leaf samples were washed with water. They were cleared with chloral hydrate solution **(**4 g of chloral hydrate / 1 ml of ultrapure water**)** under low heat afterward.

Leaf sample was kept on slide, mounted with a few drops of water then cover slip was placed on top. The appropriate eyepiece and objective lens of microscope were used, image labeling was taken. The slide was placed on the stage and the selected cells were traced. Each sample was counted for 30 fields. The average of 90 fields from three locations per cultivar was carried out.

Molecular characteristics

DNA extraction and electrophoresis

Genomic DNA was extracted from the fresh young leaf tissues following a CTAB method as described previously by Doyle and Doyle [142] with a minor modification. One gram of cleaned leaf sample was rapidly ground in liquid nitrogen to a fine powder with mortar and pestle followed by transferred that powder into microcentrifuge tube with 500 µl of CTAB extraction buffer (2% (W/V) CTAB, 100 mM Tris–HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% (W/V) β-mercaptoethanol). The mixture was incubated at 65°C for 1 hour then centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was transferred to a new microcentrifuge tube, added 500 µl of chloroform and centrifuged at 10,000 rpm for 10 minutes. The same phase was transferred to a new microcentrifuge tube, added 500 µl of chloroform / isoamyl alcohol (24:1) and centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was transferred again to a new microcentrifuge tube, added 1:10 volume of 3M Sodium acetate pH 5.0 followed by added 2 volume of cold absolute ethanol (-20°C), inverted tube and kept at -20°C for 1 hour. It was centrifuged at 10,000 rpm for 10 minutes, The supernatant was gently discarded. DNA pellet was washed using 1 ml of cold 70% ethanol (4°C) and centrifuged at 10,000 rpm for 10 minutes. The supernatant was smoothly discarded. DNA pellet was dried at room temperature, dissolved in 200 µl of TE buffer and stored at -20°C. The quantity and quality of genomic DNA was determined by spectrophotometry and 1% agarose gel stained with 2 mg / ml of ethidium bromide, respectively [143]. Fragment size was also estimated using GeneRuler 1 kb DNA ladder.

ISSR amplification

ISSR amplification was performed as stated by Bornet and Branchard [23]; Forty-five primers were screened. PCR amplifications were performed in 20 µl reaction mixtures; containing a final concentration about 50 ng of DNA, 2.5 mM of $MgCl_2$, 1X of PCR buffer, 0.1 µM of primer, 0.1 µM of each dNTP and 0.5 unit of *Taq* DNA polymerase. ISSR amplifications were performed using a Proflex PCR system thermocycler with an initial denaturation step for 5 minutes at 95°C, followed by 45 cycles of denaturation step

45 seconds at 95 ℃, annealing step 45 seconds at annealing temperature of each primer, extension step 1 minute at 72 ℃ and completed with a final extension for 5 minutes at 72 ℃. Optimal conditions were resolved based on ISSR-PCR products. A negative control, which contained all PCR mixture except genomic DNA, was included in every testing to evaluate the mixture contamination. ISSR amplified products were visualized on 1% agarose gel stained with 2 mg / ml of ethidium bromide [143]. Fragment size was also estimated using GeneRuler 1 kb DNA ladder.

Mangiferin quantitative analysis

Fifteen mature leaf samples of *Mangifera indica* 'Okrong' were obtained from Chiang Mai, Sing Buri, Nakhon Sawan, Nakhon Pathom, Prachin Buri, Chiang Rai, Uttaradit, Lamphun, Kanchanaburi, Ratchaburi, Yasothon, Nakhon Ratchasima, Khon Kaen, Kalasin and Ubon Ratchathani provinces, Thailand. All leaf samples were collected during June to October in 2014. They were authenticated by Assoc. Prof. Dr. Nijsiri Ruangrungsi. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. Mango leaves were washed with water and dried in hot air oven at 50℃. They were pulverized and exhaustively extracted with 95 % ethanol by Soxhlet extraction apparatus. The extract was filtered through Whatman number 1 filter paper. The extract yields were weighed, recorded and dissolved in methanol to obtain a concentration of 2 mg/ml.

The stock solution of mangiferin standard (1 mg/ml) was prepared in 80% methanol in water. It was correctly diluted to obtain concentration of 0.2, 0.4, 0.6, 0.8, 1 mg/ml. The other chemicals were analytical grade and water was ultrapure water.

Determination of mangiferin content using TLC-densitometry

Five microliters of the standard and extract solutions were spotted on the same plate of silica gel 60 F_{254} then allowed to dry. The TLC plate was developed in TLC chamber saturated with a mobile phase; ethyl acetate: methanol: formic acid (3.9 : 6 : 0.1). After development, the plate was removed and allowed to dry. It was scanned by CAMAC TLC scanner 4 under 254 nm and expressed as chromatographic peak using winCATS

software. Mangiferin content was calculated by peak area. The test was done in triplicate.

Determination of mangiferin content using TLC-image analysis by ImageJ software

Developed TLC plate was photographed using digital camera under ultraviolet at 254 nm and saved as tiff files. Chromatographic peak and peak area was obtained using ImageJ software [113]. The test was done in triplicate.

Method validation [144]

Calibration range

Regression line of peak area *versus* mangiferin concentration and correlation coefficient were determined by Excel 2007 program.

Specificity

The specificity of mangiferin quantitative analysis in *Mangifera indica* cv. Okrong was determined by comparing absorption spectra of 15 sample spots to that mangiferin standard using CAMAC TLC scanner 4.

Accuracy

The accuracy of mangiferin quantitative analysis in *Mangifera indica* cv. Okrong was tested by spike method. Known amounts of mangiferin standard (0.1, 0.3 and 0.5 µg /µl) were spiked into the extract to obtain three different levels of mangiferin that were low, medium and high in calibration range and each level, three determinations were performed. The accuracy were determined as percent recovery by using following formula:

$$\% \text{ Recovery } = \frac{A}{B + C} \times 100$$

Where, A = the amount of mangiferin test in spike sample extract

B = the amount of mangiferin test un-spike sample extract

C = the amount of mangiferin standard actually add to sample

Precision

The precision of mangiferin quantitative analysis in *Mangifera indica* cv. Okrong was determined by repeatability (intra-day) and intermediate precision (inter-day) studies. Intra-day and inter-day precision were performed by analyzed sample solution of three concentrations (each one on triplicate) at same day and three different days of experiments, respectively. Precision was calculated in term % RSD of mangiferin content following formula:

$$\% \text{ RSD } = \frac{\text{SD}}{\text{Mean}} \times 100$$

Limit of detection (LOD)

The limit of detection (LOD) was the lowest concentration that could be detected but not quantified the LOQ was determined from the calibration curve using following formula:

$$\text{LOD } = \frac{3.3\ (\text{SD})}{\text{S}}$$

Where, SD = the residual standard deviation of a regression line

S = the slope of calibration curve

Limit of quantitative (LOQ)

The limit of quantitation (LOQ) was the lowest concentration that could be quantified. LOQ was determined from the calibration curve using following formula:

$$\text{LOQ } = \frac{10(\text{SD})}{\text{S}}$$

Where, SD = the residual standard deviation of a regression line

S = the slope of calibration

Robustness

Mobile phase composition was selected for robustness parameter in this study by a slight variation in a mixture ratio of mobile phase including; ethyl acetate: methanol: formic acid (4.1: 5.8: 0.1), (4.0: 5.9: 0.1), (3.8: 6.1: 0.1). The robustness was represented by % RSD of peak area of mangiferin in the extract.

Bioactive potentials

Materials and chemicals

Mangifera indica 'Okrong' leaves were collected in Lamphun, Thailand. They were authenticated by Assoc. Prof. Dr. Nijsiri Ruangrungsi. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. Leaf samples were washed with water and dried in hot air oven at 50°C. The dried leaves were pulverized and exhaustively extracted with ethanol by Soxhlet apparatus. The extract was filtered through Whatman number 1 filter paper and evaporated to dryness *in vacuo*. The yield was recorded and the extract was stored at -20°C.

Antidiabetic activities

Inhibition of yeast alpha-glucosidase activity

The enzyme inhibition activity against *Saccharomyces cerevisiae* α-glucosidase was determined using 1 mM of PNPG as substrate according to Wan *et al*. [145] with minor modifications. In 96 well plate, 30 µl of enzyme solution (0.5 U/ml), 30 µl of 0.1 M sodium phosphate buffer (pH 6.9) and 30 µl of tested inhibitors (the extract, mangiferin or acarbose) in DMSO were mixed and incubated at 37°C for 10 minutes. Next, 30 µl of substrate were added and incubated again at 37°C for 20 minutes. After incubation, 80 µl of 0.2 µM Na_2CO_3 was added to stop the reaction. The absorbance was measured at 405 nm using Anthos Zenyth 200 RT microplate reader (Biochrom, England). All tested inhibitors were analysed in triplicate. The percent inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{\left(\text{OD}_{405} \text{ control} - \text{OD}_{405} \text{ inhibitor}\right)}{\text{OD}_{405} \text{ control}} \text{ x } 100$$

Inhibition of rat alpha-glucosidase activity

The enzyme inhibition activity against intestinal acetone powders from rat was determined using 1 mM of PNPG as substrate, according to Lordan *et al*. [146] and Hemalatha *et al*. [147] with minor modifications. Intestinal acetone powders from rat (30 mg/ml) in 0.1 M sodium phosphate buffer (pH 6.9) was sonicated for 20 minutes.

The suspension was centrifuged at 3,500 rpm for 30 minutes to remove particulated matter. In 96 well plate, 50 µl of tested inhibitors in DMSO, 100 µl of substrate and 50 µl of enzyme solution (0.5 U/ml) were mixed and incubated at 37ºC for 30 minutes. The absorbance was measured at 405 nm using microplate reader. All tested inhibitors were analysed in triplicate. The percent inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{\left(\text{OD}_{405} \text{ control} - \text{OD}_{405} \text{ inhibitor}\right)}{\text{OD}_{405} \text{ control}} \text{ x } 100$$

Inhibition of pancreatic alpha-amylase activity

The enzyme inhibition activity against α-amylase from porcine pancreas were determined using 1 mM of CNPG-3 as substrate, following a method as described previously by Yonemoto *et al*. [148] with modifications. In 96 well plate, 30 µl of enzyme solution (25 U/ml) and 30 µl of tested inhibitors in DMSO were mixed and preincubated at 37° C for 10 minutes. Then, 30 µl of substrate were added and incubated again at 37ºC for 20 minutes. The absorbance was measured at 405 nm using microplate reader. All tests were analysed in triplicate. The percent inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{\left(\text{OD}_{405} \text{ control} - \text{OD}_{405} \text{ inhibitor}\right)}{\text{OD}_{405} \text{ control}} \text{ x } 100$$

Antimicrobial activities

Microorganisms

Bacillus cereus (ATCC 6633), *Bacillus subtilis* (ATCC 11778), *Kocuria rhizophila* (Isolates), *Staphylococcus aureus* (ATCC 6538P), *Staphylococcus epidermidis* (ATCC 9341), *Escherichia coli* (ATCC 25922), *Enterobacter aerogenes* (ATCC 13048), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella typhi* (Isolates), *Salmonella typhimurium* (ATCC 13311), *Shigella* spp. (Isolates), *Candida albicans* (ATCC 10230) and *Saccharomyces*

cerevisiae (ATCC 9763). They were obtained from Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Department of Microbiology, Faculty of Sciences and Technology, Suan Sunandha Rajabhat University and Department of Microbiology, Faculty of Sciences, Chulalongkorn University. All these microbial cultures were grown on Mueller Hinton agar or Mueller Hinton broth for bacteria and Sabouraud Dextose agar or Sabouraud Dextose broth for fungi. They were incubated at 37 ºC, for 24 hours. The turbidity of culture was adjusted about 0.5 McFarland standard and suspended in 0.85% sodium chloride.

Determination of zone of inhibition

Zone of inhibition was determined following agar disk diffusion assay as described previously by CLSI [136] and Bauer *et al.* [149] with a minor modifications. It was performed using the double agar layer technique. One hundred microliters of the suspension were added to 5 ml of sterile seed agar then poured on sterile base agar. All plates were allowed to dry at room temperature. Twenty microliters of tested solutions (extract (200 mg/ml of *Mangifera indica*), standard (200 mg/ml of mangiferin) or positive control (1 mg/ml of ampicllin sodium or 1 mg/ml of amikacin sulfate) in DMSO were dropped on the 6 mm paper disk. DMSO was used as a negative control. The plates were incubated at 37 °C for 24 hours. The diameters of inhibition zone were measured in millimeter. All tested solutions were analysed in triplicate.

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

Determination of minimum inhibitory concentration was performed following a microbroth dilution assay as described previously by CLSI [136] with a minor modifications. In 96 well plate, column 1st to 10th were for tested solutions using *two-fold* dilutions, column 11th was for negative control and column 12th was for broth only. Each well was filled with 50 µl of tested solutions in broth and 50 µl microbial suspended in broth and incubated at 37 ºC, for 24 hours. The last well which was shown in clear solution was recorded as minimum inhibitory concentration. Streaked

clear microbial suspended broth on agar then incubated at 37 ºC, for 24 hours. Minimum bactericidal concentration and minimum fungicidal concentration of the extract were evaluated from the agar plate with no appeared microbial growth. All tested solutions were analyzed in triplicate.

Anticancer activity

Cell cultures

The human cancer cell lines; ductal carcinoma (BT474, ATCC HTB20), bronchogenic carcinoma (Chago K-1, ATCC HTB-168TB), liver hepatoblastoma (Hep-G2, ATCC HB8065), gastric carcinoma (Kato-III, ATCC HTB103) and colon adenocarcinoma (SW 620, ATCC CCL227); The human normal cell lines; skin fibroblast (CCD-986SK, ATCC CRL1947) and lung fibroblast (WI-38 VA-13 subline 2RA, ATCC CLS 300421) were obtained from the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University. BT474, Chago K-1, Hep-G2, Kato-III, SW 620 and WI-38 cell lines were cultured in RPMI 1640 medium containing 5% fetal calf serum and CCD-986SK cell line was cultured in DMEM medium. They were incubated at 37 ºC in a 5% (v/v) CO_2 atmosphere.

MTT cell proliferation assay

Cell viability using MTT assay were determined as described previously by Mosmann [139] with minor modifications. In 96 well plate, 198 µl of 5,000 cells in cultured medium were added and incubated at 37ºC in a 5% (v/v) CO_2 atmosphere for 24 hours. Then, 2 µl of tested inhibitors (mango extract, mangiferin, or doxorubicin), or 2 µl of negative control (DMSO for mango extract and mangiferin; or water for doxorubicin) were added and incubated at 37 ºC for 48 hours. Ten microliter of MTT solution (5mg/ml) were added into each well and incubated at 37 ºC for 4 hours. The media were removed. A mixture of 150 µl of DMSO and 25 µl of glycine (0.1 mol/l) were added into each well and mixed thoroughly to dissolve the formazan crystals. The absorbance was measured at 540 nm using microplate reader. All tests were analysed in quadruplicate. The percent survival was calculated as follows:

$$\% \text{ Survival} = \frac{\text{absorbance intensity of tested sample}}{\text{absorbance intensity of negative control}} \text{ x } 100$$

Data analysis

For macroscopic characteristics, leaf microscopic characteristics, antidiabetic activities, antimicrobial activities, and anticancer activity, all data were expressed as mean ± standard deviation (SD). For molecular characteristics, reproducible amplified bands were chosen for analysis. Agarose gels were photographed and fragment sizes were estimated. Amplification profiles were scored in binary code as present (1) or absent (0). A similarity matrix was analysed and a pairwise distance matrix was also generated a dendrogram by cluster analysis using Unweighted Pair Group Method with Arithmetic Average (UPGMA) based on character differences. The mangiferin contents between TLC-densitometric and TLC-image analysis were compared by Wilcoxon signed-rank test statistical analysis. Values of $p < 0.05$ was considered to statistically significant.

CHAPTER IV
RESULTS

Scientific Name	*Mangifera indica* L.
Common Name	Ma-muang
English Name	Mango
Family	Anacardiaceae
Distribution	Throughout the world, mainly in tropical, subtropical and temperate areas
Used part	Leaf

Macroscopic characteristics

Thai mango cultivars have been classified according to plant germplasm database for mango [150] using fruit and leaf macroscopic characteristics as main criteria to separate all of Thai mango cultivars into seven groups including Nangklangwan, Namdokmai, Okrong, Roundish, Keao, Khiaosawoey and miscellaneous groups. Nangklangwan group is cylindrical fruit shape with oblong leaf shape, attenuate leaf apex, and entire leaf margin. Namdokmai group is elliptical fruit shape with elliptical leaf shape, acuminate leaf apex, acute leaf base and undulate leaf margin. Okrong group is elliptical fruit shape with lanceolate leaf shape, acuminate leaf apex, acute leaf base and entire leaf margin. Roundish group is roundish fruit shape with elliptical leaf shape, attenuate leaf apex, acute leaf base and entire leaf margin. Keao group is obovate fruit shape with lanceolate leaf shape, attenuate leaf apex, acute leaf base and entire leaf margin. Khiaosawoey group is oblong fruit shape with oblong leaf shape, attenuate leaf apex, attenuate leaf base and entire leaf margin.

Seventeen *M. indica* cultivars were selected from each group; Nangklangwan group (‘Nga Khao’, ‘Nangklangwan’ and ‘Mahacharnok’), Namdokmai group (‘Khiaoyai’, ‘Mankhunsi’ and ‘Namdokmai’), Okrong group (‘Kaemdaeng’, ‘Okrong’, ‘Chok Anan’ and ‘Raet’), Roundish group (‘Talapnak’), Keao group (‘Kaeo’, ‘Phetbanlat’ and

‘Nongsaeng’) and Khiaosawoey group (‘Tongdam’, ‘Khiaosawoey’ and ‘Falan’). This six groups have clear macroscopic characters listed in table 5.

Table 5 Macroscopic characteristic comparisons of selected Thai *Mangifera indica* cultivars and outgroups

Group	Samples	Leaf shape			
		Leaf shape	Leaf apex	Leaf base	Leaf margin
Nangklangwan	*M. indica* ‘Nga Khao’	Elliptical	Acute	Acute	Entire
Nangklangwan	*M. indica* ‘Nangklangwan’	Elliptical	Acute	Acute	Undulate
Namdokmai	*M. indica* ‘Khiaoyai’	Lanceolate	Acute	Obtuse	Undulate
Namdokmai	*M. indica* ‘Mankhunsi’	Oblong	Acute	Acute	Undulate
Namdokmai	*M. indica* ‘Namdokmai’	Oblong	Acuminate	Obtuse	Undulate
Nangklangwan	*M. indica* ‘Mahacharnok’	Linear-oblong	Acuminate	Obtuse	Undulate
Okrong	*M. indica* ‘Kaemdaeng’	Lanceolate	Acute	Obtuse	Undulate
Okrong	*M. indica* ‘Okrong’	Oblong	Acuminate	Acute	Undulate
Okrong	*M. indica* ‘Chok Anan’	Elliptical	Attenuate	Acute	Undulate
Okrong	*M. indica* ‘Raet’	Oblong-lanceolate	Attenuate	Obtuse	Undulate
Roundish	*M. indica* ‘Talapnak’	Oblong	Acute	Acute	Undulate
Kaeo	*M. indica* ‘Kaeo’	Elliptical	Acuminate	Acute	Entire
Khiaosawoey	*M. indica* ‘Tongdam’	Elliptical	Acute	Acute	Undulate
Khiaosawoey	*M. indica* ‘Khiaosawoey’	Oblong	Attenuate - acuminate	Attenuate	Undulate
Khiaosawoey	*M. indica* ‘Falan’	Linear-oblong	Acute	Acute	Entire
Kaeo	*M. indica* ‘Phetbanlat’	Oblong-lanceolate	Acuminate	Acute	Entire
Kaeo	*M. indica* ‘Nongsaeng’	Oblong-lanceolate	Acuminate	Acute	Entire
-	*M. caloneura*	Oblong	Acute	Acute	Undulate
-	*B. macrophylla*	Ovate-oblong	Acute-acuminate	Acute-cuneate	Entire

Microscopic characteristics

All nineteen leaf samples were similar in that their stomata were anomocytic type, which bordered by a varying number of cells and not different from the epidermis. They were small size and presented only in the lower surface of the leaf. The epidermal cells were oval or round shaped. Stomatal numbers were slightly to moderately differed among different *M. indica* cultivars, totals ranging from 515.11 stomata / 1 mm^2 to 954.58 stomata / 1 mm^2, with an average of 695.82 stomata / 1 mm^2. 'Namdokmai' had lowest stomatal number and 'Reat' had highest stomatal number (Table 6). This numbers of *M. caloneura and B.macrophylla* were slightly to moderately differed from *M. indica* cultivars too, they were found to be 562.09 and 550.53 stomata / 1 mm^2, respectively. The stomatal numbers also varied within same cultivar located on the different environmental conditions.

Mango leaf veins are reticulate veins patterns, small veins forming a network**.** From the finding, veinlet termination number were slightly to moderately differed among different *M. indica* cultivars, totals ranging from 24.69 veinlet terminations / 1mm^2 to 45.08 veinlet terminations / 1mm^2, with an average of 36.41 veinlet terminations / 1mm^2. 'Khiaoyai' had lowest veinlet termination number and 'Chok Anan' had highest veinlet termination number (Table 6). *M. caloneura* was quite similar regarding to both veinlet termination patterning and density of that termination. The abundant fibers covering on *B.macrophylla* leaf caused their veinlet termination could not be detected.

Mango palisade cells lie between upper and lower epidermis. They consist of one or two layers of elongated, closely arranged columnar cells. Palisade ratio was not varied based on geographical variation. It was slightly differed among different *M. indica* cultivars, totals ranging from 2.92 to 3.72, with an average of 3.23. 'Raet' had lowest palisade ratio and 'Mankhunsi' had highest palisade ratio (Table 6). *M. caloneura* was slightly differed, whereas *B. macrophylla* were highly differed from *M. indica* cultivars in that palisade ratio.

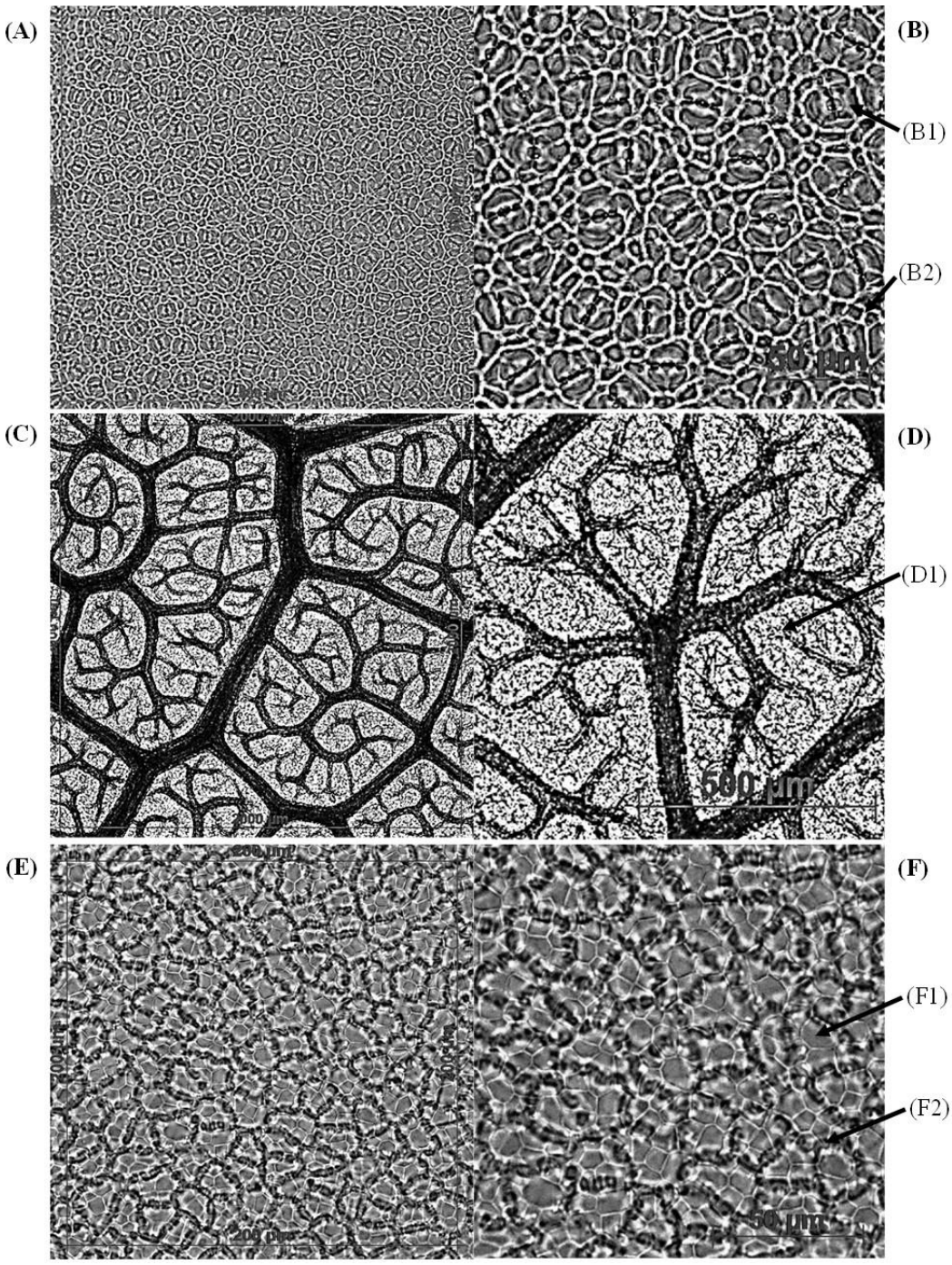


Figure 18 Images of *Mangifera indica* leaves showing **(A)** mango stomata at a magnification of 200X, scale 500X500 µm; **(B)** (B1) stomata cell and (B2) epidermal cell, scale 50 µm; **(C)** veinlet terminations at a magnification of 50X, scale 2000X2000 µm; **(D)** (D1) veinlet termination, scale 500 µm; **(E)** palisade and epidermal cells at a magnification of 400X, scale 200X200 µm; **(F)** (F1) stomata cell and (F2) epidermal cell, scale 50 µm

Table 6 Leaf constant values of selected *Mangifera indica* cultivars and outgroups

Leaf Samples	Stomatal number* (stomata/1mm^2)	Veinlet termination number* (veinlet termination/1mm^2)	Palisade ratio*
M. indica 'Nga Khao'	722.58 ± 43.50	32.92 ± 5.98	2.94 ± 0.34
M. indica 'Nangklangwan'	594.76 ± 166.21	36.51 ± 3.80	3.36 ± 0.32
M. indica 'Khiaoyai'	668.80 ± 57.80	24.69 ± 4.67	3.38 ± 0.38
M. indica 'Mankhunsi'	622.22 ± 42.47	32.47 ± 4.35	3.72 ± 0.42
M. indica 'Namdokmai'	515.11 ± 33.37	29.35 ± 3.45	2.98 ± 0.44
M. indica 'Mahacharnok'	595.29 ± 36.69	24.92 ± 5.27	3.10 ± 0.37
M. indica 'Kaemdaeng'	902.27 ± 65.71	43.34 ± 8.00	3.14 ± 0.34
M. indica 'Okrong'	659.16 ± 161.94	37.63 ± 4.99	3.13 ± 0.43
M. indica 'Chok Anan'	710.36 ± 50.43	45.08 ± 4.67	3.07 ± 0.28
M. indica 'Raet'	954.58 ± 52.41	43.13 ± 4.36	2.92 ± 0.30
M. indica 'Talapnak'	549.87 ± 91.03	39.27 ± 4.62	3.66 ± 0.52
M. indica 'Kaeo'	803.38 ± 125.90	44.56 ± 10.24	3.14 ± 0.35
M. indica 'Tongdam'	601.60 ± 57.44	41.23 ± 9.08	3.38 ± 0.35
M. indica 'Khiaosawoey'	643.07 ± 36.47	33.79 ± 3.25	3.25 ± 0.42
M. indica 'Falan'	844.09 ± 53.67	33.80 ± 3.30	3.55 ± 0.46
M. indica 'Phetbanlat'	670.44 ± 48.31	36.76 ± 3.81	3.13 ± 0.35
M. indica 'Nongsaeng'	771.42 ± 56.21	39.48 ± 5.26	3.11 ± 0.38
M. caloneura	562.09 ± 35.00	40.80 ± 2.92	2.81 ± 0.27
B. macrophylla	550.53 ± 31.86	ND	1.94 ± 0.21

* means ± SD. ND = could not detect. Data were the average of 90 determinations from three different locations per sample.

Molecular characteristics

In this study, forty-five ISSR primers, comprising di-, tri-, and tetra- nucleotide repeat primers were screened to amplify DNA fragments against all selected Thai mango genomic DNA, then seven primers that amplified the reproducible band patterns were selected to analyze. They were confirmed with repeated reactions using genomic DNA from three different locations per sample (n=57) and same selected primers.

From seven ISSR primers, they amplified 78 bands from *M. indica* cultivars, which 64 bands were polymorphic. Primer 'ISSR19' generated the smallest number of bands and primer ISSR03 generated the largest number of bands ranging from 8 bands to 13 bands, with an average of 11.14 bands per primer. Band size ranged from 190 bps to 2660 bps. Most of the AG, GA and TG dinucleotide repeat sequences and GGAT tetranucleotide repeat sequences were also successful in amplifying bands. Both primer 'ISSR 02' and primer 'ISSR 31' had lowest polymorphic percentage and primer 'ISSR 03' had highest polymorphic percentage ranging from 75.00% to 92.30%, with an average of 82.05 %. No ISSR primer amplified a unique band pattern among *M. indica* cultivars. No band was found in negative control amplification. Annealing temperatures of each primer were optimized listed in table 7.

Table 7 Summary of ISSR markers

Primer	Primer sequence	Annealing Tm (℃)	Fragment size range (bps)	Total bands	Polymorphic fragment	Polymorphic percentage
ISSR02	AGAGAGAGAGAGAGAGC	50	380-2360	12	9	75.00
ISSR03	GAGAGAGAGAGAGAGAT	46	640-2560	13	12	92.30
ISSR13	AGAGAGAGAGAGAGAGYA	50	480-1760	9	7	77.78
ISSR19	ACACACACACACACACYT	54	650-1910	8	7	87.50
ISSR22	TGTGTGTGTGTGTGTGRC	54	360-2070	13	11	84.62
ISSR27	GGATGGATGGATGGAT	48	190-2660	11	9	81.82
ISSR31	AGAGAGAGAGAGAGT	44	570-2520	12	9	75.00
	Total		**190-2660**	**78**	**64**	**82.05**

*Single letter abbreviations for mixed-base positions: Y=(C,T), R=(A,G)

The genetic similarity coefficients were calculated using Jaccard's coefficient. Among *M. indica* cultivars, the highest genetic similarity value of 0.6985 was found between *M. indica* 'Nga Khao' (Nangklangwan group) and *M. indica* 'Nangklangwan' (Nangklangwan group); however, the lowest genetic similarity value of 0.0858 was found between *M. indica* 'Mahacharnok' (Nangklangwan group) and *M. indica* 'Talapnak' (Roundish group) (Table 8).

Table 8 Similarity index (S.I.) of *Mangifera indica* cultivars and outgroups

	M. indica 'Nga Khao'	*M. indica* 'Nangklangwan'	*M. indica* 'Khiaoyai'	*M. indica* 'Mankhunsi'	*M. indica* 'Namdokmai'	*M. indica* 'Mahacharnok'	*M. indica* 'Kaemdaeng'	*M. indica* 'Okrong'	*M. indica* 'Chok Anan'	*M. indica* 'Raet'	*M. indica* 'Talapnak'	*M. indica* 'Kaeo'	*M. indica* 'Tongdam'	*M. indica* 'Khiaosawoey'	*M. indica* 'Falan'	*M. indica* 'Phetbanlat'	*M. indica* 'Nongsaeng'	*M. caloneura*	*B.macrophylla*
M. indica 'Nga Khao'	1.0000																		
M. indica 'Nangklangwan'	0.6985	1.0000																	
M. indica 'Khiaoyai'	0.5119	0.6475	1.0000																
M. indica 'Mankhunsi'	0.5468	0.4889	0.3811	1.0000															
M. indica 'Namdokmai'	0.4222	0.3703	0.4812	0.4066	1.0000														
M. indica 'Mahacharnok'	0.3054	0.1867	0.3676	0.4293	0.2822	1.0000													
M. indica 'Kaemdaeng'	0.2733	0.2519	0.3718	0.3253	0.2516	0.5188	1.0000												
M. indica 'Okrong'	0.3128	0.2328	0.2893	0.3979	0.3117	0.3941	0.3439	1.0000											
M. indica 'Chok Anan'	0.3620	0.1937	0.2532	0.3036	0.2104	0.3525	0.3667	0.3683	1.0000										
M. indica 'Raet'	0.2942	0.2056	0.3054	0.3274	0.1576	0.3680	0.3563	0.4106	0.4921	1.0000									
M. indica 'Talapnak'	0.1335	0.1448	0.1406	0.1447	0.2577	0.0858	0.1284	0.2311	0.1852	0.1133	1.0000								
M. indica 'Kaeo'	0.3352	0.3619	0.3629	0.3377	0.4138	0.3177	0.3889	0.3066	0.2105	0.2475	0.2544	1.0000							
M. indica 'Tongdam'	0.2274	0.2226	0.2644	0.1792	0.3029	0.2785	0.2665	0.1871	0.2061	0.1542	0.3382	0.3555	1.0000						
M. indica 'Khiaosawoey'	0.2121	0.1613	0.2241	0.1997	0.2282	0.2517	0.2475	0.3533	0.2517	0.2161	0.4051	0.3585	0.3229	1.0000					
M. indica 'Falan'	0.1227	0.1141	0.1867	0.1622	0.2983	0.1905	0.1745	0.3513	0.2078	0.1717	0.3269	0.4483	0.3877	0.6397	1.0000				
M. indica 'Phetbanlat'	0.3026	0.2360	0.2225	0.2567	0.3222	0.2134	0.2527	0.3012	0.2294	0.1419	0.3946	0.3807	0.4725	0.5125	0.5712	1.0000			
M. indica 'Nongsaeng'	0.2703	0.2453	0.2602	0.2242	0.2015	0.2274	0.2039	0.1985	0.1421	0.1367	0.2843	0.2696	0.4654	0.4427	0.4078	0.6213	1.0000		
M. caloneura	0.1821	0.1539	0.1781	0.1900	0.1103	0.2401	0.2440	0.1787	0.3791	0.2673	0.1512	0.1171	0.1510	0.1376	0.0824	0.1722	0.1361	1.0000	
B.macrophylla	0.0925	0.0253	0.0384	0.0974	0.0510	0.0788	0.0798	0.0702	0.1235	0.1074	0.1235	0.1283	0.1319	0.1430	0.0853	0.0416	0.0702	0.0623	1.0000

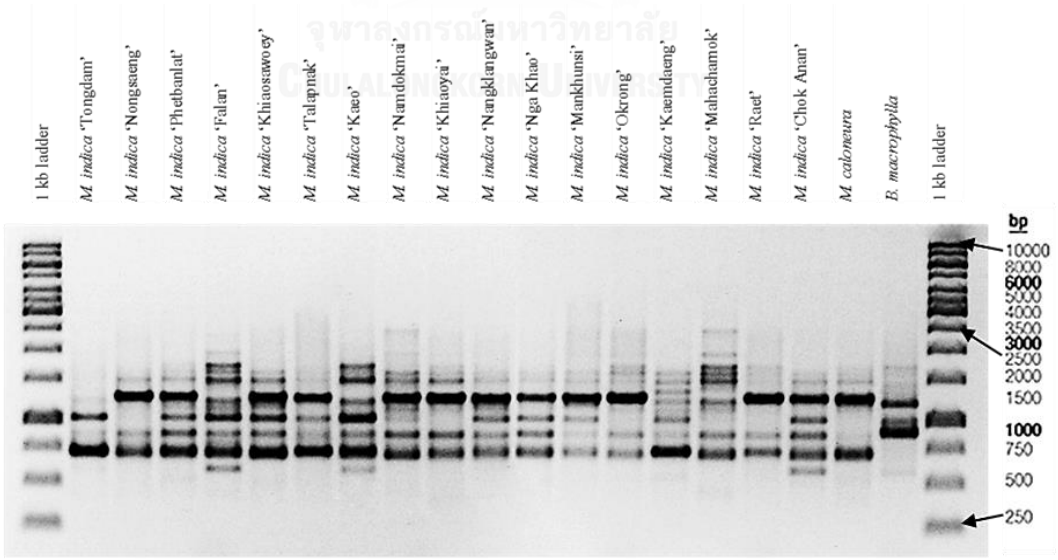


Figure 19 ISSR fingerprint of selected *Mangifera indica* cultivars and outgroups obtained from primer ISSR 31

The similarity coefficients generated the dendrogram, which separated different *M. indica* cultivars then grouped them into two major clusters. For the cluster I, the highest genetic similarity value of 0.6985 was found between *M. indica* "Nga Khao" and "Nang klang wan;" whereas, the lowest genetic similarity value of 0.1576 was found between *M. indica* "Namdokmai" and "Raet." For the cluster II, the highest genetic similarity value of 0.6397 was found between *M. indica* "Khiaosawoey" and "Falan;" whereas, the lowest genetic similarity value of 0.2544 was found between *M. indica* "Kaeo" and "Talapnak." *M. caloneura* and *B. macrophylla*, which were outgroups in this current study, were clearly separated from *M. indica* cultivars listed as the cluster III and IV, respectively (Figure 20).

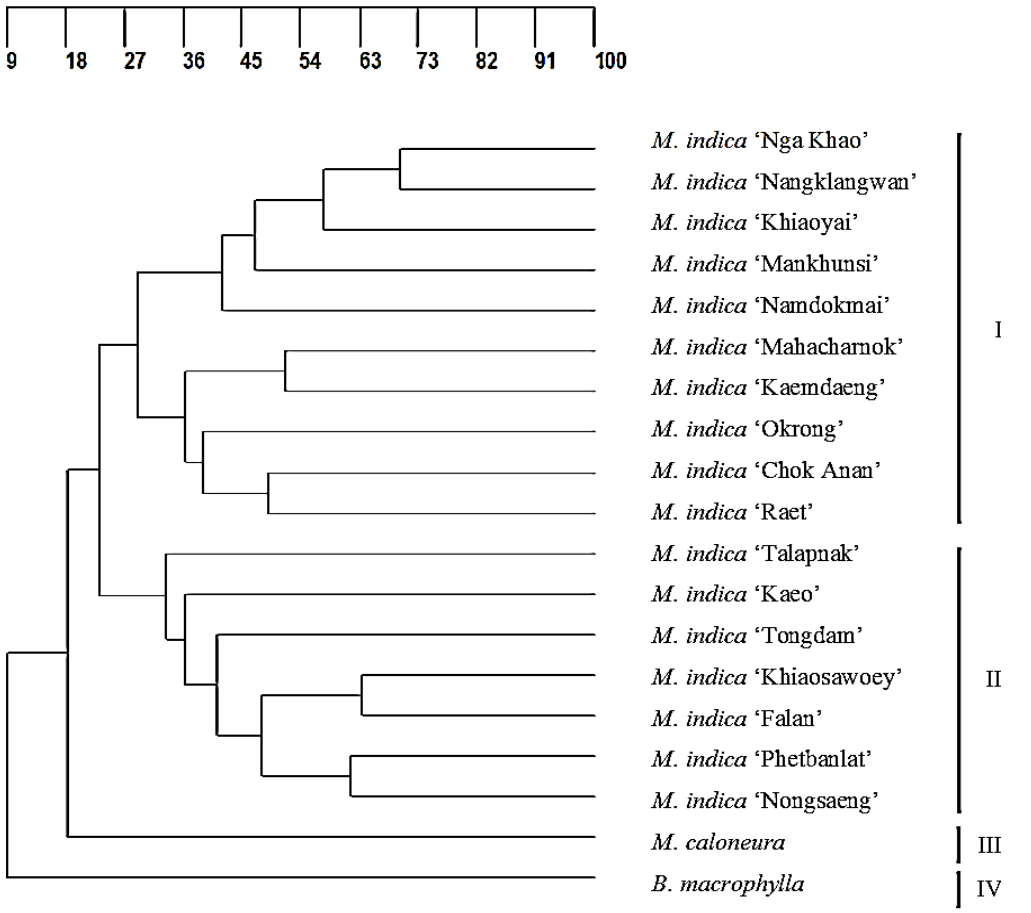


Figure 20 Dendrogram of *Mangifera indica* cultivars and outgroups using UPGMA cluster analysis based on genetic similarities from selected seven ISSR primer

Mangiferin quantitative analysis

Mangifera indica from 15 sources were pulverized and exhaustively extracted with 95 % ethanol by Soxhlet extraction apparatus. The percent yields of crude extracts were shown in Table 9. The average percent yield of *M. indica* ethanolic extract was 27.22±3.45 g/ 100 g by dry weight.

Table 9 The percent yield of *Mangifera indica* ethanolic extract from 15 different locations in Thailand

Source	Crude drug (g)	Ethanolic extract (g)	% yield
Chiang Mai	5.0005	1.2983	25.96
Sing Buri	5.0000	1.2208	24.42
Nakhon Sawan	5.0009	1.4306	28.61
Nakhon Pathom	4.9998	1.3278	26.56
Prachin Buri	5.0013	1.2887	25.77
Chiang Rai	5.0004	1.3030	26.06
Uttaradit	5.0000	1.2854	25.71
Lamphun	5.0004	1.2898	25.79
Kanchanaburi	5.0002	1.2849	25.70
Ratchaburi	5.0008	1.5750	31.49
Yasothon	5.0009	1.6244	32.48
Nakhon Ratchasima	5.0005	1.3441	26.88
Khon Kaen	4.9999	1.7704	35.41
Kalasin	5.0007	1.0885	21.77
Ubon Ratchathani	4.9996	1.2871	25.74
		Average	**27.22±3.45**

TLC-densitometry

Calibration curve

The calibration curve of mangiferin ranged from 1 to 5 µg/spot was shown on figure 21. The polymomial equation was $y = -816.05x^2 + 12393x - 9489.5$ and the coefficient of determination (R^2) of the curve was 0.9998 (Figure 21).

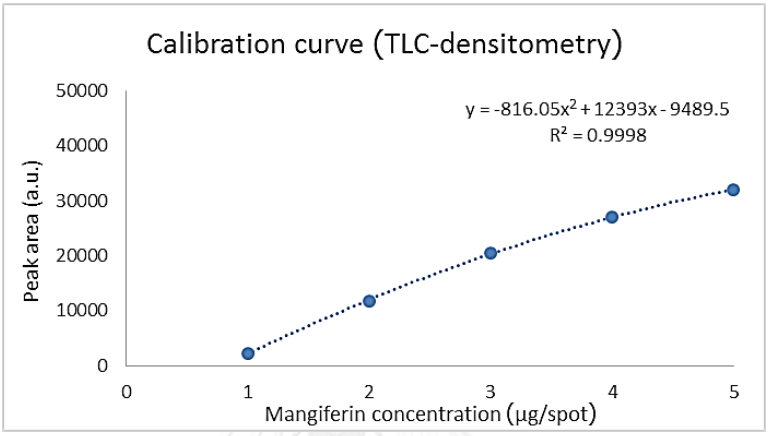


Figure 21 Calibration curve of mangiferin standard by TLC-densitometry

Specificity

TLC densitogram scanned to compare spectrum in range of 200 to 700 nm indicated that mangiferin had three maximum absorbances at the wavelength of 258, 323 and 366 nm, respectively (Figure 22).

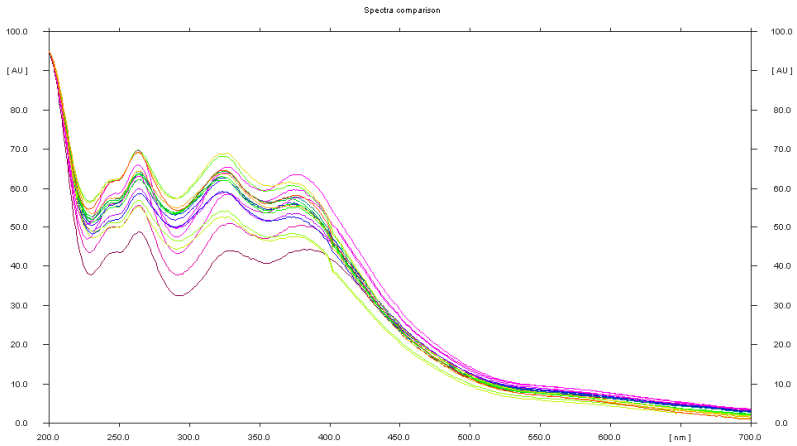


Figure 22 Absorbance spectra of mangiferin among standard and the extracts

Accuracy

The accuracy was determined by spiking mangiferin standard (0.5, 1.5, 2.5 µg) in the sample. They were judged in percent of recovery. The recovery of mangiferin spiked into the sample at three different concentrations were between 91.35 to 96.86 % (Table 10).

Table 10 Recovery of mangiferin by TLC-densitometry

Mangiferin added (µg/spot)	Mangiferin found (µg/spot)	% Recovery
0.0	1.518	-
0.5	1.867	92.54
1.5	2.923	96.86
2.5	3.670	91.35

Precision

The repeatability and intermediate precision were performed on sample with different concentrations of mangiferin at same day and three different days of experiments, respectively. The results were shown as % RSD. The repeatability and intermediate precision were between 1.67 to 7.43 % RSD and 1.03 to 11.50 % RSD, respectively (Table 11).

Table 11 Precision of mangiferin quantitation by TLC-densitometry

Repeatability		Intermediate precision	
Mangiferin (µg/spot)	%RSD	Mangiferin (µg/spot)	%RSD
1.518±0.11	7.43	1.523±0.02	1.03
1.867±0.12	6.40	1.910±0.19	9.98
2.923±0.05	1.67	3.097±0.23	7.48
3.670±0.11	2.94	3.944±0.45	11.50

Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection and limit of quantitation were calculated by residual standard deviation of regression line and slope of calibration curve. LOD and LOQ were 0.13 and 0.40 µg/spot, respectively.

Robustness

The robustness was determined in slight variation in mobile phase ratio. The result of robustness was 3.74 % RSD of peak area of mangiferin in the extract (Table 12).

Table 12 Robustness of mangiferin quantitation by TLC-densitometry

Mobile phase ratio (v/v)			Mangiferin peak area
Ethyl acetate	Methanol	Formic acid	
4.1	5.8	0.1	32628.99
4.0	5.9	0.1	34667.72
3.8	6.1	0.1	34976.68
		Average	**34091.13±1275.64**
		% RSD	**3.74**

Mangiferin quantification by TLC-densitometry

Mangiferin contents in each *Mangifera indica* ethanolic extract and in each crude drug were shown in table 13. Mangiferin contents in *Mangifera indica* leaves evaluated using TLC-densitometry were 4.992 ± 1.025 g/100 g of dried crude drug.

Table 13 The content of mangiferin in *M. indica* crude drug by TLC-densitometry

Source	Mangiferin in ethanolic extract (g/g)	Yield of ethanolic extract (g/100g of dried crude drug)	Mangiferin in *M. indica* leaves (g/100g of dried crude drug)
Chiang Mai	0.208	25.963	5.407
Sing Buri	0.193	24.416	4.710
Nakhon Sawan	0.140	28.607	4.005
Nakhon Pathom	0.153	26.557	4.058
Prachin Buri	0.177	25.767	4.555
Chiang Rai	0.185	26.058	4.812
Uttaradit	0.190	25.708	4.882

Table 13 (Cont.) The content of mangiferin in *M. indica* crude drug by TLC-densitometry

Source	Mangiferin in ethanolic extract (g/g)	Yield of ethanolic extract (g/100g of dried crude drug)	Mangiferin in *M. indica* leaves (g/100g of dried crude drug)
Lamphun	0.197	25.794	5.093
Kanchanaburi	0.265	25.697	6.801
Ratchaburi	0.188	31.495	5.928
Yasothon	0.164	32.482	5.320
Nakhon Ratchasima	0.182	26.879	4.897
Khon Kaen	0.195	35.409	6.900
Kalasin	0.134	21.767	2.913
Ubon Ratchathani	0.179	25.744	4.601
	Average		**4.992 ± 1.025**

TLC-image analysis by Image J software

Calibration curve

The calibration curve of mangiferin ranged from 1 to 5 µg/spot was shown on figure 23. The polymomial equation was y= - 1194.2x^2 + 19236x - 16661 and the coefficient of determination (R^2) of the curve was 0.9991 (Figure 23).

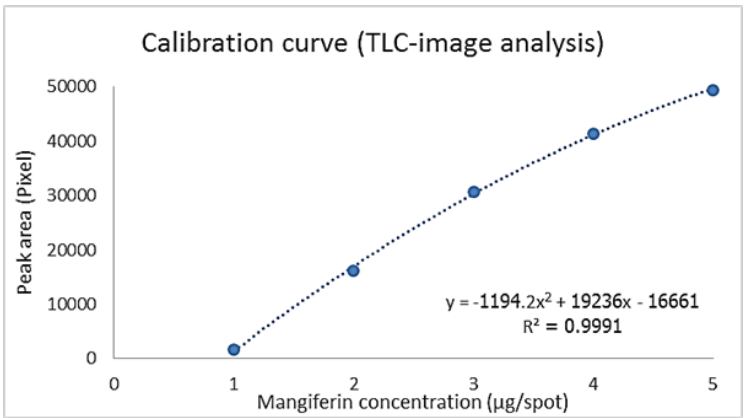


Figure 23 Calibration curve of mangiferin standard by TLC-image analysis

Accuracy

The accuracy was determined by spiking mangiferin standard (0.5, 1.5, 2.5 µg) in the sample. They were judged in percent of recovery. The recovery of mangiferin spiked into the sample at three different concentrations were between 84.46 to 106.24 % (Table 14).

Table 14 Recovery of mangiferin by TLC-image analysis

Mangiferin added (µg/spot)	Mangiferin found (µg/spot)	% Recovery
0.0	1.298	-
0.5	1.519	84.46
1.5	2.701	96.53
2.5	4.036	106.24

Precision

The repeatability and intermediate precision were performed on sample with different concentrations of mangiferin at same day and three different days of experiments, respectively. The results were shown as % RSD. The repeatability and intermediate precision were between 2.06 to 6.44 % RSD and 3.53 to 6.10 % RSD, respectively (Table 15).

Table 15 Precision of mangiferin quantitation by TLC-image analysis

Repeatability		Intermediate precision	
Mangiferin (µg/spot)	%RSD	Mangiferin (µg/spot)	%RSD
1.298±0.08	6.44	1.211±0.07	6.10
1.519±0.04	2.51	1.542±0.12	7.55
2.701±0.06	2.06	2.807±0.10	3.53
4.036±0.14	3.36	3.761±0.23	6.05

Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection and limit of quantitation were calculated by residual standard deviation of regression line and slope of calibration curve. LOD and LOQ were 0.03 and 0.09 µg/spot, respectively.

Robustness

The robustness was determined in slight variation in mobile phase ratio. The result of robustness was 3.40 % RSD of peak area of mangiferin in the extract (Table 16)

Table 16 Robustness of mangiferin quantitation by TLC-image analysis

Mobile phase ratio (v/v)			Mangiferin peak area
Ethyl acetate	Methanol	Formic acid	
4.1	5.8	0.1	63083.89
4.0	5.9	0.1	59662.55
3.8	6.1	0.1	63516.19
		Average	**62087.54±2111.20**
		% RSD	**3.40**

Mangiferin quantification by TLC-image analysis

Mangiferin contents in each *Mangifera indica* ethanolic extract and in each crude drug were shown in table 17. Mangiferin contents in *Mangifera indica* leaves were evaluated using TLC- image analysis with an average value of 4.311 ± 0.987 g/100 g of dried crude drug.

Table 17 The content of mangiferin in *M. indica* crude drug by TLC-image analysis

Source	Mangiferin in ethanolic extract (g/g)	Yield of ethanolic extract (g/100g of dried crude drug)	Mangiferin in *M. indica* leaves (g/100g of dried crude drug)
Chiang Mai	0.180	25.963	4.677
Sing Buri	0.140	24.416	3.421
Nakhon Sawan	0.120	28.607	3.439
Nakhon Pathom	0.125	26.557	3.311
Prachin Buri	0.146	25.767	3.750
Chiang Rai	0.146	26.058	3.794
Uttaradit	0.142	25.708	3.662

Table 17 (Cont.) The content of mangiferin in *M. indica* crude drug by TLC-image analysis

Source	Mangiferin in ethanolic extract (g/g)	Yield of ethanolic extract (g/100g of dried crude drug)	Mangiferin in *M. indica* leaves (g/100g of dried crude drug)
Lamphun	0.163	25.794	4.201
Kanchanaburi	0.215	25.697	5.513
Ratchaburi	0.172	31.495	5.411
Yasothon	0.142	32.482	4.599
Nakhon Ratchasima	0.183	26.879	4.926
Khon Kaen	0.184	35.409	6.500
Kalasin	0.133	21.767	2.904
Ubon Ratchathani	0.177	25.744	4.549
	Average		**4.311 ± 0.987**

The mangiferin contents in *Mangifera indica* 'Okrong' leaves by TLC-densitometry was a few higher than TLC-image analysis. These contents in mango leaves were 4.992 ± 1.025 and 4.311 ± 0.987 g/100 g of dried crude drug, respectively ($p<0.05$ by Wilcoxon signed-rank test).

Antidiabetic activities

Antidiabetic activities of mango leaf extract, mangiferin and acarbose showed a dose-response relationship (Figure 24). For yeast α-glucosidase, mango leaf extract showed the greatest inhibition with the IC_{50} of 0.050 mg/ml, rat α-glucosidase, mangiferin showed the greatest inhibition with the IC_{50} of 0.433 mg/ml and pancreatic α-amylase, mangiferin also showed the most inhibition with the IC_{50} of 1.049 mg/ml (Table 18). Acarbose was used as a positive control in this study.

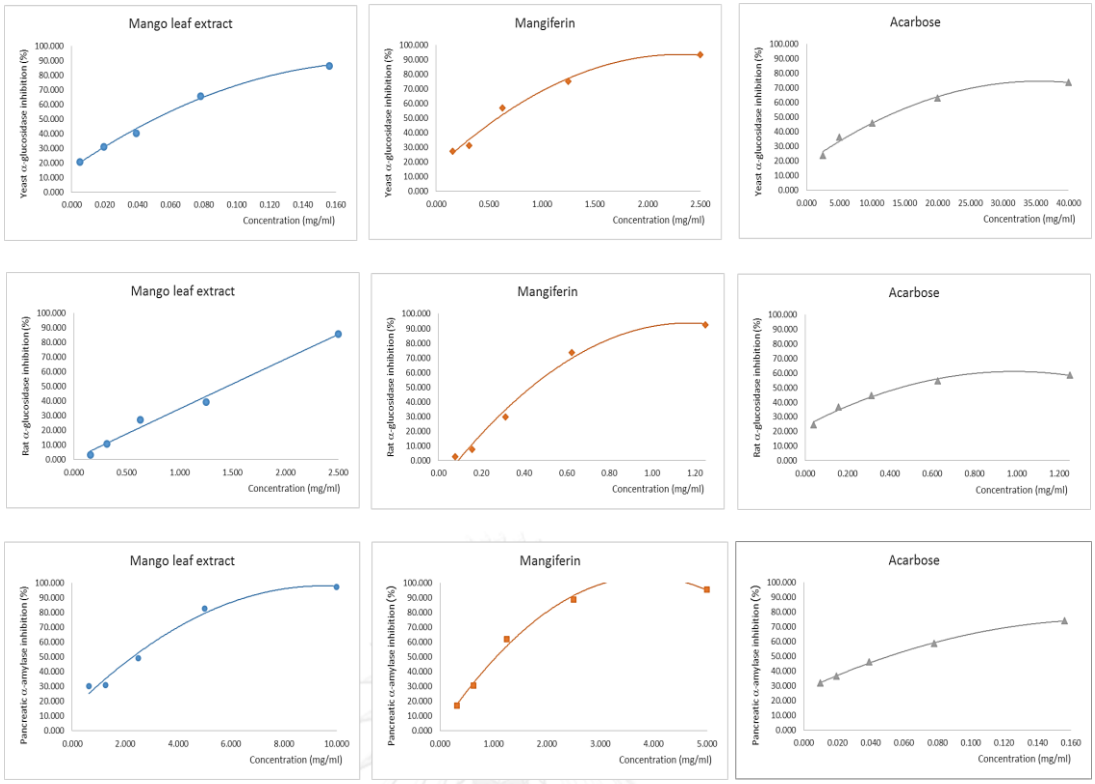


Figure 24 Yeast alpha-glucosidase, rat alpha-glucosidase and pancreatic alpha-amylase inhibitions of mango leaf extract, mangiferin and acarbose at different concentrations

Table 18 Antidiabetic activities of mango leaf extract, mangiferin and acarbose

	IC_{50} (mg/ml)*		
	Yeast α-glucosidase	Rat α-glucosidase	Pancreatic α-amylase
Mango leaf extract	0.050	1.453	2.284
Mangiferin	0.581	0.433	1.049
Acarbose	11.929	0.449	0.051

* The tests were done in triplicate.

Antimicrobial activities

For disk diffusion, mango leaf extract showed inhibition zones against tested Gram-positive bacteria except *Staphylococcus epidermidis* ranging from 11.00 to 12.67 mm, the widest inhibition zones were found against both *Bacillus cereus* and *Kocuria rhizophila* of 12.67 mm. Mangiferin showed inhibition zones against some tested bacteria ranging from 6.00 to 11.67 mm, the widest inhibition zone was also found against *Kocuria rhizophila* of 11.67 mm (Table 19).

Table 19 Antimicrobial activities of mango leaves extract, mangiferin, ampicillin and amikacin using disk diffusion method

Microorganisms	Inhibition zone (mm)*			
	Mango leaf	Mangiferin	Ampicillin	Amikacin
Staphylococcus aureus	11.00 ± 0.00	NA	35.00 ± 0.00	10.67 ± 0.57
Staphylococcus epidermidis	NA	7.00 ± 0.00	23.67 ± 0.57	15.33 ± 0.57
Bacillus subtilis	11.00 ± 0.00	NA	15.00 ± 0.00	13.33 ± 0.57
Bacillus cereus	12.67 ± 0.58	NA	16.67 ± 0.57	15.67 ± 0.57
Kocuria rhizophila	12.67 ± 0.58	11.67 ±0.57	43.33 ± 0.57	20.33 ± 0.57
Enterobacter aerogenes	NA	6.00 ± 0.00	7.00 ± 0.00	9.00 ± 0.00
Escherichia coli	NA	NA	18.33 ± 0.57	9.00 ± 1.00
Pseudomonas aeruginosa	NA	6.00 ± 0.00	NA	10.33 ± 0.57
Salmonella typhi	NA	NA	24.33 ± 0.57	10.00 ± 0.00
Salmonella typhimurium	NA	6.00 ± 0.00	28.33 ± 0.57	10.33 ± 0.57
Shigella spp.	NA	NA	24.33 ± 0.57	12.33 ± 0.57
Candida albicans	NA	NA	NA	NA
Saccharomyces cerevisiae	NA	NA	NA	NA

*mean ± SD, NA = no activity, Ø 6 mm of disk. The tests were done in triplicate.

For broth microdilution, mango leaf extract showed the most potent inhibition against *Kocuria rhizophila* with MIC and MBC values of 15.63 and 2000 µg/ml, respectively; however, mangiferin showed the most potent inhibition against *Kocuria rhizophila* with MIC values of 62.5 µg/ml (Table 20). Ampicillin and amikacin were used as a positive control comparable in these studies. There were no activities against yeast.

Table 20 Antimicrobial activities of mango leaf extract, mangiferin, ampicillin and amikacin using broth microdilution method

Microorganisms	Mango leaf		Mangiferin		Ampicillin		Amikacin	
	MIC (µg/ml)	MBC/ MFC (µg/ml)	MIC (µg/ml)	MBC/ MFC (µg/ml)	MIC (µg/ml)	MBC/ MFC (µg/ml)	MIC (µg/ml)	MBC/ MFC (µg/ml)
Staphylococcus aureus	250	>2000	NA	NA	3.13	≥100	100	100
Staphylococcus epidermidis	NA	NA	500	>2000	25	≥100	50	50
Bacillus subtilis	1000	>2000	NA	NA	100	≥100	25	100
Bacillus cereus	2000	>2000	NA	NA	12.5	≥100	12.5	≥100
Kocuria rhizophila	15.63	2000	62.5	>2000	0.78	3.13	0.78	25
Enterobacter aerogenes	NA	NA	2000	>2000	100	≥100	12.5	100
Escherichia coli	NA	NA	NA	NA	100	≥100	100	≥100
Pseudomonas aeruginosa	NA	NA	2000	>2000	NA	NA	50	100
Salmonella typhi	NA	NA	NA	NA	25	≥100	50	50
Salmonella typhimurium	NA	NA	1000	>2000	3.13	100	12.5	≥100
Shigella spp.	NA	NA	NA	NA	25	≥100	25	100
Candida albicans	NA	NA	NA	NA	NA	NA	NA	NA
Saccharomyces cerevisiae	NA	NA	NA	NA	NA	NA	NA	NA

* NA = no activity. The tests were done in triplicate.

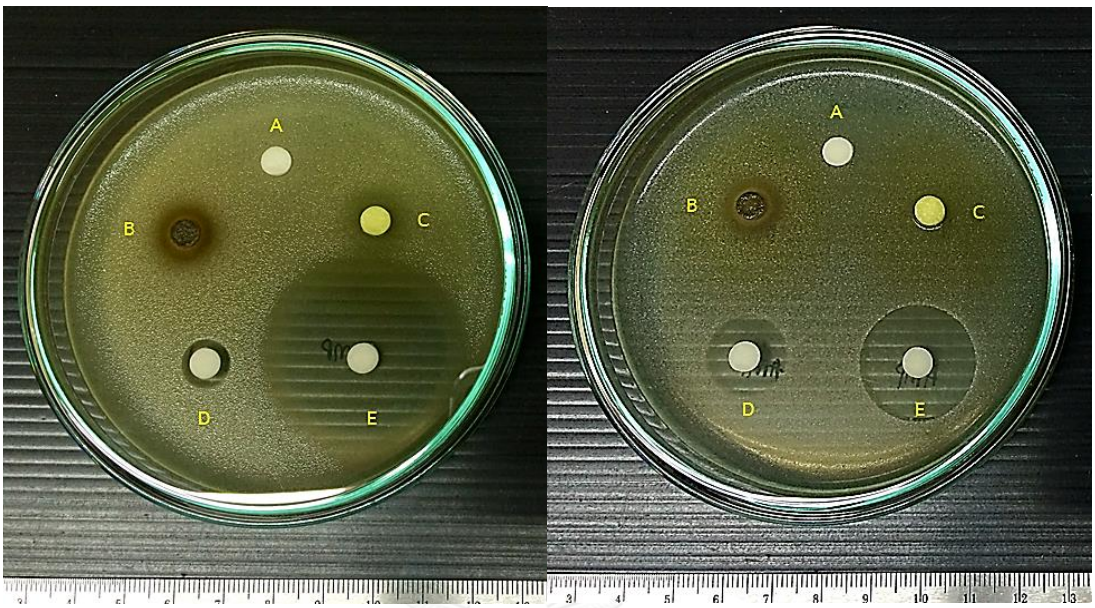


Figure 25 (Left) The inhibition zone of *Staphylococcus aureus* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate; (Right) The inhibition zone of *Staphylococcus epidermidis* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate

Figure 25 (Continue) (Left) The inhibition zone of *Bacillus cereus* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate; (Right) The inhibition zone of *Bacillus subtilis* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate

Figure 25 (Continue) The inhibition zone of *Kocuria rhizophila* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate

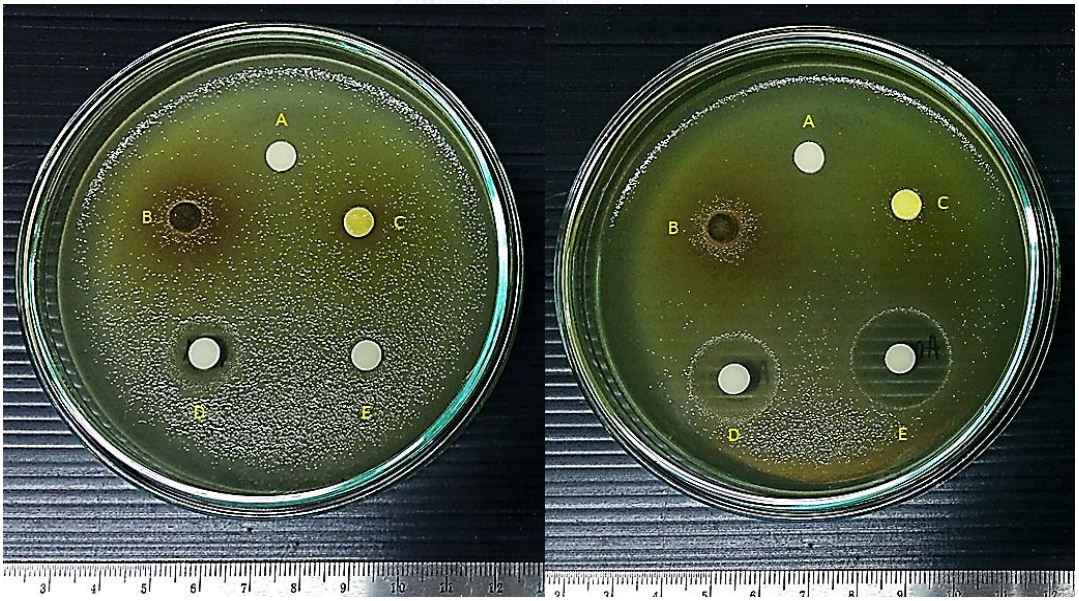


Figure 25 (Continue) (Left) The inhibition zone of *Enterobacter aerogenes* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate; (Right) The inhibition zone of *Escherichia coli* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate

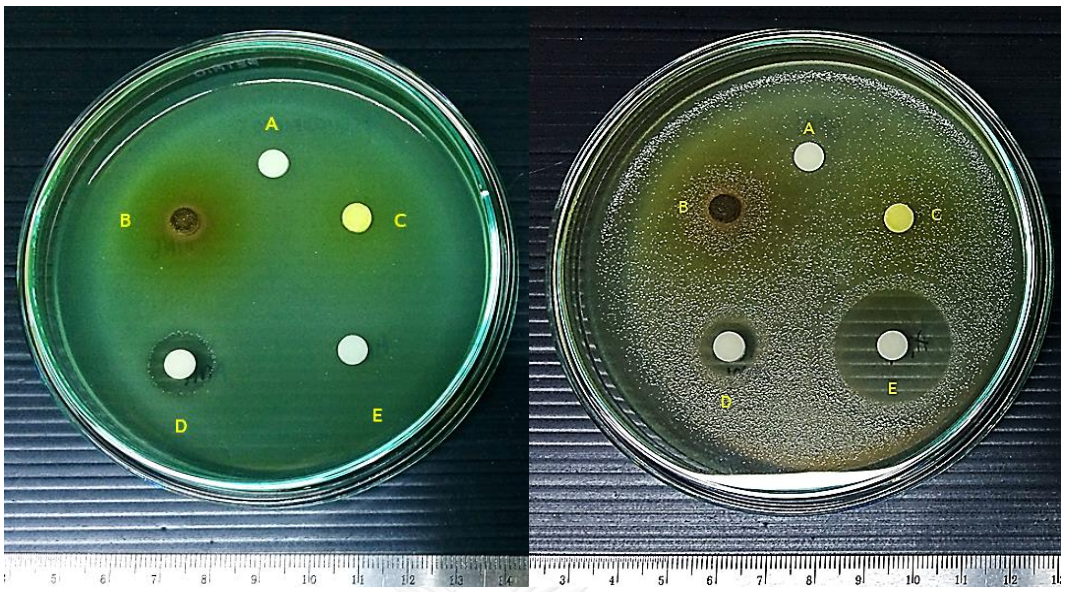


Figure 25 (Continue) (Left) The inhibition zone of *Pseudomonas aeruginosa* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate; (Right) The inhibition zone of *Salmonella typhi* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate

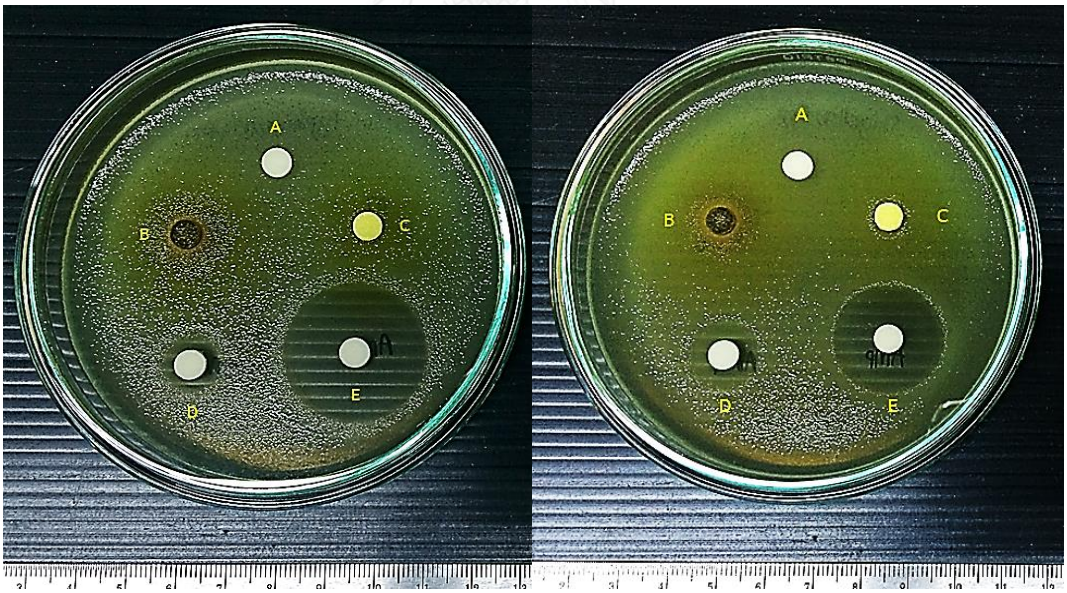


Figure 25 (Continue) (Left) The inhibition zone of *Salmonella typhimurium* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate; (Right) The inhibition zone of *Shigella* spp. from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate

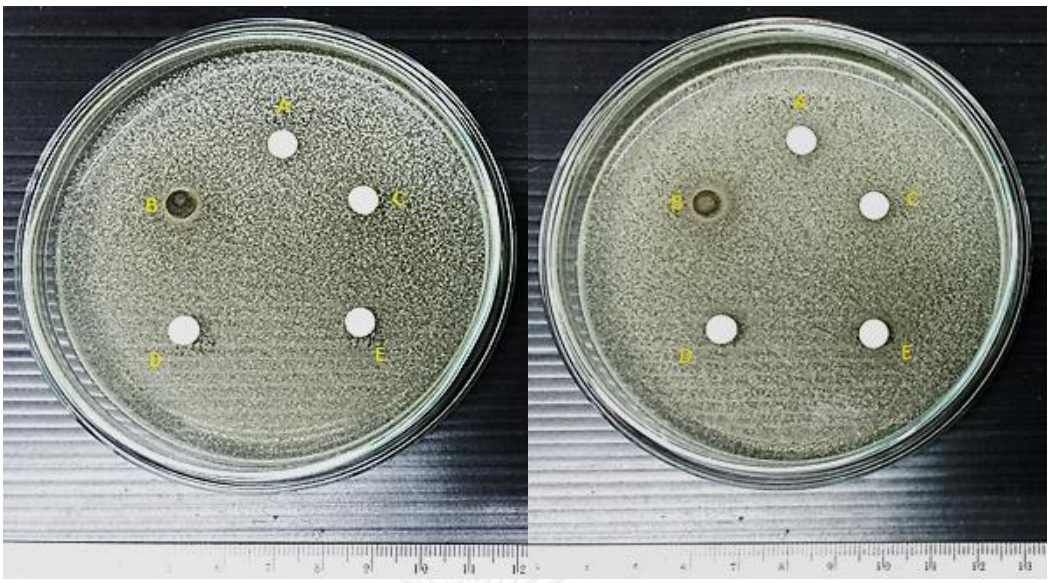


Figure 25 (Continue) (Left) The inhibition zone of *Candida albicans* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate; (Right) The inhibition zone of *Saccharomyces cerevisiae* from: (A) DMSO, (B) *Mangifera indica*, (C) Mangiferin, (D) Amikacin sodium, (E) Ampicillin sulfate

Anticancer activity

Mango leaf extract, at 200 µg/ml, showed cytotoxicity against all tested cancer cell lines. Mangiferin did not significantly affect % survival of tested cancer cells (Figure 26). Doxorubicin was used as a positive control; normal skin fibroblast (CCD) and normal lung fibroblast (Wi-38) were comparable cell lines in this study. Mango leaf extract, at high dose, also showed toxicity on lung fibroblast. On the contrary, the extract increased % survival of skin fibroblast. At high dose, mangiferin tended to increase the survival of skin and lung fibroblasts (Figure 26). The IC_{50} for cytotoxic activities of the extract, mangiferin and doxorubicin were shown in Table 21.

Table 21 Cytotoxic activities of mango leaf extract, mangiferin and doxorubicin

	IC_{50} (µg/ml)						
	BT474	Chago-K1	Hep-G2	Kato-III	SW620	CCD	Wi-38
Mango leaf extract	>200	>200	>200	>200	>200	>200	>200
Mangiferin	>200	>200	>200	>200	>200	>200	>200
Doxorubicin	0.80	0.65	0.12	0.71	2.57	>10	0.22

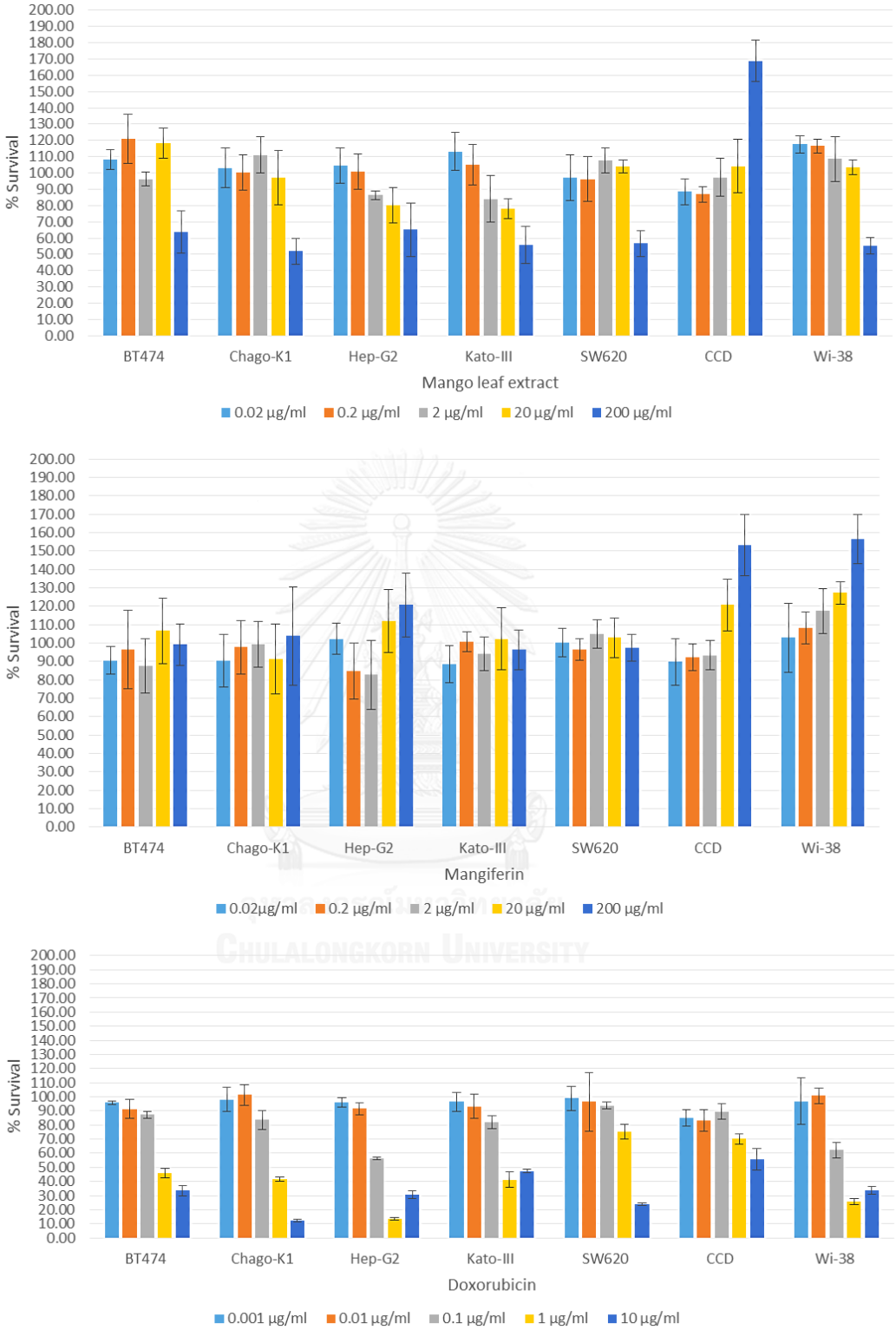


Figure 26 Inhibition of cancer cell growth by mango leaf extract, mangiferin and doxorubicin

CHAPTER IV
DISCUSSION AND CONCLUSION

People have been interested in the complementary and alternative use of medicinal plants since the last decade due to their safer and less destructive to the body. Mango (*Mangifera indica* L.) leaf, as one of the medicinal plant, was used to relieve the symptoms of diabetes, to lower blood pressure, to strengthen the blood vessels, to cure cough effectively, to cure voice loss, to stop bleeding dysentery, to heal burns on the skin and scalds and to help prevent various stomach ailments. In Thailand, besides consuming as vegetable, mango leaf was used for treatment of dysentery and flatulence [4].

Due to over 1,000 known cultivars, mangoes have confronted with confusions about numerous synonym nomenclatures and needed to be correctly identified [151, 152]. The identification of mango cultivars is conventionally based on morphological characteristics. In this study, the macroscopic evaluation of selected seventeen Thai mango cultivars that popularly cultivated in Thailand were observed on fruit shape, leaf shape, leaf apex, leaf base and leaf margin, which clearly reported in table 5. Macroscopic characteristics assessments in fruit crops including mango typically requires the presence of fruit. However, in off-fruiting season, they still need to differentiate among those cultivars. Leaf microscopic and molecular characteristics can be used despite in off-fruiting season [152].

For leaf microscopic characteristics, all of selected seventeen Thai mango cultivars had anomocytic stomata type, which bordered by a varying number of cells and not different from the epidermis. They were small size and presented only in the lower surface of the leaf. The epidermal cells were oval or round shaped. Mango leaf veins are reticulate veins patterns, small veins forming a network**.** Mango palisade cells appeared below the upper epidermis. They formed one or two layers of cells with columnar in shape, contained plentiful chloroplasts, elongated at right angles to the surface and arranged parallel to one another.

Leaf constant numbers could be used as distinguished characteristics of plant. Among selected seventeen Thai mango cultivars, they were quite similar in both leaf patterning distribution and leaf constant values. There were not differences between closely related *M. indica* cultivars, but differences between *M. indica* cultivars and outgroups.

Mango is a tropial diploid fruit crop (2n = 40 chromosomes), its genome size is about $4.39x10^8$ base pairs [153]. Many AG, GA, AC and CA dinucleotide repeat sequences were possible to exist in the mango genome because that repeat primer produced larger number of bands and polymorphic fragments [154]. GA and GT dinucleotide repeat sequences were also plenty present in the mango genome which could be effective to evaluate mango genetic diversity [155]. GACT and GGAT tetranucleotide repeat sequences were also found in the mango genome. In this study, most of the AG, GA and TG dinucleotide repeat sequences and GGAT tetranucleotide repeat sequences were also successful in amplifying bands. The average polymorphic percentage (82.05 %) in this study was higher than the other ISSR markers among mango cultivars in India (71.06%) and China (56.79%) [154, 156]. Although ISSR marker provided highly polymorphic percentage among these selected Thai mango cultivars, the number of total fragments amplified was relatively low. This might be because of electrophoretic gel types or staining technique influencing both number of total amplified band and polymorphic percentage detected. Polyacrylamide gel with silver staining may give more resolution [22]. No ISSR primer amplified a unique band pattern among *M. indica* cultivars. RAPD primer was alike, it was not amplified a unique band pattern also [23].

Macroscopic characters together with the dendrogram were sufficient to support dendrogram **(**figure 20**)**. ISSR had a potential to identify among seventeen Thai mango cultivars. The dendrogram showed two major clusters. Cluster 'I' was composed of 10 *M. indica* cultivars from 3 macroscopic characteristic groups; Nangklangwan group ('Nga Khao', 'Nangklangwan', 'Mahacharnok'); Namdokmai group ('Khiaoyai', 'Mankhunsi', 'Namdokmai'); Okrong group, ('Kaemdaeng', 'Okrong', 'Chok Anan', 'Raet'). The highest genetic similarity of 0.6985 in cluster I was found between 'Nga Khao' (Nangklangwan group) and 'Nangklangwan' (Nangklangwan group); whereas, the lowest

genetic similarity of 0.1576 was found between ‘Namdokmai’ (Namdokmai group) and ‘Raet’ (Okrong group). Cluster ‘II’ consisted of 7 cultivars from 3 macroscopic characteristic groups; Roundish group (‘Talapnak’); Keao group (‘Kaeo’, ‘Phetbanlat’, ‘Nongsaeng’); Khiaosawoey group (‘Tongdam’, ‘Khiaosawoey’, ‘Falan’). The highest genetic similarity of 0.6397 in cluster II was found between ‘Khiaosawoey’ (Khiaosawoey group) and ‘Falan’ (Khiaosawoey group); whereas, the lowest genetic similarity of 0.2544 was found between ‘Kaeo’ (Kaeo group) and ‘Talapnak’ (Roundish group).

TLC-densitometry is a high reliability quantitative technique with a very sensitive to measure in both UV and visible ranges. TLC-image analysis could be used as an alternative method to TLC-densitometry to quantitate mangiferin content in *Mangifera indica* leaves due to its convenient and cost-effective**.** Mango leaves, which are waste material gained from timming of post-harvest, are considered to be the good reasonable source of mangiferin.

There had many parameter such as solvent selections, temperatures, various parts of plants or different cultivars influenced on a mangiferin quantitative analysis of mango. Mangiferin could be very slightly soluble in most of the solvents [37]. It showed a maximum extraction in methanol and it decreased in ethanol to acetone with percent extractions of 59.00, 30.68 and 10.93, respectively. Increased in extraction temperature, percent recovery of mangiferin was increased [157]. Different parts of plants gave different mangiferin contents for example, mangiferin contents in *Mangifera indica* ‘Van Dyke’ peels, kernels, bark, old leaves and young leaves were 0.49, 0.64, 1.83, 3.69 and 5.81 g/100 g dry weight, respectively [158] . Three Thai mango cultivars leaf extracts (‘Namdokmai’, ‘Khiaosawoey’ and ‘Kaeo’) were also reported their mangiferin contents in different solvent extractions. Corresponding, they possessed a maximum extraction in methanol and it decreased in ethanol to 70% acetone. In methanol extract, mangiferin contents in *Mangifera indica* ‘Namdokmai’, ‘Khiaosawoey’ and ‘Kaeo’ leaves were 2.80, 2.40 and 1.30 g/100 g dry weight, respectively. In ethanol extract, mangiferin contents were 1.00, 0.30 and 0.90 g/100 g dry weight, respectively.

In 70% acetone, mangiferin contents were 0.66, 0.15 and 0.13 g/100 g dry weight, respectively [159].

In this study, TLC-densitometry and TLC-image analysis using image J software were performed and validated to confirm these analytical techniques provided reliable and accurate results. The specificity of TLC method indicated that the maximum absorbances of mangiferin were at the wavelength of 258, 323 and 366 nm, respectively. To compare both method, mangiferin spots were selected to detect under same wavelength of 254 nm. Accuracy and precision were in acceptable ranges. Accuracy were within range of 80 to 120% [160]. Repeatability and intermediate precision were less than 15 % RSD [161]. LOD and LOQ values demonstrated adequate methods sensitivity. Robustness showed that varying mobile phase composition was not significant influenced on both methods. However, the mangiferin contents in *Mangifera indica* 'Okrong' leaves by TLC-densitometry was a few higher than TLC-image analysis. These contents in mango leaves were 4.992 ± 1.025 and 4.311 ± 0.987 g/100 g of dried crude drug, respectively (p<0.05 by Wilcoxon signed-rank test).

α-Glucosidase may be largely divided into two types due to the difference in primary structure, types I (yeast) and II (mammals) [162]. Previous studies reported that various foods were active for yeast α-glucosidase, they had the potential to inhibit yeast α-glucosidase more than rat α-glucosidase and had inhibited those α-glucosidase more than α-amylase. On the contrary, acarbose which was anti-diabetic drug, had more potential to inhibit α-amylase than α-glucosidase and had slightly or no ability to inhibit yeast α-glucosidase relative to rat α-glucosidase [44, 162]. The similar results were found that both mango peels and mango seeds extracts had potential to inhibit α-glucosidase more than α-amylase with the IC_{50} of 3.5, 4.0 and 0.34, 0.71 μg/ml, respectively [43, 162]. Their leaf extract inhibited α-glucosidase with the IC_{50} of 59.0 μg/ml. They were active for yeast α-glucosidase, these dose-dependent inhibitory activity were significantly higher than acarbose [40, 44]. Different solvent extractions gave different inhibited potency. As an example, mango stem barks ethanolic extract showed the maximum inhibitory effects with the IC_{50} of 37.86 μg/ml; hexane extract

showed moderate inhibitory effects with the IC_{50} of 114.13 µg/ml; petroleum ether, chloroform and aqueous showed no inhibitory effects on alpha amylase activities [163]. However, the low IC_{50} value may be because the occurrence of other phenolic acids, flavonoids and carotenoids [41]. Previous study compared antidiabetic potential of mature and tender mango leaves aqueous methanolic extracts. Mature leaves extract inhibited α-glucosidase and α-amylase with the IC_{50} of 21.03 and 35.73 µg/ml, respectively due to their higher saponin, polyphenol, flavonoid contents. Tender leaves extract inhibited α-glucosidase and α-amylase with the IC_{50} of 27.16 and 22.01 µg/ml, respectively. They concluded that mango mature leaf had more potential to inhibit α-glucosidase; whereas, mango tender leaf had potential to inhibit α-amylase when compared to each other [41]. Mangiferin had more potent to inhibit α-glucosidase than α-amylase with the IC_{50} of 41.88 and 74.35 µg/ml, respectively [164]. In addition, many flavonoids were weakly inhibiting rat α-glucosidase. Our findings, mango leaf extract had strong potential to inhibit yeast α-glucosidase when compared to acarbose and mangiferin. It had the potential to inhibit α-glucosidase more than α-amylase. Mangiferin had strong potential for rat α-glucosidase when compared to acarbose and mango leaves extract. It also had more potent to inhibit α-glucosidase than α-amylase. Acarbose had strong potential to inhibit α-amylase compared to α-glucosidase.

Earlier, mango extracts and mangiferin have been reported to possess antibacterial and antifungal activity. Doughari *et al.* mentioned antibacterial activity of mango leaf extracts that they had more potent to inhibit Gram-positive bacteria than Gram-negative bacteria [165]. In case of Gram-negative bacteria, mango extracts most inhibited bacteria in the Enterobacteriaceae family. For example, Anand *et al.* screened antimicrobial properties of mango leaf ethanol extract against *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli* and *Candida albicans* using agar well diffusion and microbroth dilution. The extract showed inhibition zones against all of selected pathogen strains ranging from 11.00 to 20.33 mm. MIC varied from 39.06 to 1,250 µg /ml (312.5, 156.25, 39.06, 39.06 and 1,250

μg/ml, respectively). MBC and MFC varied from 78.12 to 2,500 μg/ml (1,250, 312.5, 156.25, 78.12 and 2,500 μg/ml, respectively). Mango leaf extract showed the most potent inhibition against *Escherichia coli* with the inhibition zone, MIC and MBC values of 20.33 mm, 39.06 μg/ml and 78.12 μg/ml, respectively [166].

Doughari *et al.* also mentioned the different degrees of antimicrobial properties may be because of the different solvents used. From their study, the highest activity against tested microorganisms was acetone extract followed by methanol extract, while water extract had no antimicrobial activity [165]. However, Poongothai *et al.* compared the antimicrobial activities of methanol to water extract of mango flower using disk diffusion and agar dilution. Methanol extract had more potent inhibiting than water extract, but there was not in agreement with Doughari *et al.* study because water extract still had a potential to inhibit bacteria. The extracts with the concentration of 250 μg/disc inhibited *Escherichia coli* at the inhibition zones of 22.6 and 18.9 mm, respectively. They possessed MIC values of 55 and 180 μg/ml, respectively [167]. El-Gied *et al.* investigated the antimicrobial activities of methanol and ethanol mango fruit seed extracts against 25 representatives gram positive, gram negative, acid fast bacteria and fungi using disk diffusion. Methanol had more potent to inhibit microorganisms than ethanol extracts. They showed the inhibition zones against most of selected pathogen strains ranging from 5 to and 18 mm, except *Bacillus cereus* and *Rhodococcus equi* which had no inhibition zones Nonetheless, methanol had high toxic, while ethanol had less toxic and more probable to be selected for biological testing [168].

Some studies have been screened mango extracts antimicrobial activities against drug resistant strains such as Hannan *et al.* reported the inhibitory effect of mango leaf extract using well diffusion, both antibiotic sensitive and multi-drug resistant *Salmonella typhi* was inhibited at the inhibition zones of 18 mm [169]. Kaur *et al.* reported the antibacterial activity of the mango seed kernel extract using disk diffusion. Methicillin resistant *Staphylococcus aureus*, *Escherichia coli* and *Vibrio vulnificus* were inhibited at the concentration of 100 mg/ml [170].

Mango extract had small inhibition zones; it may be due to low diffusion rate in agar medium. Bbosa *et al.* observed the antibacterial activity of mango leaf extract using well diffusion and gradient serial dilution. The extract possessed weak antibacterial activity compared to gentamycin against *Staphylococcus aureus, Esherichia coli* and *Pseudomonas aeruginosa* with MIC values ranging from 5.48 to 43.75 mg/ml [171].

Singh *et al.* argued inhibitory effect of mangiferin which isolated from mango stem bark ethanolic extract against bacteria namely *Bacillus pumilus*, *Bacillus cereus*, *Salmonella virchow* and *Pseudomonas aeruginosa*, and fungi namely *Thermoascus aurantiacus* and *Aspergillus flavus* using disk diffusion. Mangiferin had wide inhibition zones against *Bacillus pumilus, Bacillus cereus*, *Salmonella virchow*; only at high concentrations mangiferin or its derivatives effected against *Pseudomonas aeruginosa* and both fungi [172]. Biswas *et al.* reported antibacterial activity of mangiferin which isolated from mango flowering buds ethanolic extract against against various strains of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Salmonella typhi*) bacteria. Mangiferin had inhibition zones against all strains at concentrations of ≥5 mg/ml [173].

This study investigated mango leaf ethanolic extract against gram-positive bacteria (non-spore-forming and spore-forming groups), gram-negative bacteria (Enterobacteriaceae, klebsiella-enterobacter-serratia and pseudomonads groups) and fungi (yeast and yeastlike fungi groups). For disk diffusion, mango leaf extract inhibited most of tested Gram-positive bacteria except *Staphylococcus epidermidis*, no activities against Gram-negative bacteria and fungi, corresponded with previous study that mango leaf extract had more potent to inhibit Gram-positive bacteria. Mangiferin inhibited some of tested bacteria namely *Staphylococcus epidermidis*, *Kocuria rhizophila*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. In contrast to previous studies that mangiferin had no activities against *Staphylococcus aureus, Bacillus cereus* and *Salmonella typhi*.

For microbroth dilution, According to Holetz *et al.,* the extracts displayed an MIC less than 100 µg/ml, the antimicrobial activity was good; from 100 to 500 µg/ml the antimicrobial activity was moderate; from 500 to 1000 µg/ml the antimicrobial activity was weak; over 1000 µg/ml the extract was consider inactive [174]. Mango leaf extract

possessed strong inhibitory effect against *Kocuria rhizophila*, moderate inhibitory effect against *Staphylococcus aureus* and mild inhibitory effect against *Bacillus subtilis*. There were no activities against tested gram-negative bacteria and yeast. Mangiferin possessed strong inhibitory effect against *Kocuria rhizophila*, moderate inhibitory effect against *Staphylococcus epidermidis* and mild inhibitory effect against *Salmonella typhimurium*. There were no activities against yeast.

Both mango leaf extract and mangiferin had most potent to inhibit against *Kocuria rhizophila* with the widest inhibition zone sizes 12.67 and 11.67 mm, the MIC values of 15.63 and 62.5 µg/ml and the MBC values of 2,000 and >2,000 µg/ml, respectively.

MTT assay, as one of the most often used as an accurate and uncomplicated screening method, provides a useful preliminary quantitative data on the viable eukaryotic cell proliferation or cytotoxic potential of natural product extracts.

Abdullah *et al*. reported cytotoxic effects of mango kernel extract on human breast cancer cell lines compared to human breast normal cell lines that mango kernel extract significantly possessed cytotoxic effects towards breast cancer cell lines (MDA-MB-231 with the IC_{50} values of 30 µg/ml and MCF-7 with the IC_{50} values of 15 µg/ml); while, it showed low cytotoxic effects towards normal breast cell lines (MCF-10A (the IC_{50} values of 149 µg/ml)) [39]. Kim *et al*. founded antiproliferative properties of mango fruit peel extracts, It had cytotoxic against human gastric cancer cell lines (AGS), cervical cancer cell lines (HeLa) and hepatocarcinoma cell lines (HepG2) in a dose-dependent manner at the concentration of 125-1000 µg/ml; whereas, it showed no significant cytotoxic effects towards lung fibroblasts normal cell line (CCD-25Lu) [175]. Timsina *et al*. stated about anticancer activity of mango fruit seed extract that it had a dose-dependent inhibitory effect on human cervical cancer cell line (HeLa) with the IC_{50} value of 25 µg/ml, but had no cytotoxic effects to Chinese hamster epithelial cell line (CHO) [176]. Joona *et al*. mentioned that mango leaf extract showed cytotoxicity against gastric adenocarcinoma cell line (AGS) with the IC_{50} value of 166.9 µg/ml [177]. Ramos *et al*. argued that the mango fruit essential oils had cytotoxic against human larynx carcinoma cell line (HEp-2), colon adenocarcinoma cell line (HT-29), lung mucoepidermoid carcinoma cell line (NCIH292), and promyelocytic leukemia cell line

(HL- 60). Mango cv. Rosa and Espada were most effective against the promyelocytic leukemia cell line, with IC_{50} values of 12.3 and 3.6 μg/ml, respectively [178]. Noratto *et al*. studied anticancer effects of various mango cultivars fruit extracts on cancer cell lines, including leukemia (Molt-4), lung (A-549), breast (MDA-MB-231), prostate (LnCap), and colon (SW-480) cancer cell lines compared to colon normal cell line (CCD-18Co) and found that all of mango cultivar extracts inhibited all tested cancer cell lines. Colon cancer cell lines were most affected; whereas, colon normal cell lines was not inhibited at the same concentration by most of extracts, except Ataulfo cultivars that inhibited normal cell line at only high concentration [179].

Fruit peel, fruit seed, fruit essential oils, kernel or leaf from several mango cultivars showed toxic effect on cancer cell lines, including human breast (MDA-MB-231 and MCF-7), gastric (AGS), cervical (HeLa), hepatoma (HepG2), cervix (HeLa), stomach (AGS) larynx (HEp-2), colon (HT-29 and SW-480), lung (NCIH292 and A-549), and leukemia (HL-60 and Molt-4) and prostate (LnCap) cancer cell lines. They had low cytotoxicity against normal cell lines, including breast (MCF-10A) and colon (CCD-18Co) normal cell lines, and no toxicity effect on lung fibroblast normal cell line (CCD-25Lu). They suggested mango extracts to be used in chemoprevention.

In this study, mango leaf was used. The leaf extract at high dose ($IC_{50} > 200$ μg/ml) possessed cytotoxic activities against all tested cancer cell lines (ductal carcinoma, bronchogenic carcinoma, liver hepatoblastoma, gastric carcinoma and colon adenocarcinoma). However, at that high dose, the toxicity on lung fibroblast normal cell line was also shown; while there was no toxic effect especially enhancing effect toward skin fibroblast normal cell line.

Kim *et al.* discussed that antiproliferative potential of mango extracts might be due to their bioactive compounds (polyphenols or flavonoids) synergistic actions [175], while Preedy *et al*. mentioned phenolic compounds might act additively, synnergitically, and/or antagonically with other compounds exposed to antiproliferative activities [96]. Ramos *et al*. concluded the cytotoxic effect of mango fruit essential oils towards mammalian cells may be due to the presence of phenols, aldehydes, and alcohols. It can stimulate the mitochondrial membranes depolarization by decreasing the

membrane potential, affecting Ca^{++} and other ion channels, and reducing the pH gradient, causes eukaryotic cells apoptosis and necrosis [178].

Mangiferin is one of the natural xanthone, which was extracted from mango tree. Li *et al*. investigated antiproliferative effect of mangiferin that it had a dose-dependent inhibitory effect on human prostate cancer cells line (PC3) with the IC_{50} value of >40 μM [180]. Li *et al*. concluded that mangiferin inhibited human breast cancer cell lines proliferation namely MDA-MB-231 and BT-549 with the IC_{50} of 298.6 and 273.8 μM, respectively, they also mentioned only high dose of mangiferin induced significant apoptosis cancer cell line [181]. Pan *et al*. mentioned mangiferin inhibited nasopharyngeal cancer cell lines growth (CNE2) because of inducing cell apoptosis [182]. From the findings, mangiferin did not show significantly toxicity against all tested cancer cell lines. This study found that mangiferin also had the potential on increasing the survival of skin and lung normal cell lines.

In summary, for mango cultivar identifications, they could be differentiated using fruit and leaf macroscopic characteristics as main criteria. However, in off-fruiting season, molecular characteristics (using ISSR marker system) together with macroscopic characteristics had a potential to identify among these cultivars. Microscopic characteristics, as supporting evidences, in combination with macroscopic and molecular characteristics were able to use as a helpful tool for more accurate differentiation among mango cultivars. For mangiferin quantitative analysis, TLC-densitometry can be used to measure mangiferin content of *Mangifera indica* 'Okrong' leaves. However, TLC-image analysis, which has been used as alternative method for TLC quantification, showed lower amont of mangiferin in *Mangifera indica* than TLC densitometry in this study. Mango leaf extract and mangiferin possessed biological evaluations including antidiabetic, antimicrobial and anticancer potential *in vitro*. For antidiabetic activity, mango leaf extract had more potent to inhibit yeast α-glucosidase than rat α-glucosidase. Mangiferin had more potent to inhibit rat α-glucosidase than yeast α-glucosidase. Both mango leaf extract and mangiferin had more potent to inhibit α-glucosidase than α-amylase. For antimicrobial activity, mango leaf extract had potent to inhibit tested Gram-positive bacteria except *Staphylococcus*

epidermidis. Mangiferin had potent to inhibit some tested bacteria. Both mango leaf extract and mangiferin had most potent to inhibit *Kocuria rhizophila*; whereas, there were no activity against tested yeast. For anticancer activity, mango leaf extract (≥ 200 µg/ml) showed cytotoxicity against tested cancer cell lines. Both mango leaf extract and mangiferin increased % survival of skin fibroblast. Mango leaf extract and mangiferin demonstrated *in vitro* potential to treat diabetes, infections and cancer.

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APPENDIX

APPENDIX A

Microscopic characteristics

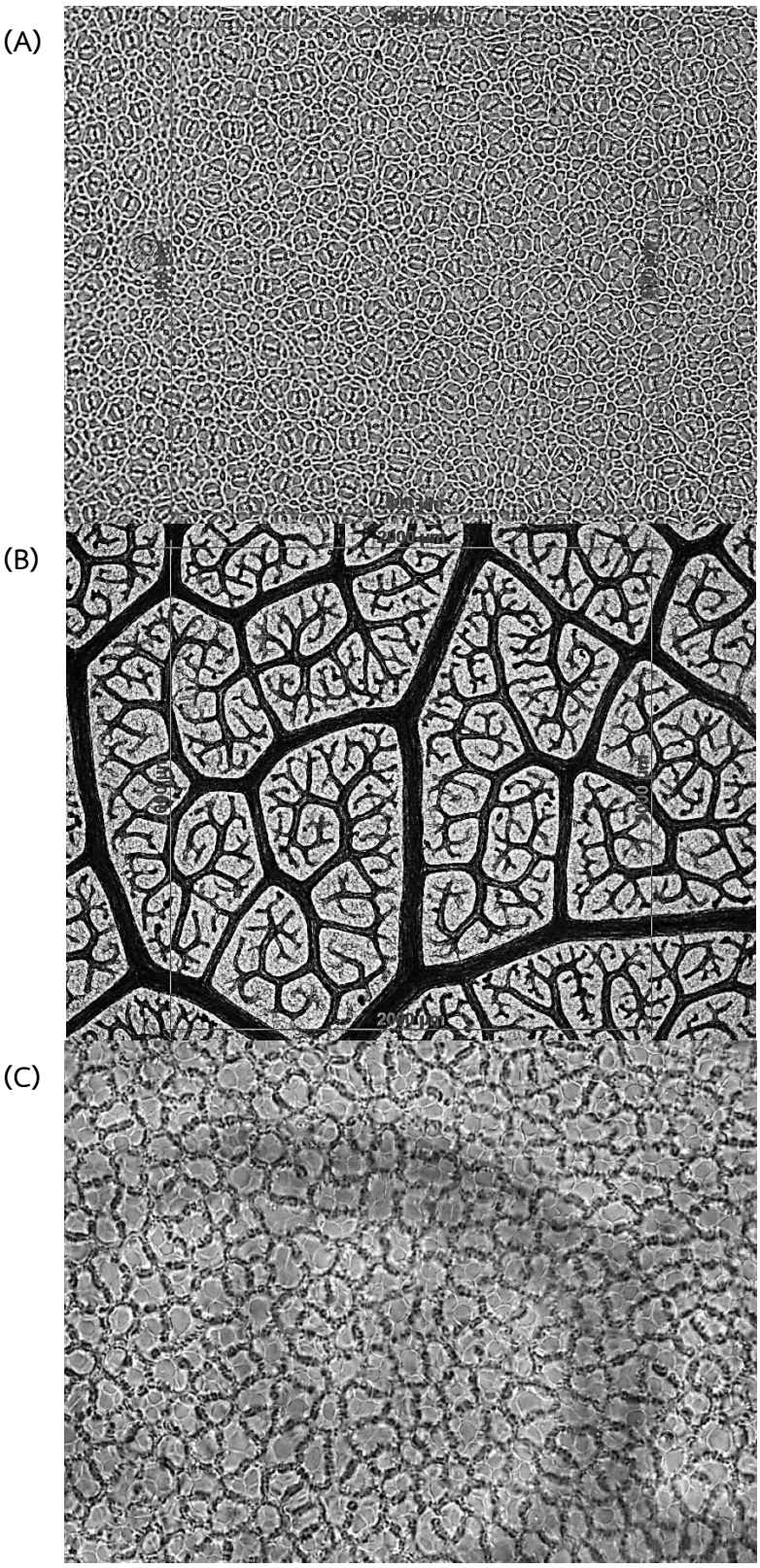


Figure 27 Images of *Mangifera indica* 'Nga Khao' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 μm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 μm; (C) palisade and epidermal cells at a magnification of 400X

Table 22 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Nga Khao'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	752	688	764	26.75	39.50	37.00	3.75	2.50	3.50
2	744	712	724	29.50	42.25	35.25	3.25	2.75	3.75
3	736	760	800	28.25	38.25	34.00	3.00	2.75	3.25
4	740	748	836	29.25	37.50	33.75	2.25	3.25	3.00
5	700	812	788	25.25	35.00	34.50	3.50	2.50	3.50
6	708	656	796	25.00	34.00	39.75	3.00	3.50	3.00
7	768	736	736	26.50	33.00	42.00	3.25	2.75	2.75
8	764	728	780	30.25	32.25	36.75	3.00	3.25	3.00
9	780	744	788	23.75	35.75	36.00	3.25	2.75	3.00
10	728	640	768	23.25	31.00	32.50	3.00	3.50	3.25
11	756	700	672	27.00	35.50	34.75	2.50	2.75	2.75
12	680	748	716	26.00	36.00	35.25	2.50	3.25	3.00
13	668	672	724	26.25	38.75	35.00	2.75	3.00	3.25
14	680	744	732	23.25	39.75	35.75	2.75	2.75	3.00
15	756	708	708	24.00	40.25	40.75	3.00	2.75	3.25
16	672	732	756	21.75	35.00	34.75	2.75	2.50	2.75
17	756	656	712	22.25	36.50	37.25	2.25	3.00	2.75
18	704	676	732	23.25	34.75	34.00	3.00	2.75	3.00
19	736	644	688	26.50	37.25	38.75	3.00	2.50	2.75
20	748	700	616	22.00	35.25	39.50	2.75	3.00	3.00
21	692	660	696	22.75	42.50	33.25	3.50	3.50	3.25
22	672	744	772	23.25	41.25	37.50	2.75	2.50	3.50
23	700	680	672	30.75	37.25	39.00	3.25	2.50	3.00
24	684	728	736	25.50	38.50	38.50	2.50	3.00	3.00
25	672	672	708	26.25	40.00	40.75	2.25	2.50	2.75
26	816	720	672	23.25	34.50	40.00	2.50	3.00	3.00
27	756	728	712	23.75	31.75	39.25	3.00	2.75	3.00
28	724	628	752	24.75	30.75	38.75	2.75	3.50	2.75
29	756	732	736	27.00	33.00	37.75	2.75	3.50	2.75
30	732	720	744	26.50	34.50	35.50	2.50	2.75	2.50
Mean	**726**	**707**	**735**	**25.46**	**36.38**	**36.92**	**2.88**	**2.91**	**3.03**
SD	**38.09**	**42.26**	**46.55**	**2.47**	**3.24**	**2.55**	**0.38**	**0.35**	**0.28**
Range	**668-816**	**628-812**	**616-836**	**21.75-30.75**	**30.75-42.50**	**32.50-42.00**	**2.25-3.75**	**2.50-3.50**	**2.50-3.75**

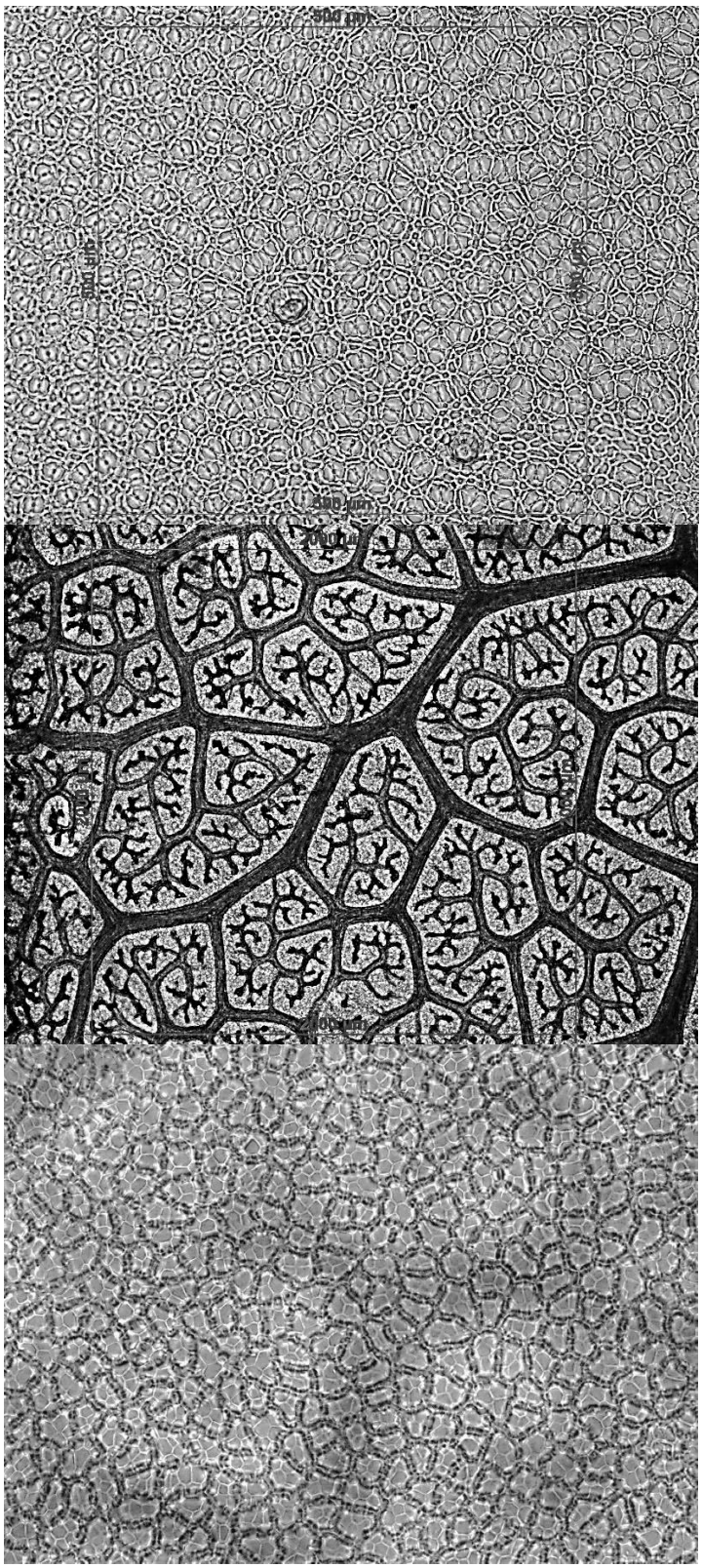


Figure 28 Images of *Mangifera indica* 'Nangklangwan' leaves showing (A) mango stomata at magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 23 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Nangklangwan'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	836	552	420	32.25	39.75	40.75	3.25	3.50	3.25
2	820	448	508	29.00	36.25	39.75	3.25	3.50	3.25
3	788	512	500	33.25	37.50	34.50	3.00	3.25	3.50
4	800	508	388	31.00	33.50	33.50	3.50	3.00	3.50
5	804	576	520	36.75	34.25	37.00	3.50	3.50	3.00
6	828	516	452	32.75	35.00	41.25	3.50	3.50	2.75
7	824	496	440	31.50	34.50	38.25	3.25	3.25	3.00
8	816	552	464	37.50	36.75	39.00	3.25	3.50	3.25
9	808	496	412	35.75	39.50	35.75	3.75	3.25	3.25
10	836	512	456	31.25	32.00	36.25	3.50	3.50	3.00
11	820	508	484	30.00	34.50	39.75	3.75	3.25	3.00
12	812	528	496	29.00	35.00	39.00	3.50	3.75	3.00
13	732	460	468	28.75	35.50	38.25	3.25	3.25	3.25
14	748	500	436	33.75	36.00	35.25	3.50	3.50	3.25
15	812	496	500	34.00	37.00	39.00	3.00	3.25	2.75
16	832	480	392	34.50	40.50	43.25	3.25	3.75	3.25
17	824	524	480	31.00	38.50	41.00	3.00	3.50	3.50
18	812	448	504	37.00	41.00	38.25	4.00	3.75	3.50
19	800	492	496	35.75	40.50	44.25	4.00	3.75	3.00
20	788	492	472	37.25	37.75	40.75	3.50	3.75	3.25
21	832	464	424	33.50	38.25	42.50	4.00	3.50	3.00
22	820	512	476	35.00	40.75	41.00	3.75	3.75	3.00
23	852	536	388	32.75	38.75	42.00	3.75	3.25	2.75
24	908	516	440	31.50	37.50	41.50	3.00	3.75	3.50
25	900	484	472	32.00	36.25	36.25	3.25	3.50	2.75
26	804	440	452	29.50	41.50	41.75	4.00	3.75	3.50
27	868	496	500	31.75	40.75	35.00	3.25	3.25	2.75
28	860	520	460	31.25	41.75	35.50	3.75	2.75	2.75
29	856	472	472	32.50	39.50	40.25	3.75	3.50	3.50
30	828	496	456	31.50	42.00	40.00	3.50	3.25	3.25
Mean	**822**	**501**	**461**	**32.78**	**37.74**	**39.02**	**3.48**	**3.45**	**3.14**
SD	**36.11**	**31.53**	**36.52**	**2.53**	**2.74**	**2.80**	**0.31**	**0.25**	**0.27**
Range	**732-908**	**440-576**	**388-520**	**28.75-37.50**	**32.00-42.00**	**33.50-44.25**	**3.00-4.00**	**2.75-3.75**	**2.75-3.50**

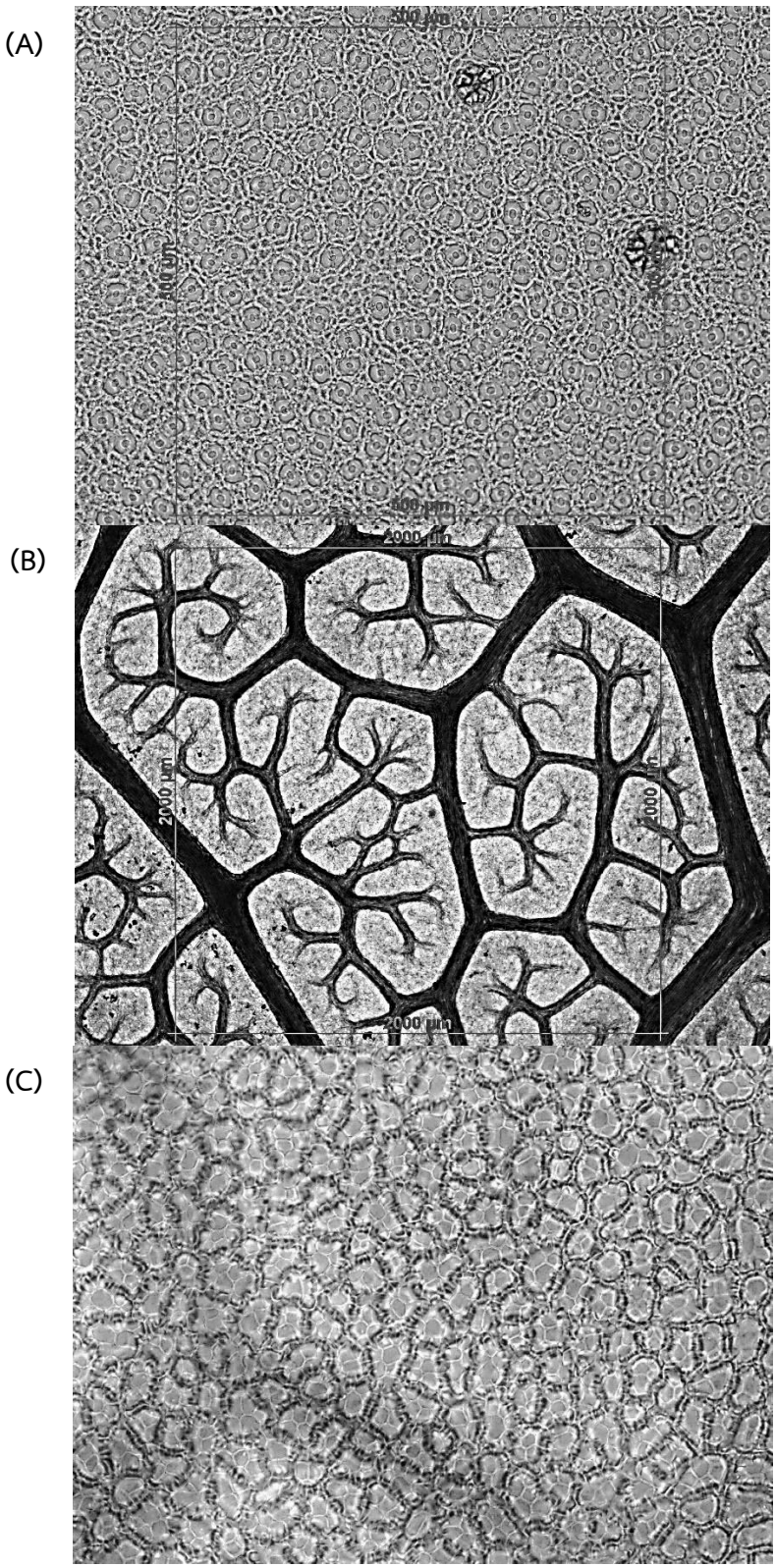


Figure 29 Images of *Mangifera indica* 'Khiaoyai' leaves showing showing (A) mango stomata at magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 24 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Khiaoyai'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	668	804	704	20.00	28.75	33.75	4.00	3.75	3.75
2	672	652	720	19.50	28.00	27.50	4.25	3.25	3.00
3	668	704	656	18.00	29.25	30.75	4.00	3.00	3.25
4	628	736	640	18.50	28.50	27.00	4.00	3.00	2.75
5	616	788	596	14.75	30.25	27.25	3.50	3.75	2.75
6	644	756	636	15.75	26.75	31.75	3.50	3.25	3.25
7	648	784	648	16.50	24.75	34.25	3.00	4.00	3.00
8	644	768	656	19.00	27.00	33.25	3.25	3.75	2.75
9	620	652	604	18.75	25.00	30.50	3.00	4.00	3.50
10	624	704	672	17.50	28.00	30.00	3.00	3.75	3.25
11	708	676	696	18.00	26.25	28.25	3.75	3.50	3.00
12	660	744	628	20.75	30.50	25.50	3.00	3.50	2.75
13	712	716	636	20.00	26.50	26.00	3.00	3.25	3.25
14	656	684	560	17.50	29.00	26.50	3.25	3.75	3.50
15	660	796	640	18.75	25.50	29.50	4.00	3.25	3.00
16	636	704	656	19.25	27.00	28.25	3.75	3.50	3.25
17	696	748	680	19.00	24.25	28.75	4.00	3.25	3.25
18	680	680	688	17.50	25.50	29.25	3.50	3.75	3.50
19	660	712	732	19.25	26.25	26.75	3.50	3.25	3.00
20	596	692	676	21.25	25.75	27.50	4.25	3.00	3.25
21	568	672	684	18.50	25.00	26.25	4.00	3.25	3.25
22	580	660	584	16.50	23.50	27.75	3.75	3.00	3.00
23	592	716	640	17.50	23.75	29.25	3.50	3.50	3.00
24	564	672	616	19.50	24.25	29.75	3.50	3.50	2.75
25	596	660	684	21.50	27.25	29.25	3.00	3.75	3.00
26	608	584	760	20.75	25.25	27.00	3.25	3.00	3.25
27	596	688	660	20.25	26.25	28.00	3.25	3.25	3.50
28	592	596	728	21.25	26.25	27.50	3.75	3.00	3.25
29	604	672	740	20.50	23.75	28.75	3.50	3.25	3.75
30	600	744	812	20.00	25.50	27.00	4.25	3.25	2.75
Mean	**633**	**705**	**668**	**18.86**	**26.45**	**28.76**	**3.58**	**3.41**	**3.15**
SD	**40.51**	**54.38**	**54.33**	**1.68**	**1.91**	**2.25**	**0.42**	**0.31**	**0.29**
Range	**564-712**	**584-804**	**560-812**	**14.75-21.50**	**23.50-30.50**	**25.50-34.25**	**3.00-4.25**	**3.00-4.00**	**2.75-3.75**

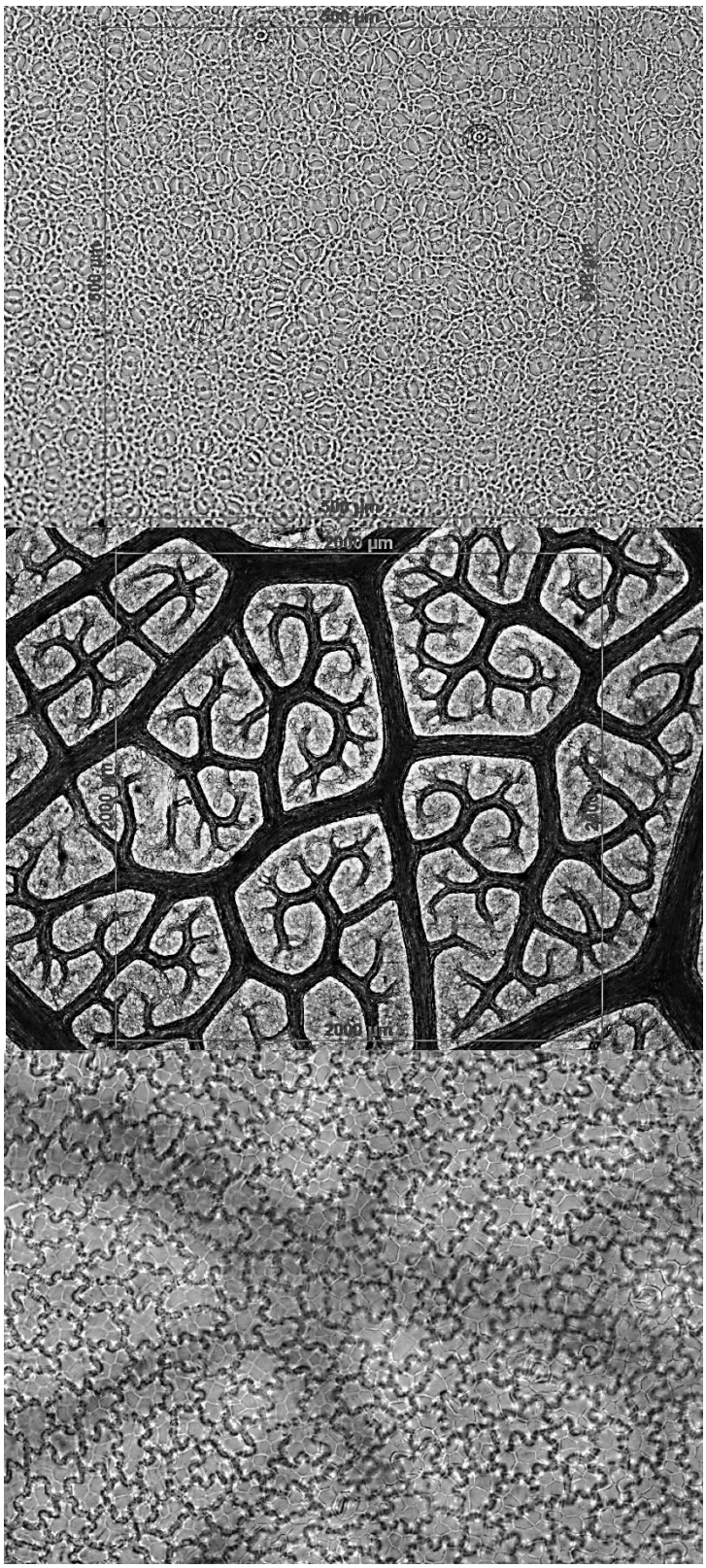


Figure 30 Images of *Mangifera indica* 'Mankhunsi' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 25 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Mankhunsi'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	648	616	676	29.00	36.25	40.50	3.25	3.75	4.00
2	632	668	580	27.50	37.25	40.00	3.00	4.25	3.75
3	628	600	588	28.50	32.50	41.25	3.75	3.75	3.50
4	620	644	632	30.00	34.50	36.25	3.00	4.00	3.50
5	624	628	648	25.75	35.75	35.50	3.75	4.25	3.75
6	636	588	592	29.75	32.00	33.50	4.00	3.50	4.00
7	588	604	604	26.50	30.75	39.25	3.75	3.50	3.50
8	616	584	656	26.00	31.25	42.25	4.50	3.50	3.25
9	616	608	692	26.50	34.25	40.75	4.25	3.75	3.50
10	604	660	664	31.50	35.50	43.75	5.00	3.75	3.75
11	584	676	600	31.75	30.50	41.00	4.25	3.25	3.50
12	588	576	700	30.25	28.75	33.75	3.50	3.50	3.75
13	592	636	576	31.25	34.75	37.25	3.50	3.75	4.00
14	576	664	664	29.50	29.75	35.00	4.00	3.50	3.25
15	560	628	640	27.50	35.00	36.25	4.25	3.00	3.50
16	588	636	588	27.25	30.00	35.50	4.00	3.75	4.00
17	608	648	596	28.75	31.75	34.75	4.50	4.25	3.50
18	580	628	596	26.50	29.25	35.25	4.00	3.50	4.00
19	560	696	644	27.25	32.50	33.00	5.00	3.25	3.50
20	548	592	636	27.75	31.50	34.50	3.75	3.75	3.25
21	624	636	576	26.25	31.75	37.75	3.75	3.00	4.00
22	528	628	596	27.75	32.50	34.25	4.25	4.00	3.25
23	624	680	680	25.50	28.50	35.25	4.50	3.50	4.00
24	560	616	664	29.25	28.00	38.25	4.00	3.50	3.75
25	540	700	696	28.50	29.00	35.75	3.75	3.25	3.25
26	552	680	672	25.00	30.00	33.00	3.50	3.00	4.00
27	552	696	680	32.75	28.50	34.75	4.25	3.25	3.75
28	592	592	668	29.75	31.00	36.75	3.50	3.50	3.50
29	588	692	688	30.00	32.75	40.25	3.50	3.50	3.75
30	584	660	608	30.25	32.00	35.75	4.00	2.75	4.00
Mean	**591**	**639**	**637**	**28.46**	**31.93**	**37.03**	**3.93**	**3.56**	**3.67**
SD	**31.60**	**36.91**	**41.14**	**2.03**	**2.54**	**2.98**	**0.50**	**0.38**	**0.27**
Range	**528-648**	**576-700**	**576-700**	**25.00-32.75**	**28.00-37.25**	**33.00-43.75**	**3.00-5.00**	**2.75-4.25**	**3.25-4.00**

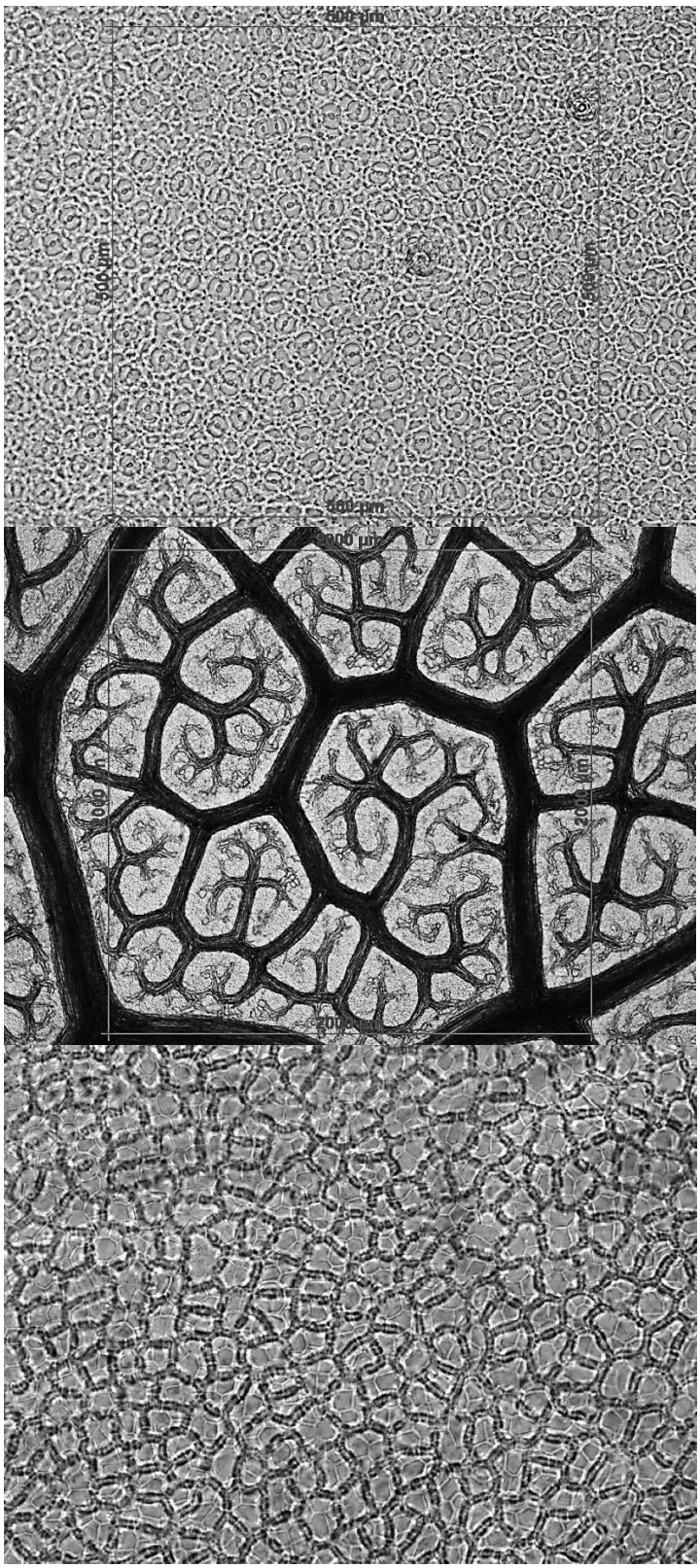


Figure 31 Images of *Mangifera indica* 'Namdokmai' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 26 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Namdokmai'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	504	448	584	28.00	33.00	31.75	3.25	3.25	2.75
2	572	520	552	25.50	26.00	31.25	2.50	2.75	2.25
3	516	524	508	23.50	22.25	33.00	3.00	3.00	2.75
4	508	516	540	28.00	23.25	30.25	3.00	3.75	2.50
5	488	572	564	28.25	27.75	32.75	3.50	3.00	2.25
6	520	548	540	30.00	29.25	31.25	3.75	3.00	2.25
7	512	508	496	26.75	32.75	34.75	3.50	2.50	2.25
8	488	592	592	25.75	35.50	31.00	3.25	3.25	3.00
9	540	472	528	24.00	31.75	33.00	3.25	2.50	2.50
10	516	464	544	23.25	28.00	37.00	3.75	2.50	2.50
11	492	452	556	25.75	26.00	25.25	3.75	3.25	3.25
12	508	468	540	26.50	29.50	31.25	2.75	2.75	2.00
13	484	464	492	25.50	30.00	36.75	3.00	2.50	3.00
14	444	528	536	27.50	28.00	32.75	3.75	3.25	3.00
15	460	504	508	26.75	30.25	29.25	3.25	2.75	3.25
16	484	488	552	24.50	29.75	31.00	4.00	3.50	3.25
17	520	536	496	28.25	31.00	35.25	3.25	2.50	2.50
18	508	476	524	26.50	31.75	28.50	3.75	3.25	3.50
19	568	516	468	29.50	28.25	30.75	3.25	3.50	2.50
20	524	460	516	26.50	32.25	31.75	3.50	2.50	2.75
21	564	512	524	26.00	31.00	33.00	3.00	3.00	2.25
22	512	528	532	25.50	33.00	36.50	2.75	2.75	3.00
23	536	516	508	27.00	26.00	30.50	3.00	3.00	3.25
24	504	504	536	25.75	29.75	33.75	3.00	3.25	2.50
25	484	496	528	24.00	26.25	34.25	3.75	3.25	2.25
26	472	508	572	28.50	30.25	35.25	3.25	3.25	2.50
27	460	504	544	24.25	27.50	33.25	2.75	2.75	2.50
28	516	508	536	28.50	27.25	31.25	3.50	3.00	3.00
29	484	500	504	27.50	30.25	32.50	3.25	3.25	2.75
30	524	512	584	25.75	28.50	33.50	3.00	3.00	2.50
Mean	**507**	**505**	**533**	**26.43**	**29.20**	**32.41**	**3.28**	**2.99**	**2.68**
SD	**30.53**	**33.29**	**29.18**	**1.75**	**2.94**	**2.52**	**0.37**	**0.34**	**0.39**
Range	**444-572**	**448-592**	**468-592**	**23.25-30.00**	**22.25-35.50**	**25.25-37.00**	**2.50-4.00**	**2.50-3.75**	**2.00-3.50**

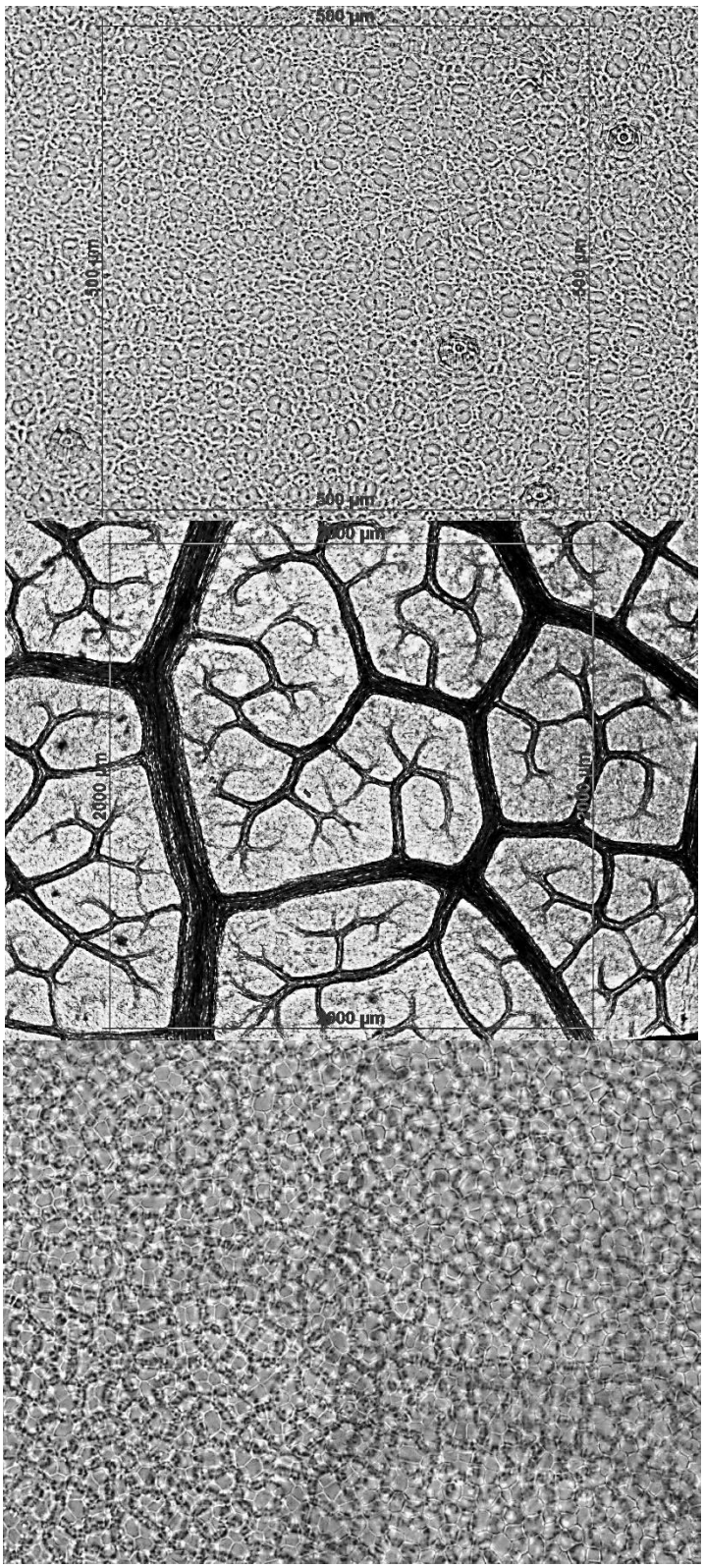


Figure 32 Images of *Mangifera indica* 'Mahacharnok' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 27 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Mahacharnok'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	576	612	648	18.75	26.75	32.25	2.50	3.00	3.00
2	588	600	608	19.75	22.50	28.50	2.25	3.50	3.50
3	572	592	684	22.25	30.75	31.25	2.50	3.00	3.75
4	632	676	548	20.25	28.00	30.75	3.00	3.00	3.25
5	644	656	612	19.50	26.00	32.50	3.25	2.75	2.50
6	640	644	576	16.75	25.00	28.50	3.25	3.00	3.25
7	580	608	520	17.75	26.25	29.00	2.75	3.50	3.50
8	584	604	560	20.75	25.50	30.75	2.25	3.25	3.00
9	612	580	580	17.50	33.25	33.00	2.75	3.75	3.25
10	584	608	600	17.25	27.00	33.50	2.50	3.00	2.50
11	632	640	556	19.75	25.25	29.75	2.75	2.75	3.00
12	628	620	568	21.50	25.75	31.00	2.75	2.50	3.25
13	560	556	548	18.00	31.00	30.25	2.75	3.50	2.75
14	572	564	584	23.50	25.25	28.75	3.50	3.25	3.00
15	640	596	564	21.00	30.25	30.75	2.50	3.25	3.75
16	564	628	628	19.00	26.25	35.25	3.00	3.50	2.75
17	584	592	552	15.75	28.25	31.00	3.25	2.75	3.50
18	608	552	572	18.00	27.00	27.75	3.00	3.00	3.25
19	680	592	524	21.75	23.75	26.25	3.50	3.50	3.75
20	560	680	576	18.25	31.00	33.25	3.75	2.75	3.25
21	560	544	568	19.75	30.50	29.25	2.75	3.25	3.00
22	588	568	556	17.25	31.00	30.75	3.75	3.25	3.25
23	632	580	532	15.75	27.75	28.50	3.50	3.00	3.50
24	616	536	624	18.50	23.50	25.50	3.25	2.75	3.00
25	600	616	640	17.25	23.25	23.75	3.00	2.75	3.50
26	628	604	576	17.75	20.75	23.25	3.75	3.50	3.25
27	592	588	620	17.50	25.75	21.75	2.75	2.75	3.00
28	656	596	568	18.00	28.75	26.50	3.00	3.00	3.25
29	572	620	548	16.00	24.00	25.50	3.25	3.50	3.00
30	580	640	580	17.50	25.75	26.25	3.75	2.75	3.00
Mean	**602**	**603**	**581**	**18.74**	**26.86**	**29.17**	**3.02**	**3.10**	**3.18**
SD	**32.47**	**35.85**	**38.21**	**1.96**	**2.96**	**3.24**	**0.45**	**0.33**	**0.33**
Range	**560-680**	**536-680**	**520-684**	**15.75-23.50**	**20.75-33.25**	**21.75-35.25**	**2.25-3.75**	**2.50-3.75**	**2.50-3.75**

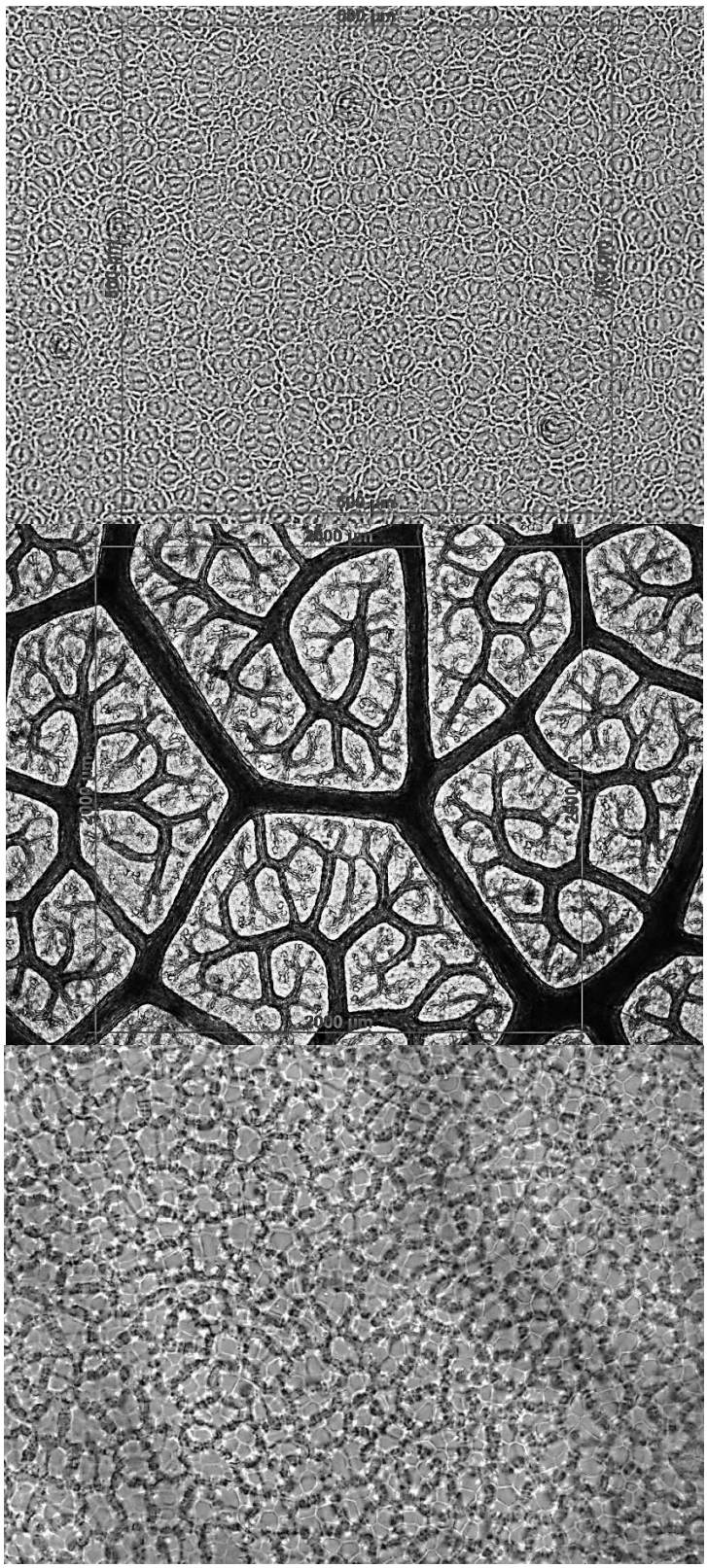


Figure 33 Images of *Mangifera indica* 'Kaemdaeng' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 28 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Kaemdaeng'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	796	964	960	38.00	48.25	51.75	3.75	3.00	2.75
2	880	980	904	35.25	43.25	47.25	2.75	3.00	2.50
3	888	1008	952	30.00	44.00	53.00	3.50	3.25	3.25
4	836	952	860	35.75	47.50	54.00	3.50	3.00	3.50
5	864	956	996	34.00	52.50	51.00	3.25	3.50	3.50
6	840	804	908	31.25	40.75	53.25	3.00	2.75	3.00
7	836	920	820	31.75	45.00	55.50	3.75	3.25	3.25
8	884	1008	876	29.75	44.50	52.75	3.00	3.25	3.50
9	828	988	956	30.75	39.25	52.00	3.50	3.00	3.25
10	848	1008	904	33.00	41.75	51.50	3.50	3.00	3.50
11	844	1020	880	33.50	49.00	53.50	3.00	3.75	3.25
12	832	952	840	37.25	42.50	54.00	3.75	3.50	2.75
13	808	960	976	29.00	39.50	54.75	3.25	3.75	3.00
14	848	1004	944	32.25	47.25	47.75	3.50	2.75	3.00
15	840	1016	792	30.25	41.50	55.00	2.75	3.00	2.75
16	764	936	896	35.00	43.25	50.25	3.25	2.75	3.25
17	868	924	832	29.00	47.50	52.50	3.25	2.50	3.00
18	792	856	956	35.75	50.00	56.00	3.75	3.00	3.25
19	828	920	884	38.00	50.50	51.50	3.25	2.75	3.00
20	780	988	900	37.50	41.75	52.00	2.75	3.25	3.50
21	792	944	996	33.25	49.25	54.00	3.75	3.00	3.25
22	788	972	888	35.00	42.50	48.00	3.00	2.75	3.25
23	880	952	908	34.25	39.50	49.25	3.75	3.00	3.25
24	888	988	936	33.00	42.75	50.25	3.00	2.75	2.75
25	872	1020	1000	35.00	43.75	49.00	2.75	3.25	2.75
26	892	944	904	33.50	43.25	47.75	3.50	2.75	3.25
27	880	948	932	37.00	48.75	49.75	2.75	2.75	2.50
28	860	916	880	33.25	45.00	52.25	3.00	3.00	2.50
29	848	860	940	34.50	38.50	50.00	3.25	3.25	2.75
30	856	920	896	35.00	48.50	49.00	3.75	3.00	3.25
Mean	**842**	**954**	**911**	**33.69**	**44.71**	**51.62**	**3.28**	**3.05**	**3.08**
SD	**35.82**	**51.10**	**52.25**	**2.63**	**3.78**	**2.46**	**0.36**	**0.30**	**0.32**
Range	**764-892**	**804-1020**	**792-1000**	**29.00-38.00**	**38.50-52.50**	**47.25-56.00**	**2.75-3.75**	**2.50-3.75**	**2.50-3.50**

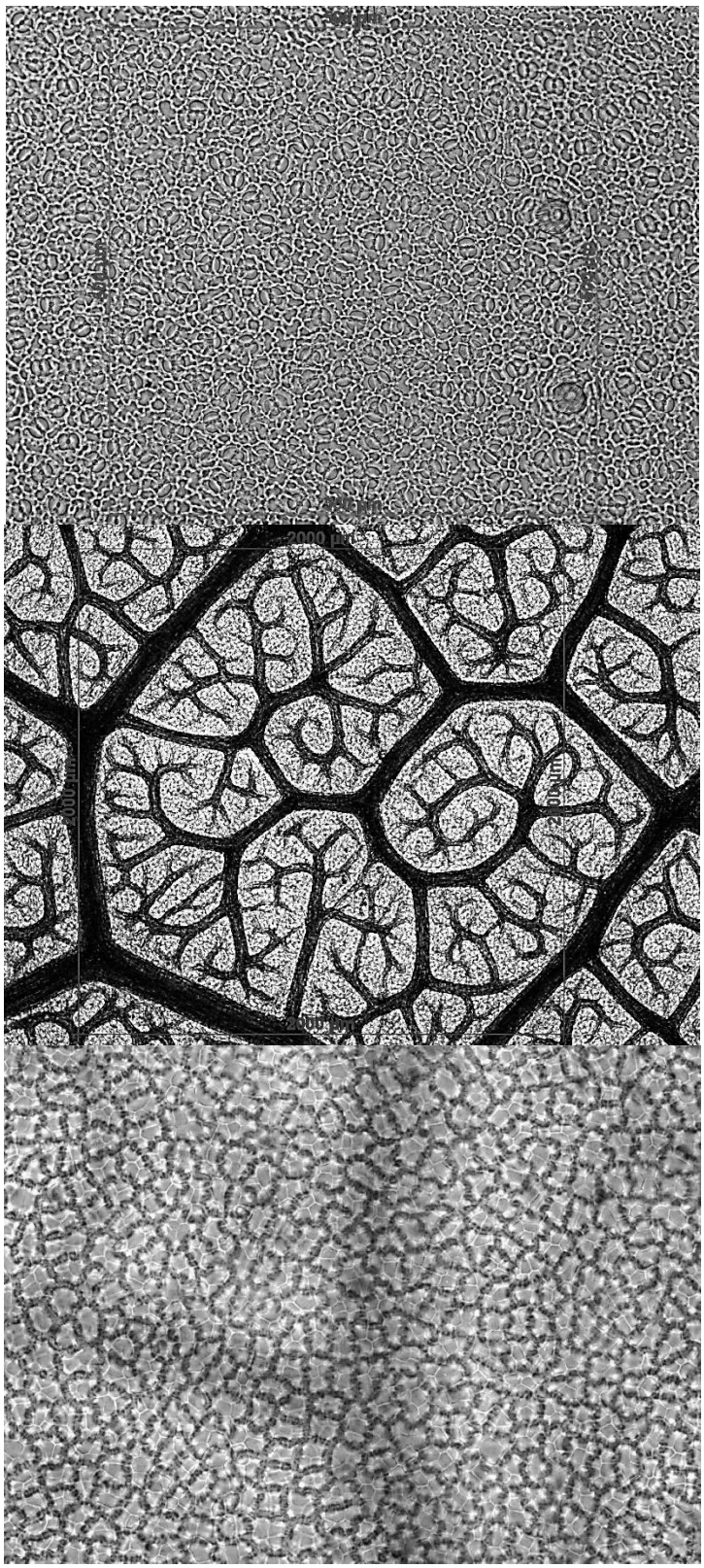


Figure 34 Images of *Mangifera indica* 'Okrong' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 29 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Okrong'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	588	596	788	51.00	35.25	45.00	3.00	3.00	2.75
2	592	588	712	50.25	31.75	44.00	3.25	3.50	3.50
3	588	584	760	45.50	32.75	40.50	2.25	3.00	3.50
4	596	588	780	37.25	30.00	43.25	3.00	3.25	3.25
5	572	580	648	40.00	37.25	46.25	2.50	3.00	3.50
6	596	572	832	45.50	32.75	42.00	3.25	2.75	4.00
7	588	580	840	46.00	33.00	40.75	3.50	2.75	3.75
8	520	480	752	40.75	32.00	44.25	3.00	2.50	3.25
9	556	552	836	39.25	28.50	38.00	3.50	3.00	4.00
10	528	536	888	42.75	29.25	41.75	3.00	3.00	3.25
11	532	564	868	41.25	31.50	35.50	2.75	2.75	3.25
12	540	536	908	43.25	29.75	39.00	2.50	2.50	4.00
13	544	560	936	35.75	34.50	44.50	2.50	2.75	3.75
14	544	504	920	35.25	29.25	41.00	3.00	2.50	3.25
15	488	516	900	41.50	31.50	37.50	2.75	2.75	3.00
16	520	556	944	39.50	33.00	36.50	2.75	3.25	4.25
17	508	540	912	40.00	29.75	36.25	2.50	3.00	3.50
18	500	516	924	37.50	36.00	40.00	3.00	3.00	3.25
19	548	564	1008	39.25	31.50	45.75	2.75	2.75	3.50
20	524	552	1032	35.75	32.50	39.75	2.75	2.50	4.00
21	548	540	788	36.00	32.75	45.50	3.00	3.00	3.75
22	548	564	896	38.50	35.25	38.50	3.50	3.25	3.50
23	536	552	932	34.50	31.75	39.50	3.25	3.25	3.50
24	564	568	848	36.50	30.25	38.25	3.00	3.25	3.75
25	560	536	904	36.00	30.75	35.00	2.75	3.00	3.25
26	568	552	884	38.50	39.75	35.50	3.00	2.75	3.75
27	556	500	892	38.25	38.75	38.25	3.00	3.50	3.25
28	548	576	936	37.00	34.50	37.50	2.75	2.50	4.00
29	592	556	996	39.25	32.00	41.25	3.00	2.75	3.75
30	576	548	936	32.50	32.50	41.75	2.75	3.00	3.50
Mean	**552**	**552**	**873**	**39.81**	**32.67**	**40.42**	**2.92**	**2.93**	**3.55**
SD	**29.69**	**28.00**	**87.37**	**4.42**	**2.76**	**3.31**	**0.31**	**0.29**	**0.34**
Range	**488-596**	**480-596**	**648-1032**	**32.50-51.00**	**28.50-39.75**	**35.00-46.25**	**2.25-3.50**	**2.50-3.50**	**2.75-4.25**

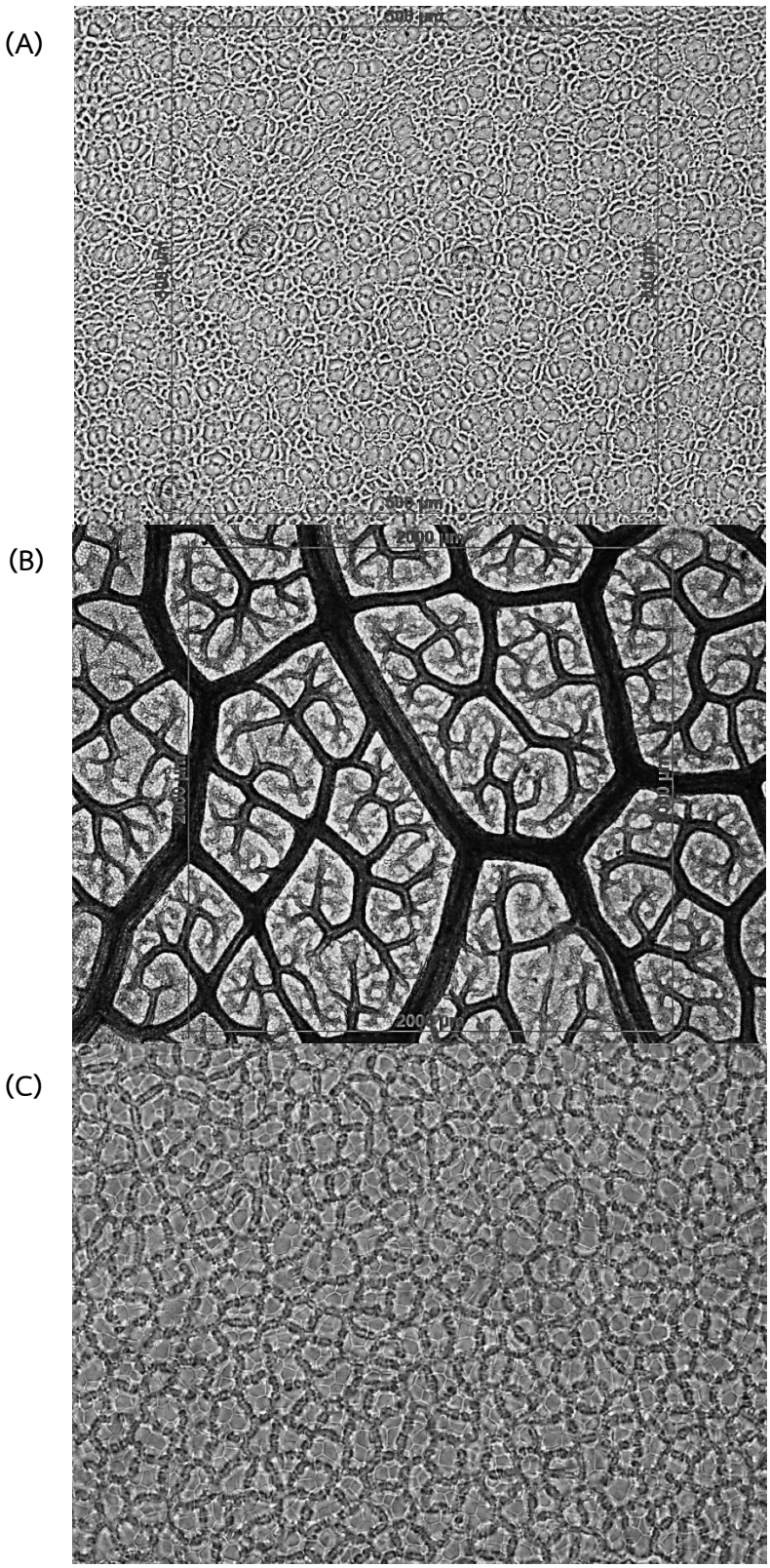


Figure 35 Images of *Mangifera indica* 'Chok Anan' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 30 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Chok Anan'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	752	744	768	50.50	45.75	50.75	2.50	3.25	3.25
2	808	784	748	45.00	39.25	60.25	3.00	3.75	3.25
3	768	772	692	44.75	36.25	56.25	2.75	3.00	2.75
4	728	688	632	47.00	38.75	52.25	3.25	3.75	3.25
5	732	644	740	45.25	37.25	47.00	3.00	3.00	3.00
6	704	736	696	42.00	40.25	48.25	3.00	3.00	3.00
7	640	760	672	42.25	37.25	45.75	3.25	3.00	3.00
8	736	708	656	50.00	40.75	44.25	2.50	3.25	3.50
9	756	608	712	54.25	38.50	48.75	3.00	3.00	3.25
10	748	764	748	42.50	39.75	51.75	2.75	2.50	3.00
11	712	772	676	46.50	42.50	45.50	2.75	2.75	2.75
12	708	708	772	48.75	42.25	42.50	3.25	3.25	3.00
13	668	740	748	52.50	42.00	44.50	3.25	3.00	2.75
14	696	604	744	48.75	38.00	40.75	2.75	3.50	3.00
15	720	744	788	41.75	45.50	37.25	3.25	2.75	3.00
16	680	732	700	48.25	47.25	42.75	3.50	3.25	3.50
17	684	680	728	51.75	45.25	42.25	3.25	2.75	3.25
18	736	676	656	51.25	44.00	43.50	3.00	3.00	2.75
19	652	740	644	44.50	45.00	42.50	3.50	3.00	3.00
20	764	676	608	44.25	41.75	39.75	2.75	3.25	3.00
21	760	760	724	50.00	43.00	42.50	3.25	3.00	3.00
22	736	756	772	53.50	45.00	40.25	3.25	3.00	3.00
23	760	644	668	50.50	42.50	41.00	3.00	2.75	3.25
24	768	620	580	51.25	41.00	46.00	2.75	2.75	3.50
25	688	768	644	43.50	45.50	43.25	3.00	3.50	3.50
26	656	748	760	50.25	47.75	50.00	3.75	2.75	3.25
27	624	760	712	48.50	42.00	44.00	3.00	2.75	3.00
28	696	668	676	48.75	43.75	39.00	3.25	3.50	3.50
29	668	688	660	51.00	44.50	42.00	2.75	2.75	3.00
30	764	664	740	48.50	41.75	41.00	3.00	3.00	3.25
Mean	**717**	**712**	**702**	**47.92**	**42.13**	**45.18**	**3.04**	**3.06**	**3.12**
SD	**44.92**	**53.24**	**53.23**	**3.60**	**3.09**	**5.21**	**0.29**	**0.31**	**0.23**
Range	**624-808**	**604-784**	**580-788**	**41.75-54.25**	**36.25-47.75**	**37.25-60.25**	**2.50-3.75**	**2.50-3.75**	**2.75-3.50**

(A)

(B)

(C)

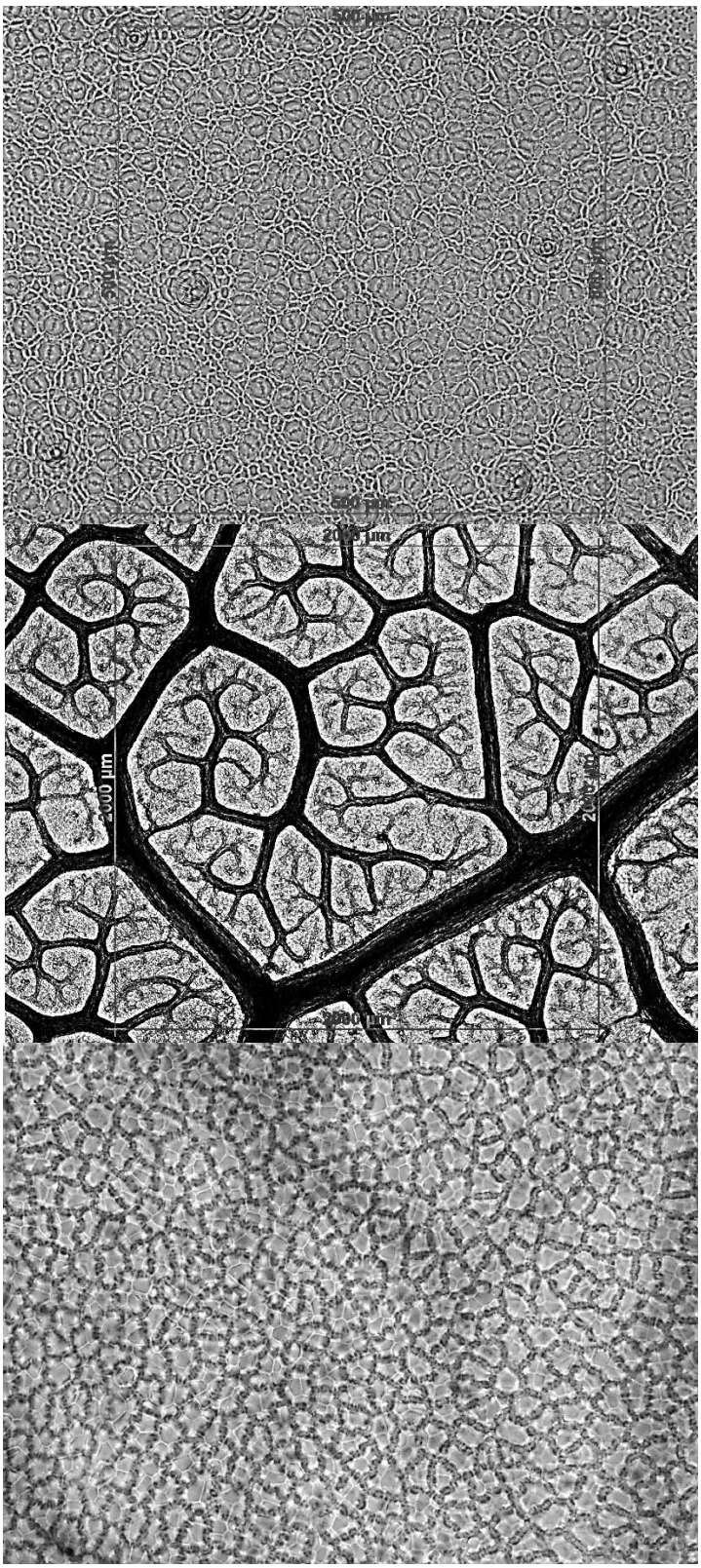


Figure 36 Images of *Mangifera indica* 'Raet' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 31 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Raet'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	960	912	968	46.75	37.25	46.75	2.75	3.50	2.50
2	944	1000	992	44.75	46.75	50.75	2.75	2.50	2.75
3	956	908	1004	44.25	45.75	48.50	2.50	3.00	3.00
4	968	964	968	43.50	43.50	39.75	3.75	3.00	3.00
5	904	920	932	43.00	50.50	46.50	2.50	3.00	3.25
6	1020	1028	988	45.50	43.25	47.50	3.00	2.75	3.00
7	940	1012	980	52.50	44.00	43.50	3.50	2.50	2.50
8	880	896	888	46.25	40.25	48.75	3.00	3.25	2.75
9	1016	956	1008	41.25	46.50	43.75	3.00	3.00	3.25
10	964	972	936	41.50	43.75	44.25	2.75	3.50	3.00
11	916	1024	992	39.00	40.25	47.50	3.25	3.00	3.00
12	952	1012	980	38.25	36.75	48.75	3.00	2.75	2.75
13	900	952	888	42.25	40.50	42.50	2.50	3.00	3.25
14	1024	968	1028	38.00	37.25	47.00	2.75	2.75	2.75
15	1020	1008	832	35.00	41.50	43.50	3.25	3.00	3.00
16	932	904	976	45.75	43.50	38.25	2.50	2.75	3.00
17	956	944	924	41.50	43.25	42.00	3.00	2.50	2.75
18	1076	976	880	48.50	43.75	35.50	3.25	3.00	3.25
19	1048	876	932	41.75	32.50	39.50	2.75	2.75	2.50
20	988	996	868	40.75	44.25	38.50	2.75	2.75	2.75
21	964	1004	1012	41.75	41.50	43.75	3.25	3.00	3.00
22	928	956	928	39.75	38.50	47.50	3.00	2.75	3.00
23	932	964	908	36.00	40.00	39.50	2.50	2.75	3.50
24	944	856	976	52.00	41.00	35.75	2.50	2.75	2.50
25	1012	848	892	53.00	48.75	44.25	3.75	3.25	2.75
26	932	876	1052	37.75	42.75	39.50	3.00	2.75	3.00
27	996	996	868	46.50	37.00	50.75	3.00	3.00	2.75
28	948	984	964	43.50	46.25	42.25	3.75	3.25	2.75
29	1020	892	868	39.25	47.25	46.75	2.50	2.75	2.75
30	880	948	1008	49.50	45.00	36.75	3.00	2.50	3.00
Mean	**964**	**952**	**948**	**43.30**	**42.43**	**43.66**	**2.96**	**2.90**	**2.90**
SD	**49.00**	**51.87**	**56.52**	**4.70**	**3.99**	**4.41**	**0.38**	**0.27**	**0.25**
Range	**880-1076**	**848-1028**	**832-1052**	**35.00-53.00**	**32.50-50.50**	**35.50-50.75**	**2.50-3.75**	**2.50-3.50**	**2.50-3.50**

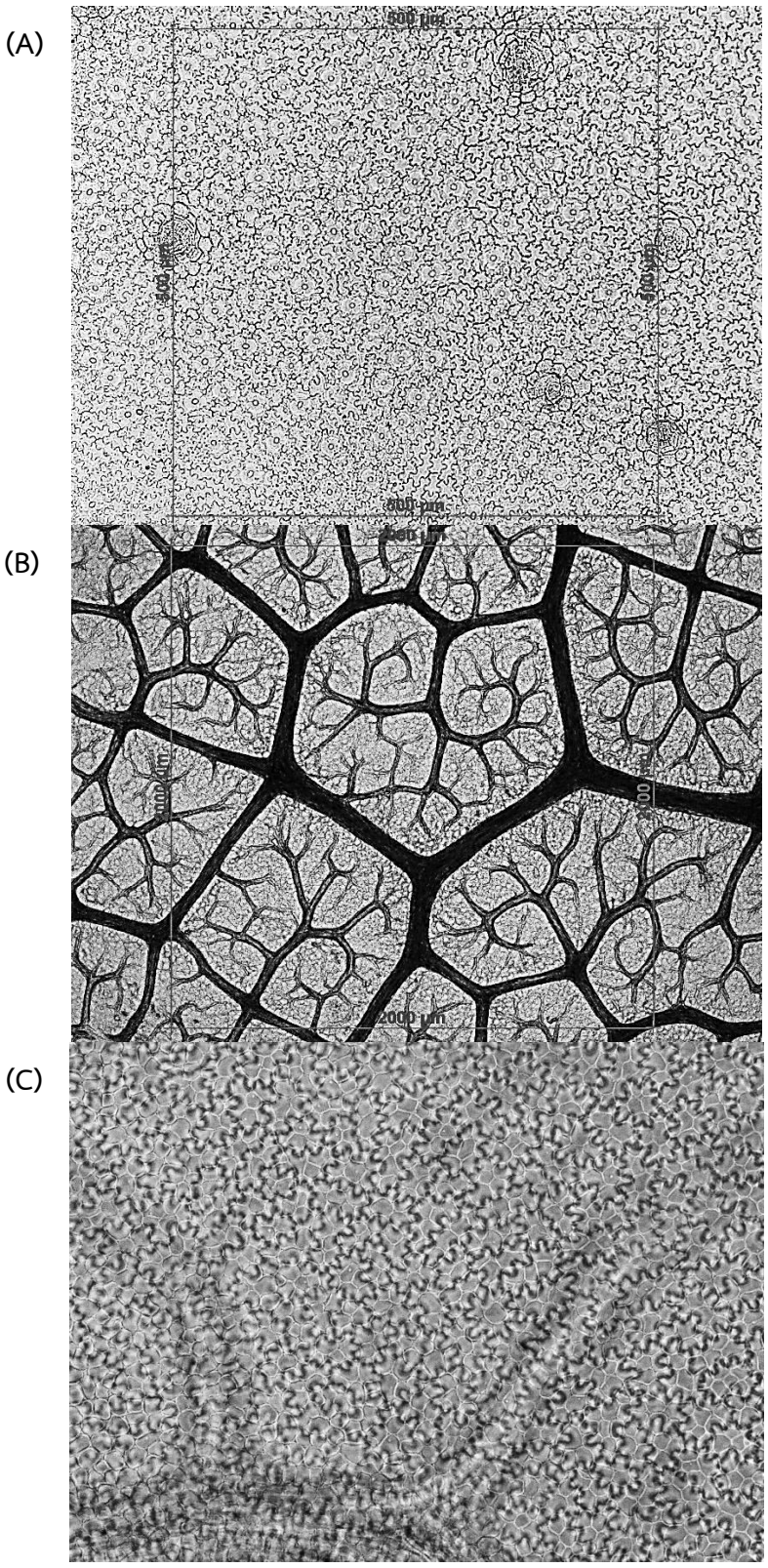


Figure 37 Images of *Mangifera indica* 'Talapnak' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 32 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Talapnak'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	452	564	676	46.00	39.50	43.25	4.75	2.75	3.75
2	440	616	664	43.00	38.75	45.75	4.50	3.00	4.25
3	416	624	640	36.50	34.00	40.50	4.50	3.75	3.25
4	440	648	616	37.00	37.25	46.25	4.50	3.00	3.50
5	412	640	664	41.50	36.75	47.50	4.00	3.00	3.50
6	416	604	672	35.75	37.50	41.25	5.00	3.25	3.25
7	404	636	632	32.00	31.25	38.50	4.00	3.25	3.75
8	432	624	596	40.50	34.50	42.00	3.75	3.75	3.75
9	392	608	568	38.25	36.00	39.75	4.25	3.50	3.25
10	404	608	684	36.50	37.25	45.50	4.25	2.75	3.25
11	444	600	596	34.75	32.25	48.00	4.50	3.50	3.50
12	412	668	572	41.50	39.00	46.75	4.00	2.75	3.50
13	460	652	564	41.00	38.50	46.25	4.00	3.50	3.75
14	444	632	552	39.50	41.00	47.25	4.75	3.00	3.50
15	448	608	580	34.75	37.75	48.50	3.75	3.00	3.50
16	440	616	588	35.75	35.50	47.75	4.25	3.50	3.50
17	444	640	540	32.50	38.00	44.50	4.25	3.50	3.25
18	412	612	624	33.75	41.25	41.75	4.00	3.00	3.50
19	424	620	568	35.00	37.50	43.00	3.50	3.25	4.00
20	452	572	600	33.50	41.00	42.50	4.00	3.00	3.25
21	428	632	596	38.25	34.75	43.25	4.00	3.00	3.25
22	408	616	564	36.50	40.00	42.50	4.25	3.25	3.50
23	412	568	584	33.25	41.50	45.25	4.00	3.25	3.75
24	440	616	600	35.00	38.50	43.00	3.75	3.75	3.25
25	444	608	572	33.50	41.00	41.75	4.00	2.75	3.50
26	452	620	604	35.25	40.00	44.25	4.50	3.50	3.25
27	436	632	536	30.25	30.25	50.00	4.25	3.25	3.75
28	400	672	584	40.50	41.00	40.75	4.50	3.00	4.00
29	416	640	604	37.00	32.50	39.50	4.75	3.25	3.25
30	456	612	560	32.50	35.50	37.25	4.25	3.50	3.50
Mean	**429**	**620**	**600**	**36.70**	**37.32**	**43.80**	**4.23**	**3.22**	**3.53**
SD	**19.18**	**25.12**	**40.94**	**3.67**	**3.13**	**3.21**	**0.35**	**0.31**	**0.27**
Range	**392-460**	**564-672**	**536-684**	**30.25-46.00**	**30.25-41.50**	**37.25-50.00**	**3.50-5.00**	**2.75-3.75**	**3.25-4.25**

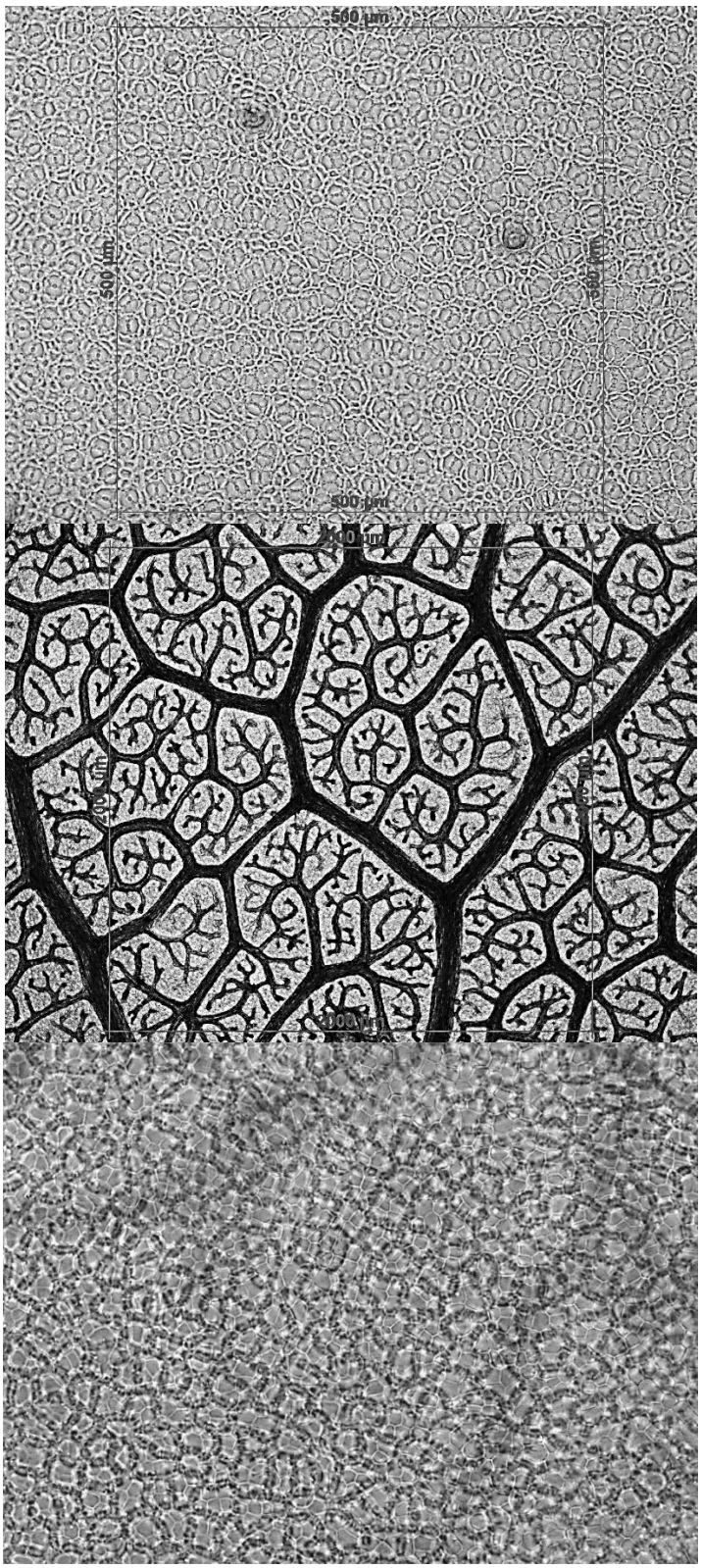


Figure 38 Images of *Mangifera indica* 'Kaeo' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 33 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Kaeo'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	628	940	876	30.25	46.50	43.50	2.75	3.25	3.75
2	632	832	880	35.75	48.50	46.00	3.25	2.75	3.00
3	672	880	844	28.50	50.75	46.75	3.50	2.75	2.75
4	636	960	852	26.75	48.25	42.00	2.75	3.25	3.50
5	616	916	888	34.50	48.75	41.50	3.25	3.00	3.75
6	648	896	796	32.75	46.75	45.00	3.25	3.50	4.00
7	644	944	836	28.75	51.50	42.50	2.50	3.25	2.75
8	632	908	900	28.50	46.50	50.75	2.75	3.25	3.00
9	656	944	816	29.00	55.75	46.75	3.25	3.00	2.75
10	676	920	856	30.00	49.50	45.50	3.50	3.00	3.75
11	616	964	816	28.50	48.75	54.75	3.25	3.25	3.50
12	668	848	868	29.25	44.50	57.75	3.25	2.75	3.25
13	644	888	884	29.00	47.75	50.00	3.25	3.00	3.75
14	644	924	800	29.50	52.75	53.25	3.00	2.50	3.00
15	668	948	900	28.75	48.75	56.25	3.25	2.75	3.75
16	664	920	840	32.50	48.00	49.75	3.00	3.25	3.25
17	608	924	808	31.50	49.00	57.75	3.00	2.75	3.75
18	612	956	852	31.75	59.25	55.25	3.25	2.75	3.00
19	608	884	748	30.00	51.75	58.50	3.25	3.50	2.75
20	628	1004	888	31.00	60.00	54.00	3.75	3.25	3.50
21	604	884	880	33.75	58.50	52.25	3.00	3.25	3.75
22	620	984	820	35.00	55.25	51.25	2.75	2.75	3.75
23	676	944	844	34.25	49.50	51.50	2.75	2.50	3.25
24	704	896	816	33.50	59.75	60.00	3.25	2.75	3.00
25	632	980	892	33.25	57.50	54.75	3.25	3.00	3.25
26	676	1016	792	31.75	47.50	50.75	3.50	2.50	3.00
27	620	880	820	35.25	58.00	55.50	2.75	2.75	3.25
28	616	944	852	36.25	48.50	56.75	3.25	3.00	3.00
29	612	824	924	35.00	45.00	54.25	3.00	3.25	2.75
30	676	956	772	31.50	44.50	52.50	3.00	3.25	3.50
Mean	**641**	**924**	**845**	**31.53**	**50.91**	**51.23**	**3.12**	**2.99**	**3.30**
SD	**26.72**	**46.79**	**42.00**	**2.65**	**4.81**	**5.29**	**0.28**	**0.29**	**0.39**
Range	**604-704**	**824-1016**	**748-924**	**26.75-36.25**	**44.50-60.00**	**41.50-60.00**	**2.50-3.75**	**2.50-3.50**	**2.75-4.00**

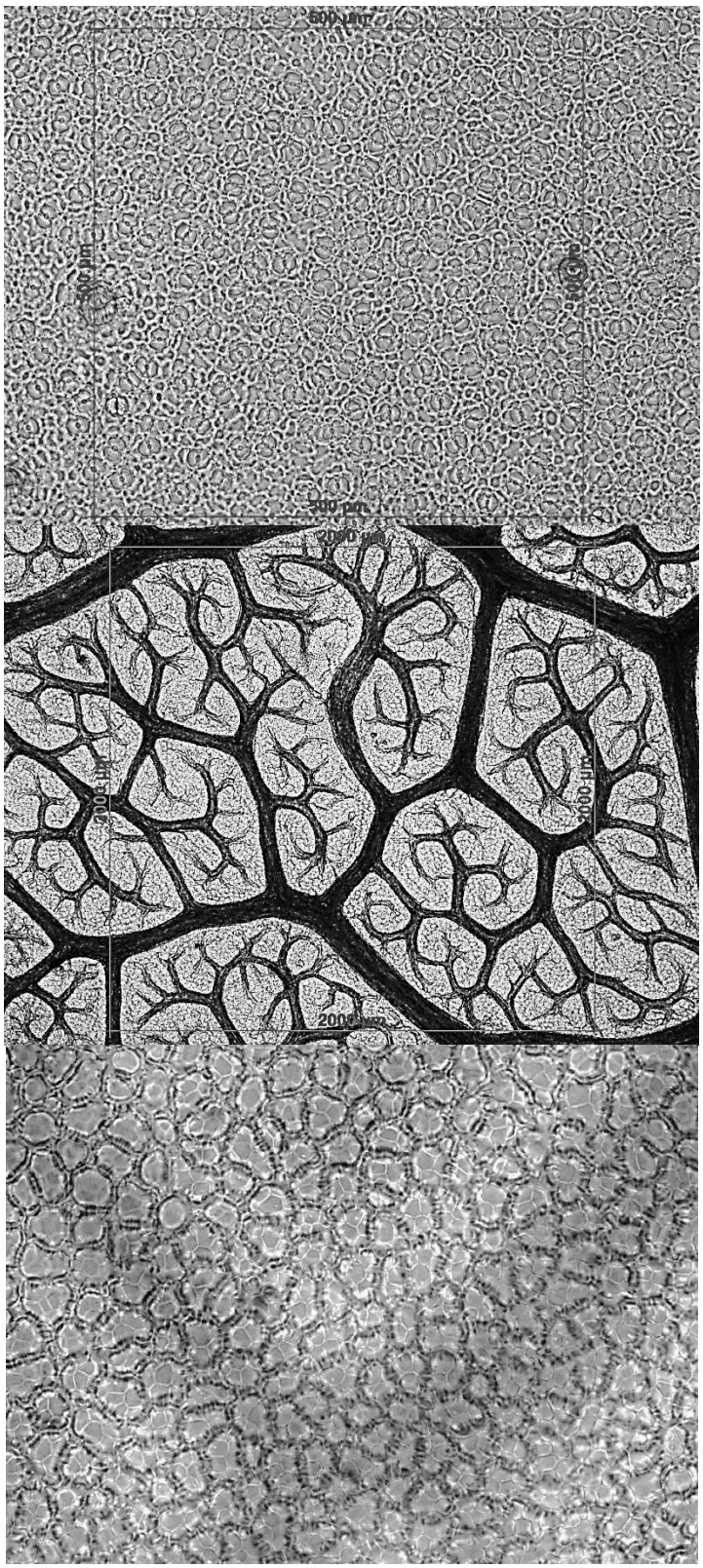


Figure 39 Images of *Mangifera indica* 'Tongdam' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 34 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Tongdam'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	672	524	576	37.50	38.75	53.75	3.50	3.50	3.50
2	680	584	596	38.25	37.25	51.25	3.25	3.00	4.00
3	672	576	580	39.75	33.50	50.75	3.25	2.50	3.75
4	636	556	600	36.50	31.75	50.00	3.75	3.25	3.50
5	700	596	588	39.50	31.50	52.00	2.75	3.00	3.50
6	684	512	612	39.25	34.75	53.50	3.00	3.00	3.75
7	648	508	592	40.00	34.00	61.00	4.00	3.25	3.50
8	704	572	552	37.50	32.50	56.00	3.25	2.75	3.75
9	704	584	532	38.50	29.00	53.25	3.50	3.00	4.00
10	672	504	544	35.00	27.75	46.00	3.25	3.75	3.25
11	672	480	572	36.75	30.00	49.25	3.50	3.25	3.00
12	656	552	560	34.50	32.75	53.25	3.50	3.25	3.50
13	672	540	596	38.50	33.75	56.75	4.00	3.00	3.50
14	688	572	588	37.50	29.25	57.50	3.00	3.00	3.25
15	680	584	580	36.50	31.50	50.00	3.50	3.00	4.25
16	664	588	528	33.50	39.75	53.25	3.50	3.50	3.75
17	632	616	576	32.75	36.75	51.75	3.00	3.00	4.00
18	656	600	584	33.75	32.75	46.25	3.25	3.50	3.75
19	692	532	556	35.75	36.50	50.25	3.50	3.50	3.50
20	680	564	592	32.75	34.25	54.25	3.50	3.75	3.50
21	656	568	548	32.50	36.00	52.25	3.25	3.25	4.00
22	696	552	596	34.75	36.75	48.50	3.50	3.50	3.75
23	668	532	584	41.25	34.50	44.75	3.25	3.50	3.50
24	648	592	564	36.25	39.25	53.25	3.75	3.00	3.75
25	664	600	596	38.75	36.00	49.50	3.50	3.00	3.25
26	720	592	532	37.25	32.25	62.00	3.00	3.50	4.00
27	672	532	524	36.00	38.75	55.75	3.25	3.25	3.25
28	696	560	548	34.25	30.25	55.00	3.00	3.00	3.25
29	628	548	592	35.50	36.50	60.25	3.00	2.75	3.50
30	672	580	572	33.75	37.75	59.25	2.75	3.00	3.50
Mean	**673**	**560**	**572**	**36.48**	**34.20**	**53.02**	**3.33**	**3.18**	**3.61**
SD	**22.09**	**33.27**	**24.27**	**2.39**	**3.26**	**4.32**	**0.32**	**0.30**	**0.29**
Range	**628-720**	**480-616**	**524-612**	**32.50-41.25**	**27.75-39.75**	**44.75-62.00**	**2.75-4.00**	**2.50-3.75**	**3.00-4.25**

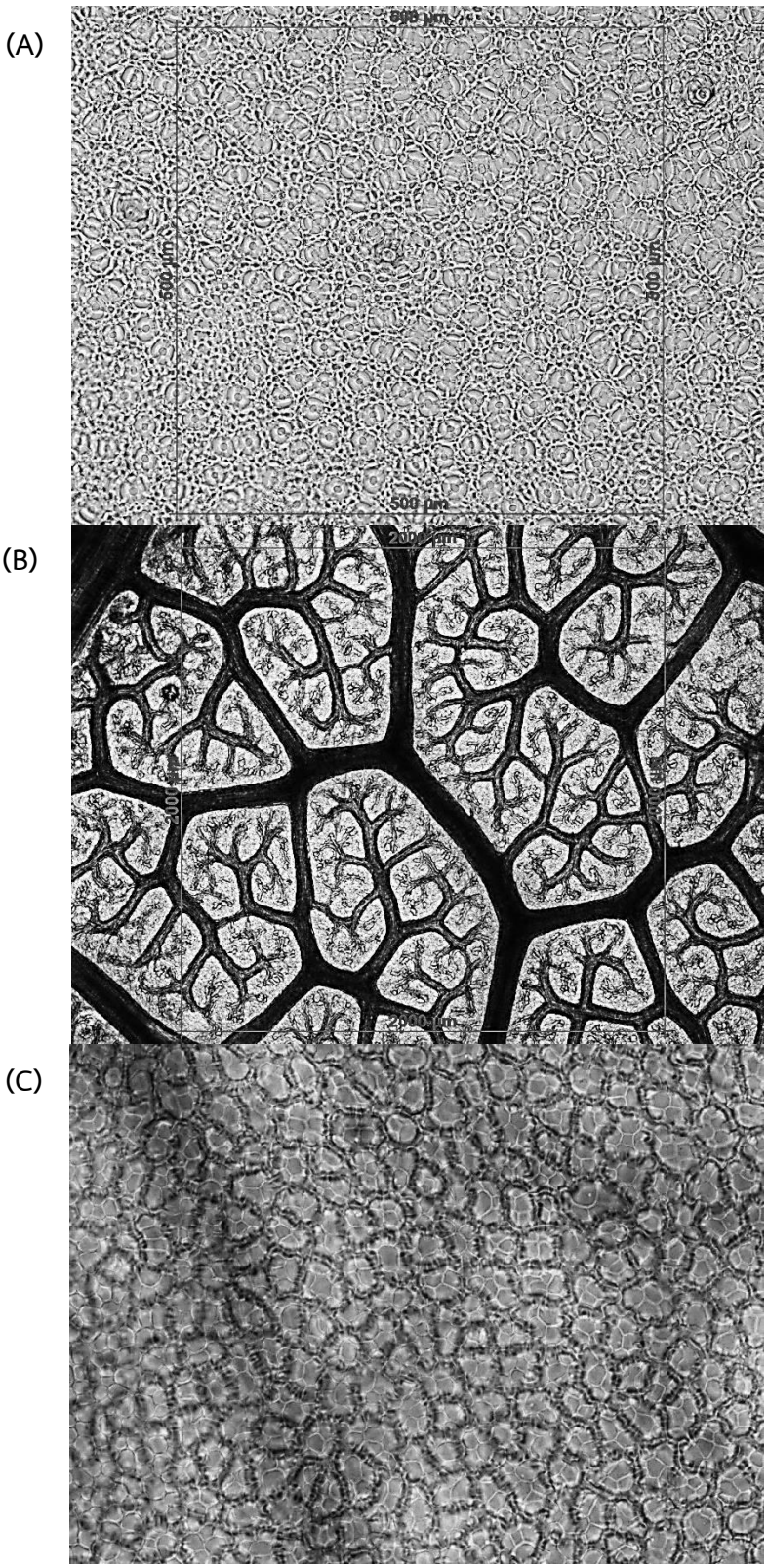


Figure 40 Images of *Mangifera indica* 'Khiaosawoey' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 35 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Khiaosawoey'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	624	712	628	36.50	30.25	37.50	3.50	3.50	3.75
2	640	700	652	37.75	30.75	30.00	3.50	2.75	3.00
3	668	576	644	38.00	32.00	34.75	3.75	3.25	3.75
4	664	584	620	36.25	31.75	37.75	3.25	2.50	3.25
5	624	636	672	34.25	30.00	32.00	3.75	2.50	4.00
6	640	628	580	36.25	28.75	38.75	3.25	3.25	2.75
7	604	580	636	32.75	26.00	33.50	3.75	3.75	3.25
8	656	708	624	38.25	29.50	32.75	3.00	3.50	3.50
9	664	640	612	37.50	30.25	35.75	3.00	2.75	3.00
10	676	716	668	35.00	36.50	36.75	3.25	2.50	3.50
11	636	692	652	36.75	29.00	32.00	3.25	3.25	4.00
12	628	640	720	34.75	30.25	32.75	3.50	2.75	3.75
13	704	680	620	33.25	32.75	35.00	3.00	3.25	2.75
14	640	672	648	33.00	31.00	34.00	3.75	2.75	3.25
15	616	696	640	34.50	35.00	36.50	3.00	3.00	4.00
16	608	572	656	34.00	36.00	33.50	3.75	3.50	4.00
17	600	652	672	35.00	31.00	34.50	3.50	3.25	3.00
18	676	716	612	36.50	27.75	37.75	3.00	3.50	2.75
19	600	684	636	36.25	33.25	37.25	3.50	3.00	3.50
20	624	620	684	40.25	34.00	34.25	3.00	2.50	3.75
21	672	620	668	36.50	29.25	33.50	3.00	2.75	3.25
22	660	568	688	39.50	33.75	35.50	3.25	2.50	4.00
23	684	608	628	38.50	28.00	31.00	3.25	2.50	3.00
24	628	584	668	32.75	31.00	34.75	3.00	2.50	4.25
25	684	668	600	37.75	28.50	33.75	3.00	3.00	3.00
26	644	620	660	41.25	30.25	31.25	3.25	2.50	4.00
27	664	648	584	36.25	27.75	34.00	3.25	2.75	3.50
28	608	600	688	37.00	29.50	31.75	3.75	3.00	3.25
29	604	624	640	36.00	27.25	35.50	3.25	3.25	3.50
30	624	616	652	34.25	31.00	34.50	3.50	3.25	3.25
Mean	**642**	**642**	**645**	**36.22**	**30.73**	**34.42**	**3.33**	**2.97**	**3.45**
SD	**28.99**	**47.28**	**31.63**	**2.17**	**2.56**	**2.21**	**0.28**	**0.39**	**0.44**
Range	**600-704**	**568-716**	**580-720**	**32.75-41.25**	**26.00-36.50**	**30.00-38.75**	**3.00-3.75**	**2.50-3.75**	**2.75-4.25**

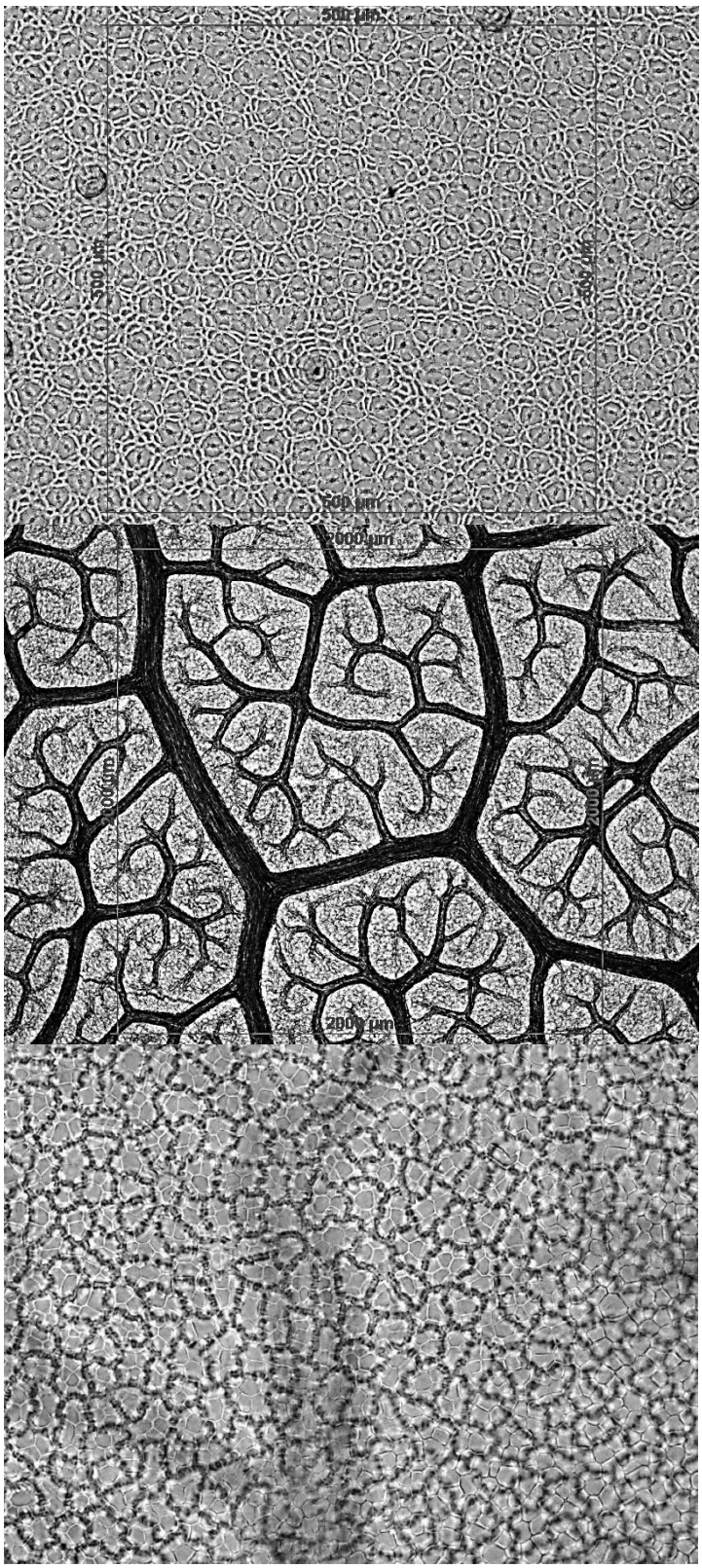


Figure 41 Images of *Mangifera indica* 'Falan' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 36 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Falan'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	924	888	856	39.00	29.50	36.75	3.50	4.25	2.75
2	916	816	908	40.50	32.25	37.25	4.00	4.25	3.25
3	824	772	708	37.25	30.75	38.00	3.50	3.25	2.75
4	884	844	836	33.75	29.25	40.25	4.25	3.00	3.50
5	880	860	792	31.50	33.25	39.75	4.25	3.75	3.50
6	860	808	936	34.50	34.00	38.25	3.50	3.75	2.75
7	940	764	904	33.00	38.00	42.00	4.25	3.75	3.25
8	872	796	756	31.50	33.75	38.75	4.00	3.50	3.00
9	920	800	844	33.25	32.50	37.25	3.75	3.00	3.25
10	852	816	868	32.50	33.25	30.50	4.50	4.00	3.00
11	888	764	748	31.75	36.25	32.75	3.75	3.50	3.50
12	864	840	732	33.25	33.50	34.50	3.25	3.75	2.75
13	872	816	788	32.50	34.00	36.00	4.25	3.00	3.50
14	828	784	892	35.00	30.50	35.50	3.50	3.00	3.25
15	912	844	868	34.00	37.25	38.00	4.00	3.25	3.50
16	892	768	888	31.50	26.50	36.25	4.50	3.75	3.00
17	784	776	880	37.25	35.00	41.75	4.25	4.25	3.00
18	804	808	808	36.75	31.75	34.75	3.75	3.50	3.25
19	828	784	944	35.00	32.75	35.25	4.25	3.75	3.50
20	840	872	904	34.25	30.25	31.00	3.50	3.50	3.50
21	808	796	956	35.25	30.00	36.25	3.75	3.00	2.75
22	796	912	816	35.75	32.75	34.50	4.00	3.50	3.75
23	788	892	856	37.00	31.25	29.25	4.00	3.25	3.00
24	840	924	840	30.50	34.25	30.50	3.75	3.00	3.75
25	900	860	868	33.50	28.50	34.50	4.00	4.00	3.25
26	868	912	840	32.00	29.25	29.75	3.50	4.00	3.25
27	920	828	824	34.25	27.00	34.75	4.00	4.25	3.75
28	868	852	864	36.25	28.75	29.00	3.50	3.50	2.75
29	800	776	832	32.75	30.50	33.00	3.25	3.00	3.75
30	768	800	900	35.00	31.25	28.25	4.00	3.00	3.50
Mean	**858**	**826**	**849**	**34.34**	**31.93**	**35.14**	**3.88**	**3.54**	**3.24**
SD	**47.49**	**47.39**	**61.40**	**2.37**	**2.80**	**3.77**	**0.35**	**0.44**	**0.34**
Range	**768-940**	**764-924**	**708-956**	**30.50-40.50**	**26.50-38.00**	**28.25-42.00**	**3.25-4.50**	**3.00-4.25**	**2.75-3.75**

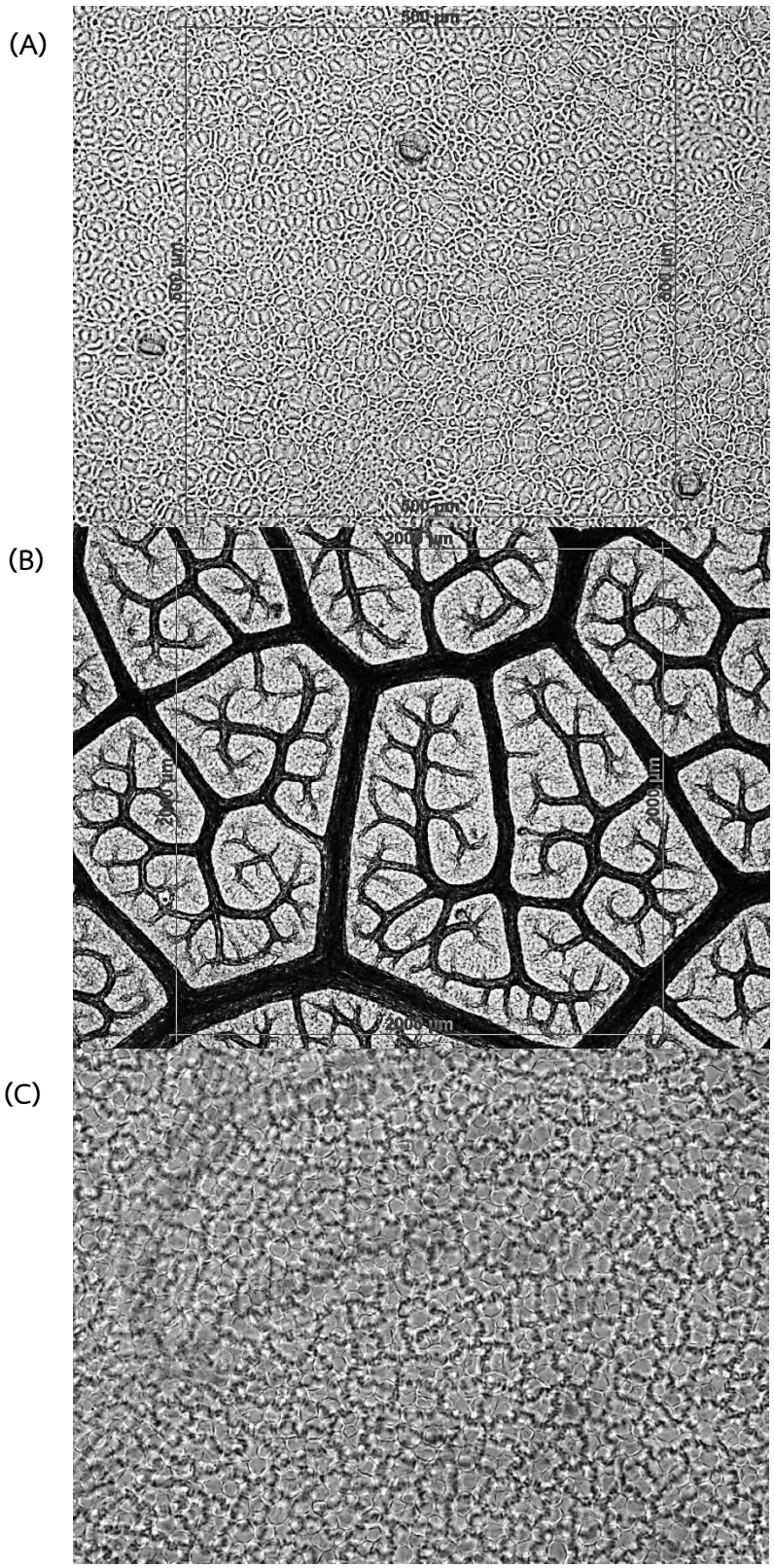


Figure 42 Images of *Mangifera indica* 'Phetbanlat' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 37 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Phetbanlat'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	748	636	668	37.25	38.00	44.00	3.00	3.50	3.25
2	760	648	712	38.00	38.75	42.00	2.75	3.00	3.25
3	768	652	672	38.75	36.50	40.25	2.75	2.75	3.50
4	768	656	608	36.50	34.50	36.00	2.75	3.00	3.00
5	704	620	636	35.75	30.00	40.75	3.00	2.75	4.00
6	752	608	708	32.00	31.50	38.75	3.00	3.00	3.25
7	696	644	676	34.75	30.75	39.75	3.00	3.25	3.25
8	688	612	612	31.75	32.75	35.00	2.50	3.75	3.25
9	764	616	644	31.50	35.25	37.00	3.25	3.00	4.00
10	752	652	676	30.50	36.00	35.25	3.50	2.75	3.50
11	692	612	616	31.00	40.75	36.75	3.25	3.00	3.00
12	684	592	684	32.75	39.50	42.25	3.00	2.50	3.75
13	708	584	716	34.25	34.50	41.00	2.50	3.00	3.50
14	712	680	656	32.25	39.00	35.50	2.75	3.25	3.00
15	724	588	568	34.50	38.50	40.25	3.50	3.50	3.00
16	696	652	652	33.25	37.75	38.00	3.75	3.00	3.50
17	732	640	616	37.00	39.25	40.00	3.25	3.25	3.50
18	764	628	644	36.00	32.25	41.00	2.75	2.75	3.25
19	692	644	632	37.75	42.50	43.50	2.50	2.75	3.25
20	680	636	652	34.50	39.50	41.25	2.75	2.75	3.00
21	740	672	684	36.00	42.00	41.75	3.25	2.75	3.00
22	712	676	664	30.75	36.00	35.00	2.50	3.25	3.50
23	724	580	676	35.00	38.25	40.75	3.00	3.00	3.50
24	676	644	712	34.50	42.75	45.25	2.75	3.50	3.75
25	696	632	612	30.75	39.75	36.50	3.00	3.00	3.75
26	720	648	660	29.75	39.25	43.75	3.00	3.25	3.00
27	776	656	632	29.50	37.50	37.50	3.25	3.75	3.00
28	712	700	660	33.75	41.25	38.75	3.00	3.50	3.00
29	664	684	636	32.75	36.50	37.25	2.75	3.00	3.25
30	704	640	616	29.25	35.00	36.25	3.00	3.50	2.75
Mean	**720**	**638**	**653**	**33.73**	**37.19**	**39.37**	**2.97**	**3.10**	**3.32**
SD	**32.03**	**29.93**	**35.53**	**2.72**	**3.44**	**2.94**	**0.31**	**0.33**	**0.32**
Range	**664-776**	**580-700**	**568-716**	**29.25-38.75**	**30.00-42.75**	**35.00-45.25**	**2.50-3.75**	**2.50-3.75**	**2.75-4.00**

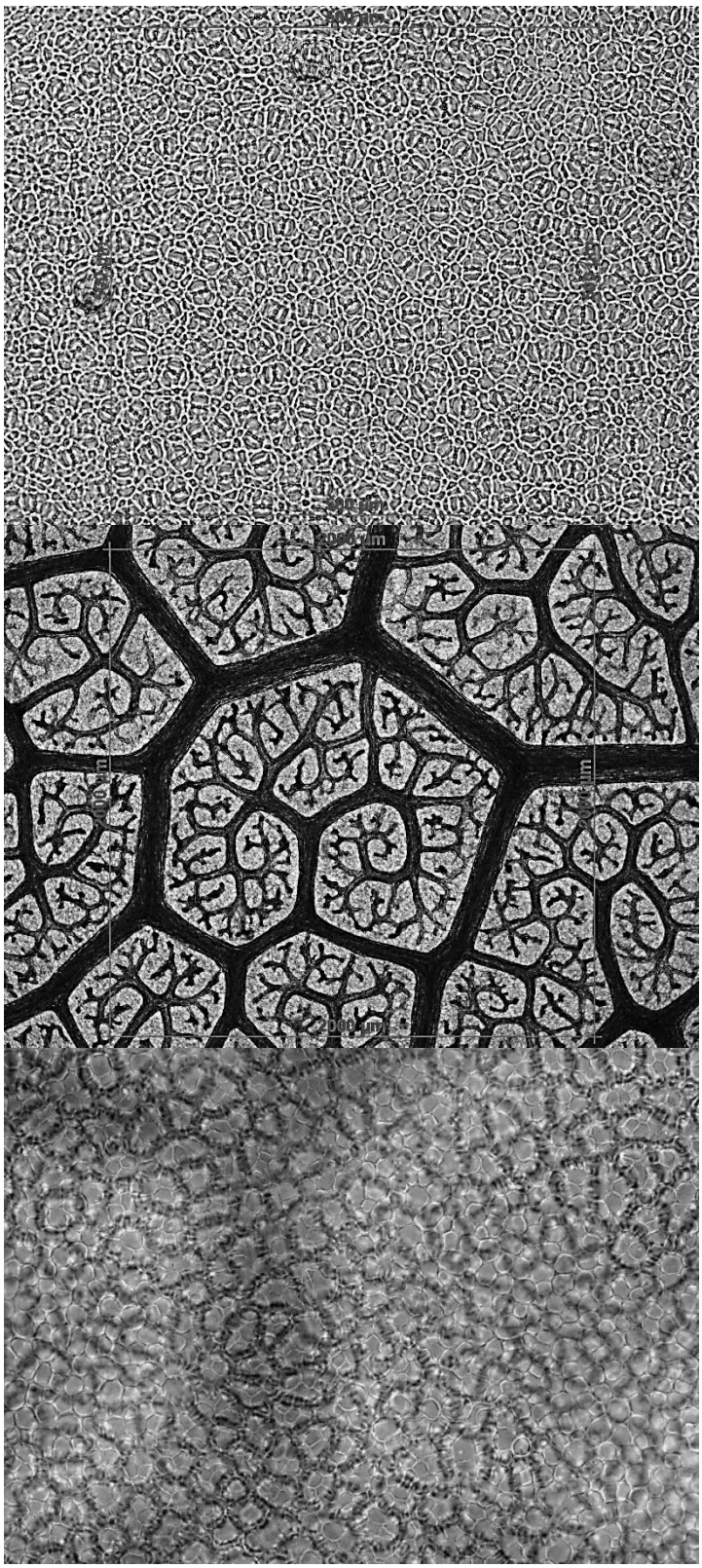


Figure 43 Images of *Mangifera indica* 'Nongsaeng' leaves showing (A) mango stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 38 Stomatal number, veinlet termination number and palisade ratio of *Mangifera indica* 'Nongsaeng'. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	724	816	844	41.75	46.00	36.25	2.75	3.50	3.50
2	688	852	804	41.00	44.50	35.50	3.00	3.25	3.75
3	756	808	912	34.00	42.25	38.50	3.00	3.25	3.25
4	680	796	796	41.25	38.00	42.50	2.50	3.50	3.25
5	660	800	776	36.75	40.00	36.25	3.25	3.50	3.50
6	756	792	708	33.75	42.75	35.00	3.00	3.25	3.25
7	736	852	804	35.00	37.50	38.50	2.50	2.75	3.25
8	744	780	816	31.75	35.25	39.00	3.25	3.00	2.50
9	712	820	756	32.50	41.00	43.75	3.00	3.25	3.00
10	764	736	768	33.00	42.00	41.25	3.25	2.50	3.50
11	716	772	784	31.50	39.25	41.50	2.75	3.00	3.25
12	712	848	852	28.25	42.75	40.00	3.00	2.75	3.00
13	748	820	864	34.75	47.00	45.00	2.75	2.75	3.50
14	724	800	696	36.00	45.00	46.25	2.75	3.00	2.75
15	712	828	800	34.25	46.25	41.50	2.50	3.50	3.50
16	708	856	808	35.75	46.75	46.00	2.50	3.00	4.00
17	680	740	828	34.50	41.25	41.00	2.75	3.25	3.75
18	672	768	728	33.25	43.50	39.25	2.75	2.50	4.00
19	708	848	760	32.75	47.50	42.50	3.00	2.75	3.00
20	712	760	712	35.25	44.75	41.25	2.50	2.50	3.75
21	732	732	752	33.50	42.25	43.75	3.00	3.00	3.50
22	680	804	852	31.50	42.00	45.00	3.25	2.75	3.25
23	740	800	764	33.50	47.50	47.00	3.50	3.00	2.75
24	676	852	760	32.25	49.50	36.25	3.00	2.75	3.75
25	736	748	692	30.00	43.50	44.75	3.25	2.75	4.00
26	728	844	792	31.75	44.00	43.50	3.25	3.00	3.50
27	736	796	756	28.75	42.75	45.75	3.00	3.25	3.75
28	736	868	764	33.75	39.75	45.25	3.25	2.75	2.75
29	752	804	852	32.25	44.75	46.75	2.75	3.00	3.00
30	748	864	848	30.75	41.25	39.00	3.50	3.50	3.50
Mean	**719**	**807**	**788**	**33.83**	**43.02**	**41.59**	**2.95**	**3.02**	**3.37**
SD	**28.51**	**39.82**	**53.75**	**3.22**	**3.26**	**3.63**	**0.30**	**0.31**	**0.40**
Range	**660-764**	**732-868**	**692-912**	**28.25-41.75**	**35.25-49.50**	**35.00-47.00**	**2.50-3.50**	**2.50-3.50**	**2.50-4.00**

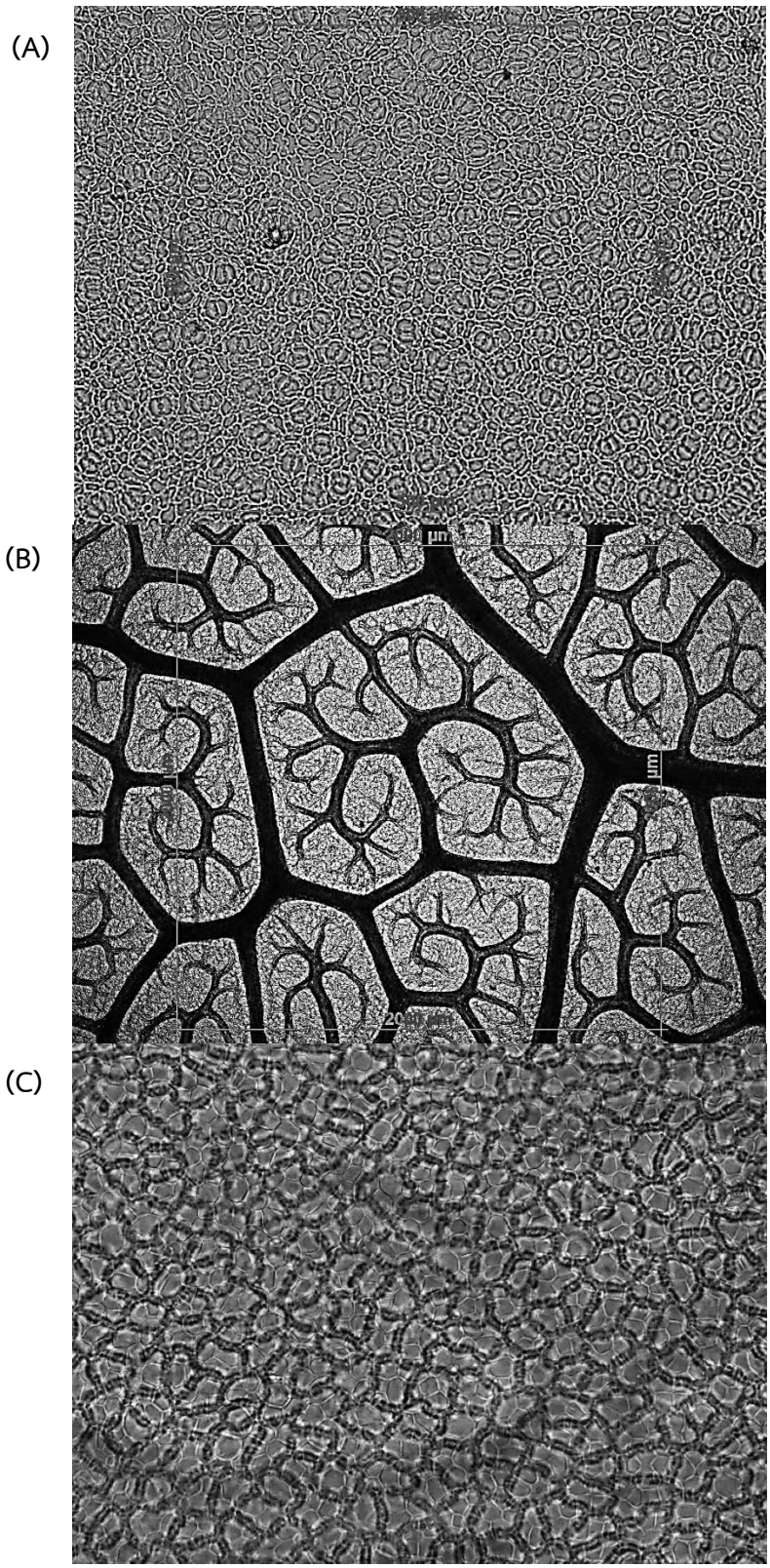
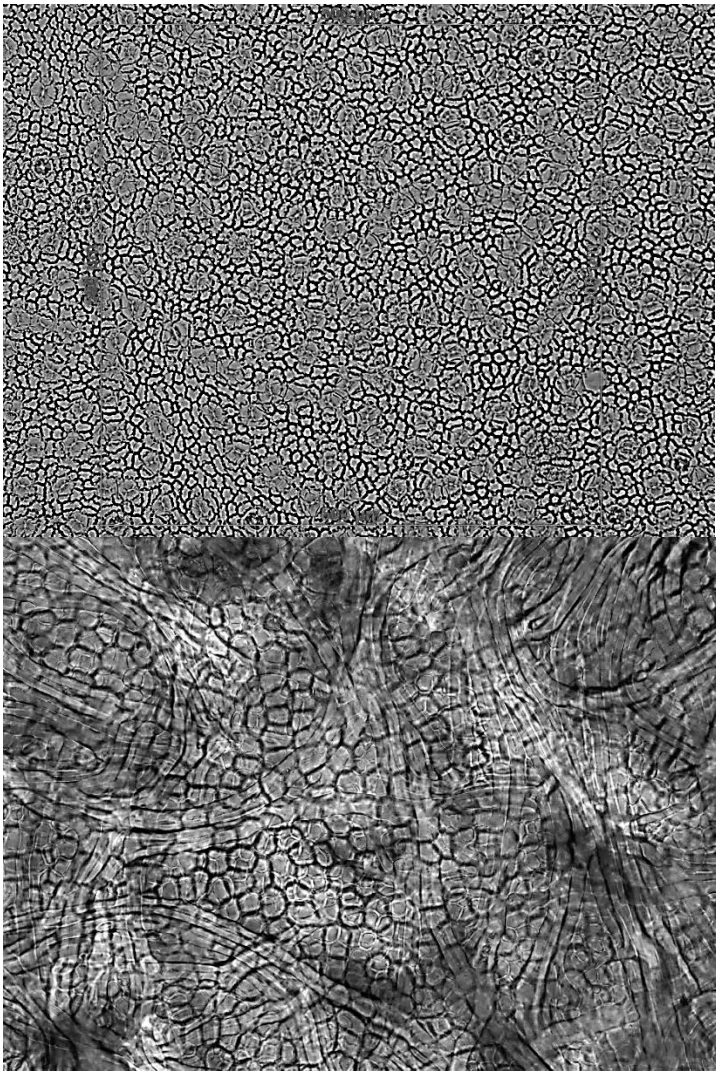


Figure 44 Images of *Mangifera caloneura* leaves; showing (A) stomata at a magnification of 200X, scale 500X500 µm; (B) veinlet terminations at a magnification of 50X, scale 2000X2000 µm; (C) palisade and epidermal cells at a magnification of 400X

Table 39 Stomatal number, veinlet termination number and palisade ratio of *Mangifera caloneura.* Samples were collected from (1) Nakhon Si Thammarat, (2) Surat Thani and (3) Songkhla provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	564	580	592	37.75	36.75	37.25	2.50	3.00	3.00
2	536	556	548	39.75	39.50	38.00	2.75	2.75	2.75
3	572	552	604	42.50	35.00	40.25	2.25	2.25	3.00
4	588	612	496	46.00	36.25	41.50	2.50	3.00	3.00
5	596	524	568	47.00	35.50	43.75	2.50	2.50	3.25
6	604	512	532	44.50	42.50	36.25	2.75	2.75	3.25
7	536	552	612	42.25	38.25	42.75	3.00	2.75	3.00
8	492	536	536	44.75	40.25	39.00	2.50	3.00	3.00
9	548	532	584	48.00	42.50	41.25	3.00	3.00	3.00
10	552	556	504	42.50	37.00	45.00	2.50	3.25	2.50
11	576	596	600	45.75	41.50	39.50	2.75	2.75	2.75
12	592	628	624	40.00	43.75	44.25	3.00	3.00	2.50
13	572	580	628	44.00	39.25	36.50	3.00	2.75	3.00
14	612	532	604	41.50	40.50	42.50	2.50	3.25	2.75
15	560	588	504	40.50	41.25	41.50	2.50	2.50	2.75
16	516	596	576	36.75	37.50	43.00	3.00	2.75	2.25
17	532	564	556	38.50	39.75	44.25	2.50	2.50	2.75
18	552	544	556	38.00	41.75	40.00	2.75	3.25	2.75
19	504	584	588	39.75	43.25	40.75	3.00	3.00	2.50
20	556	576	640	42.50	43.00	43.00	3.00	3.25	3.00
21	548	552	524	36.00	40.75	44.00	2.75	3.00	3.25
22	532	588	564	43.50	41.00	43.00	2.25	2.75	2.50
23	624	576	608	42.50	39.25	40.50	3.00	2.50	3.00
24	568	536	588	36.75	44.00	42.50	3.00	2.75	3.00
25	552	528	540	40.00	37.50	36.25	3.25	3.00	2.75
26	540	552	572	44.00	40.75	41.25	2.50	2.50	2.50
27	512	584	516	41.00	36.50	37.50	2.50	3.25	3.00
28	584	500	552	43.25	45.50	39.50	2.75	2.75	2.75
29	504	588	624	40.50	41.75	43.50	3.00	3.00	3.25
30	568	532	520	36.00	38.50	37.75	2.75	2.50	2.50
Mean	**556**	**561**	**569**	**41.53**	**40.02**	**40.87**	**2.73**	**2.84**	**2.84**
SD	**32.94**	**30.50**	**40.81**	**3.26**	**2.72**	**2.63**	**0.26**	**0.27**	**0.27**
Range	**492-624**	**500-628**	**496-640**	**36.00-48.00**	**35.00-45.50**	**36.25-45.00**	**2.25-3.25**	**2.25-3.25**	**2.25-3.25**

(A)



(B)

Figure 45 Images of *Bouea macrophylla* leaves showing (A) stomata at a magnification of 200X, scale 500X500 µm; (B) fiber, palisade and epidermal cells at a magnification of 400X

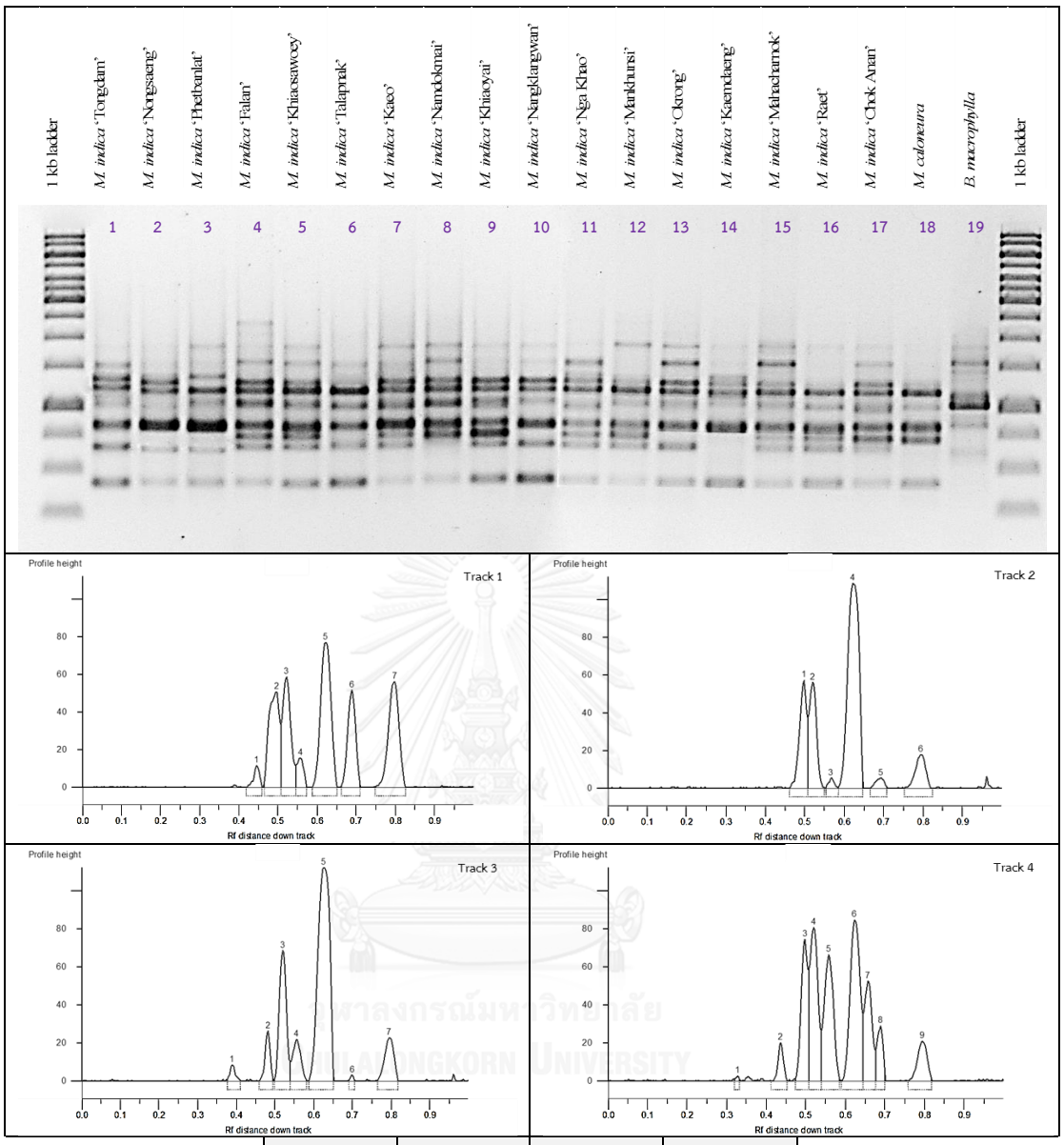
Table 40 Stomatal number, veinlet termination number and palisade ratio of *Bouea macrophylla*. Samples were collected from (1) Chiang Mai, (2) Nonthaburi and (3) Uttaradit provinces, Thailand.

Position	Stomatal number			Veinlet termination number			Palisade ratio		
	1	2	3	1	2	3	1	2	3
1	580	484	552	ND	ND	ND	2.25	2.00	2.00
2	588	540	580	ND	ND	ND	2.00	2.00	2.50
3	564	572	528	ND	ND	ND	1.75	1.75	1.75
4	524	548	540	ND	ND	ND	2.25	2.25	1.75
5	500	560	564	ND	ND	ND	2.00	2.00	2.00
6	560	568	488	ND	ND	ND	2.00	1.75	2.25
7	496	596	560	ND	ND	ND	2.00	2.00	2.00
8	544	552	528	ND	ND	ND	1.75	2.00	1.75
9	532	536	552	ND	ND	ND	1.75	1.75	1.75
10	544	544	584	ND	ND	ND	2.00	1.75	1.75
11	568	508	512	ND	ND	ND	2.00	1.75	2.00
12	532	532	536	ND	ND	ND	1.75	1.75	1.75
13	540	556	600	ND	ND	ND	2.25	2.00	1.75
14	560	580	552	ND	ND	ND	2.00	2.25	1.75
15	576	632	572	ND	ND	ND	2.00	1.75	2.00
16	540	552	496	ND	ND	ND	2.25	2.00	2.00
17	532	580	588	ND	ND	ND	1.75	2.00	1.75
18	544	484	504	ND	ND	ND	2.00	1.50	2.25
19	604	540	608	ND	ND	ND	1.75	2.25	1.75
20	592	568	524	ND	ND	ND	1.75	2.25	1.75
21	584	596	536	ND	ND	ND	2.25	1.75	2.00
22	544	588	600	ND	ND	ND	2.00	2.50	1.75
23	492	560	524	ND	ND	ND	2.00	2.25	2.25
24	576	500	500	ND	ND	ND	1.75	2.00	1.75
25	524	540	588	ND	ND	ND	2.00	1.75	1.75
26	584	588	532	ND	ND	ND	2.00	1.75	2.00
27	512	552	560	ND	ND	ND	2.00	2.00	1.75
28	556	568	540	ND	ND	ND	1.75	1.75	1.75
29	532	576	568	ND	ND	ND	1.75	2.00	2.25
30	568	496	544	ND	ND	ND	2.25	2.00	1.75
Mean	**550**	**553**	**549**	**ND**	**ND**	**ND**	**1.97**	**1.95**	**1.91**
SD	**29.35**	**34.48**	**32.46**	**ND**	**ND**	**ND**	**0.18**	**0.22**	**0.21**
Range	**492-604**	**484-632**	**488-608**	**ND**	**ND**	**ND**	**1.75-2.25**	**1.50-2.50**	**1.75-2.50**

* ND = could not detect

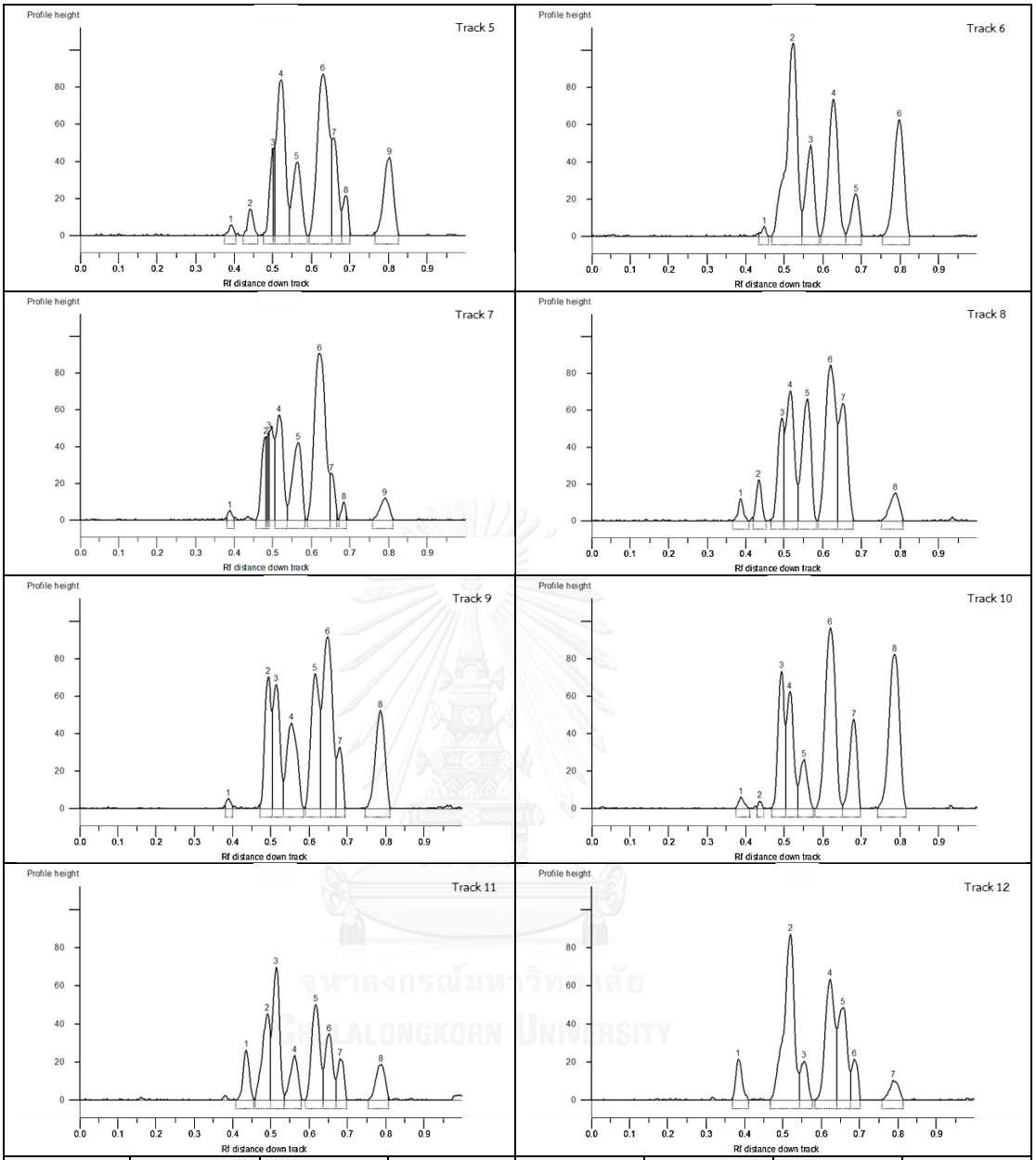
APPENDIX B

Molecular characteristics

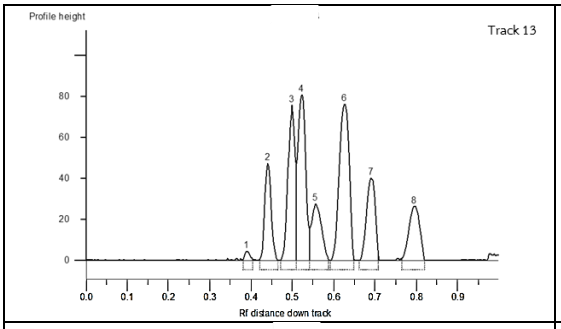


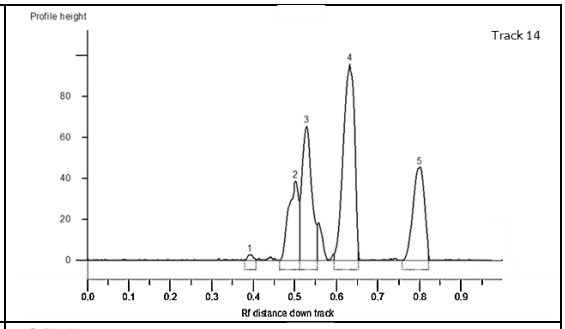
Tract 1		Tract 2		Tract 3		Tract 4	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1500	1	1270	1	1810	1	2360
2	1280	2	1180	2	1340	2	1550
3	1170	3	1020	3	1180	3	1260
4	1040	4	830	4	1050	4	1180
5	830	5	630	5	820	5	1040
6	640	6	400	6	620	6	820
7	390			7	400	7	730
						8	640
						9	400

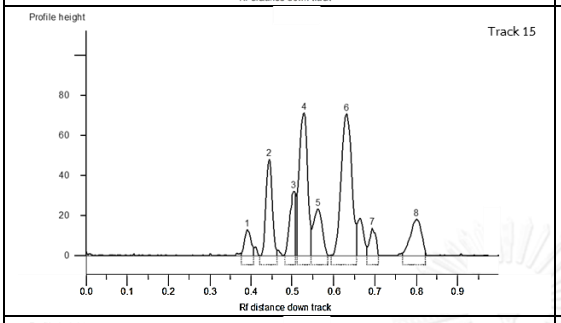
Table 41 Fingerprint and molecular weight plots of ISSR 02 (AGAGAGAGAGAGAGAGC), an annealing temperature 50 °C, fragment sizes range from 380 to 2360 bps, 75.00 % polymorphic

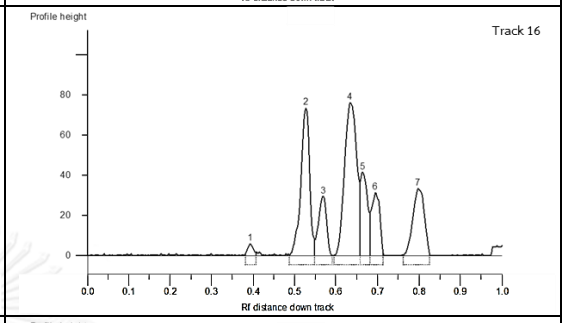


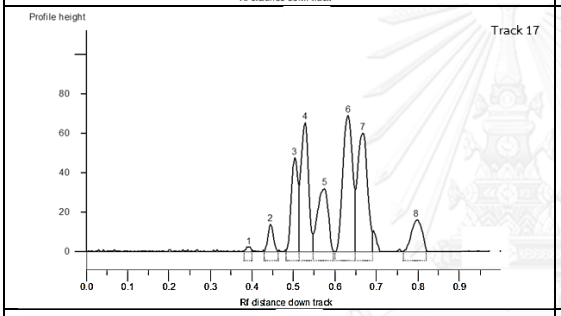
Tract 5		Tract 6		Tract 7		Tract 8		Tract 9		Tract 10		Tract 11		Tract 12	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1800	1	1490	1	1810	1	1830	1	1800	1	1830	1	1550	1	1850
2	1520	2	1170	2	1340	2	1560	2	1300	2	1400	2	1300	2	1200
3	1250	3	1010	3	1300	3	1290	3	1200	3	1300	3	1200	3	1050
4	1170	4	810	4	1190	4	1200	4	1060	4	1200	4	1030	4	820
5	1030	5	650	5	1020	5	1040	5	850	5	1060	5	840	5	730
6	800	6	390	6	830	6	840	6	750	6	840	6	740	6	650
7	720			7	740	7	740	7	660	7	670	7	660	7	420
8	640			8	650	8	420	8	420	8	420	8	420		
9	380			9	410										

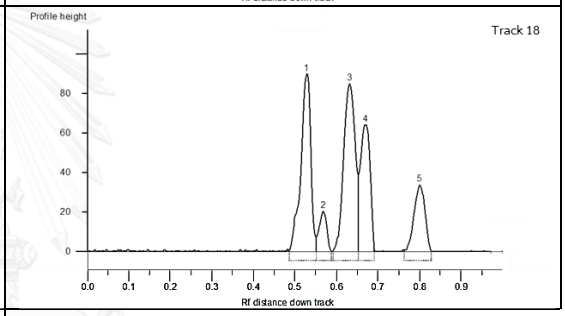


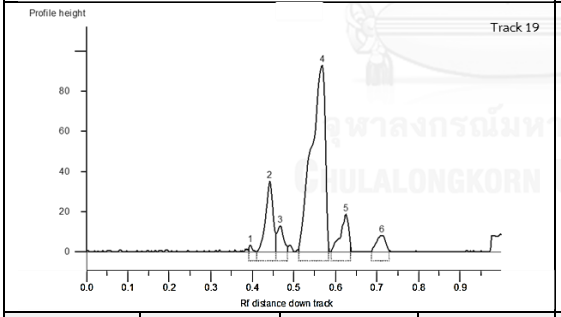




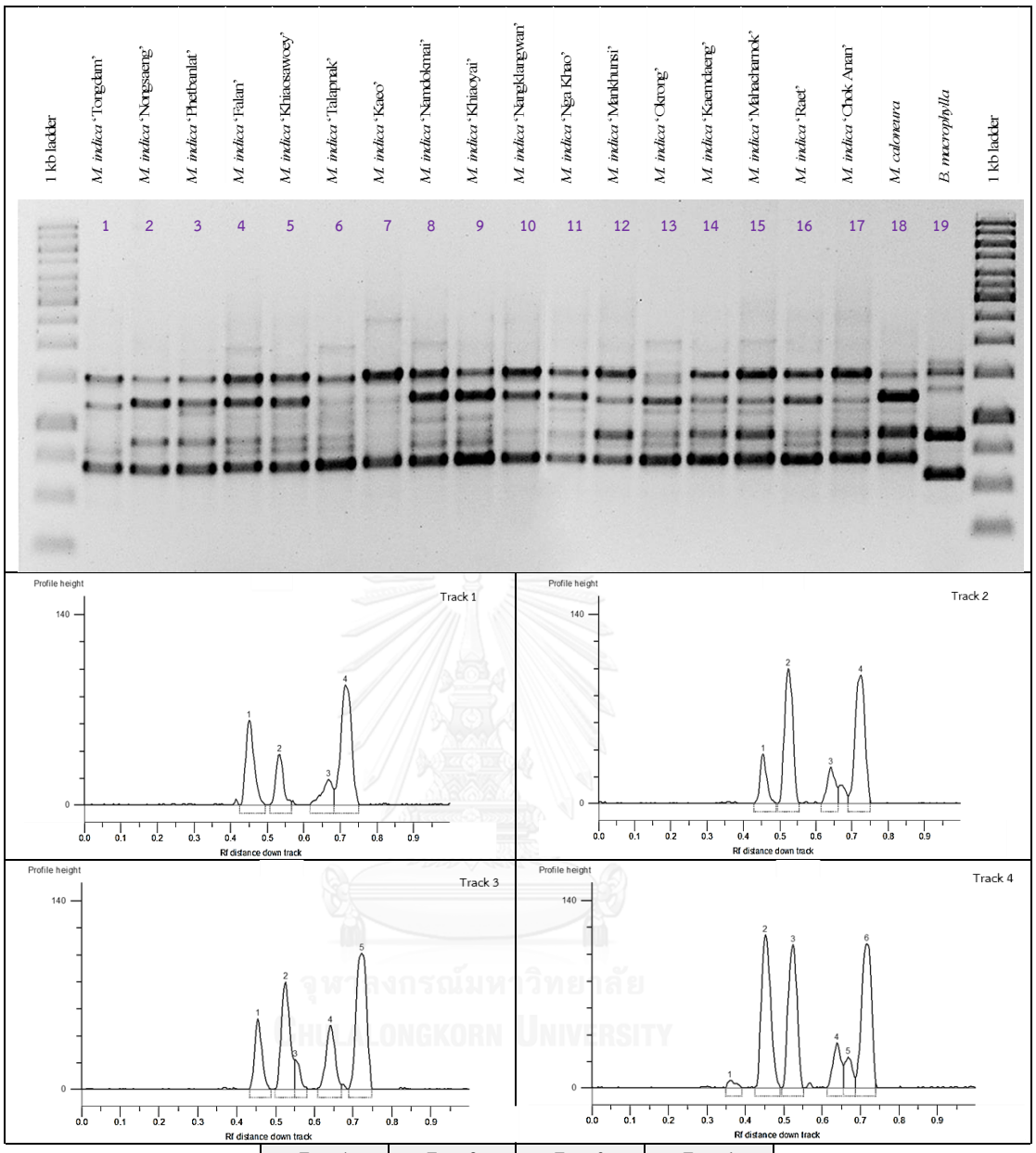






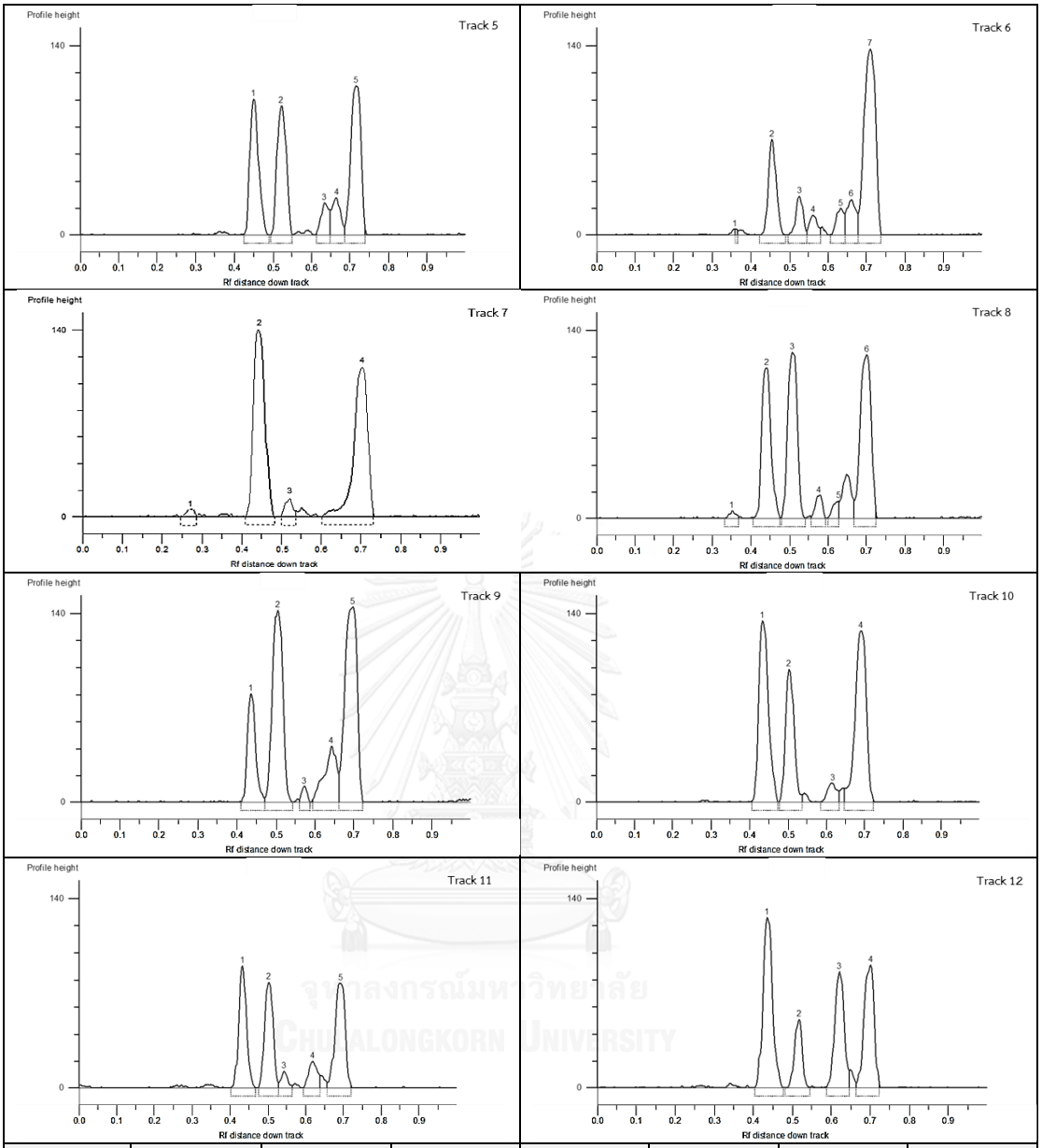


Tract 13		Tract 14		Tract 15		Tract 16		Tract 17		Tract 18		Tract 19	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1810	1	1800	1	1800	1	1790	1	1790	1	1150	1	1780
2	1530	2	1250	2	1500	2	1150	2	1500	2	1010	2	1520
3	1260	3	1140	3	1240	3	1010	3	1250	3	800	3	1400
4	1170	4	800	4	1140	4	800	4	1140	4	690	4	1010
5	1050	5	390	5	1030	5	710	5	1000	5	390	5	820
6	820			6	800	6	630	6	800			6	590
7	640			7	630	7	400	7	670				
8	400			8	390			8	400				

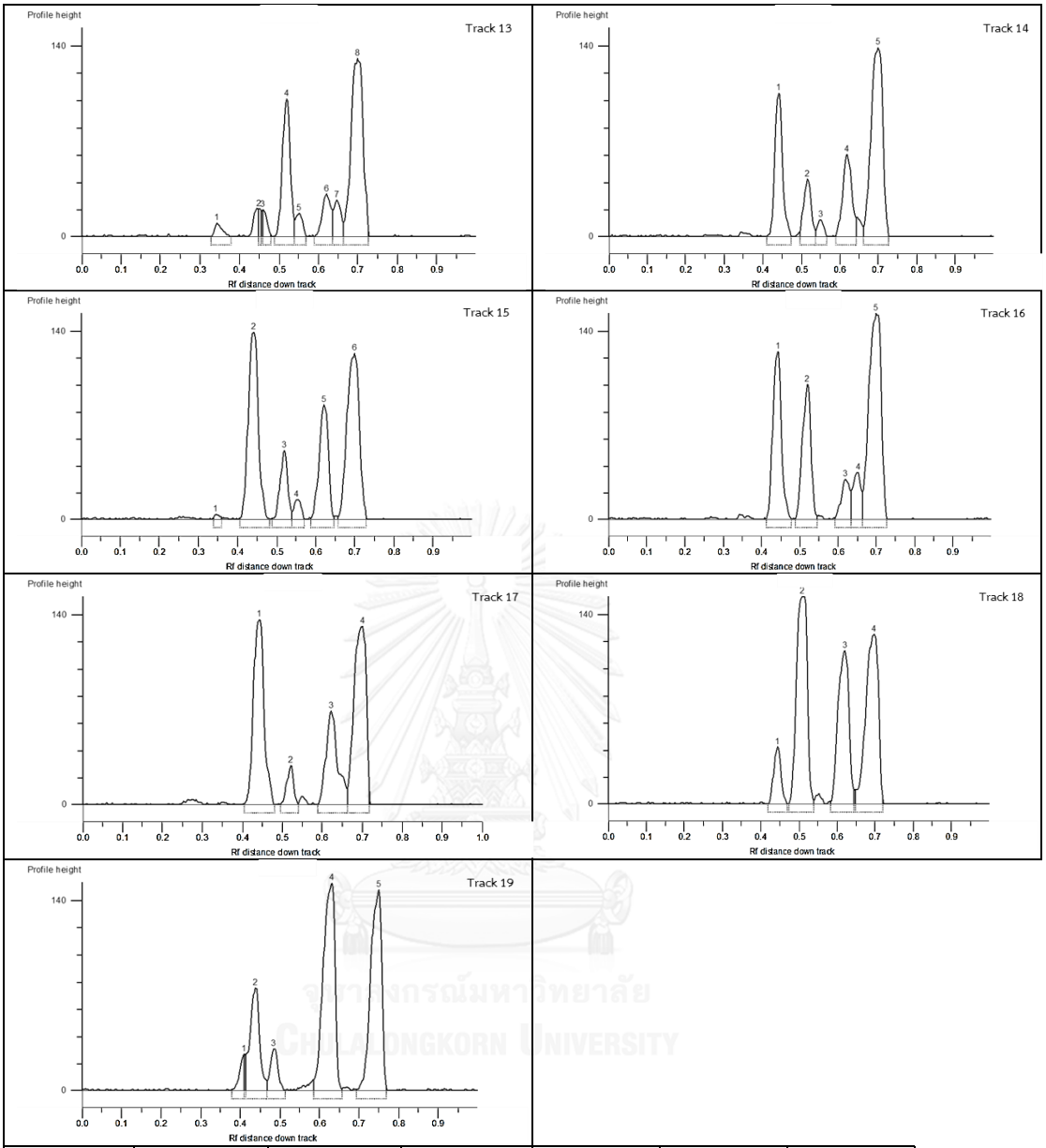


Tract 1		Tract 2		Tract 3		Tract 4	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1500	1	1480	1	1480	1	1940
2	1170	2	1200	2	1190	2	1490
3	780	3	840	3	1100	3	1200
4	670	4	640	4	840	4	850
				5	650	5	780
						6	660

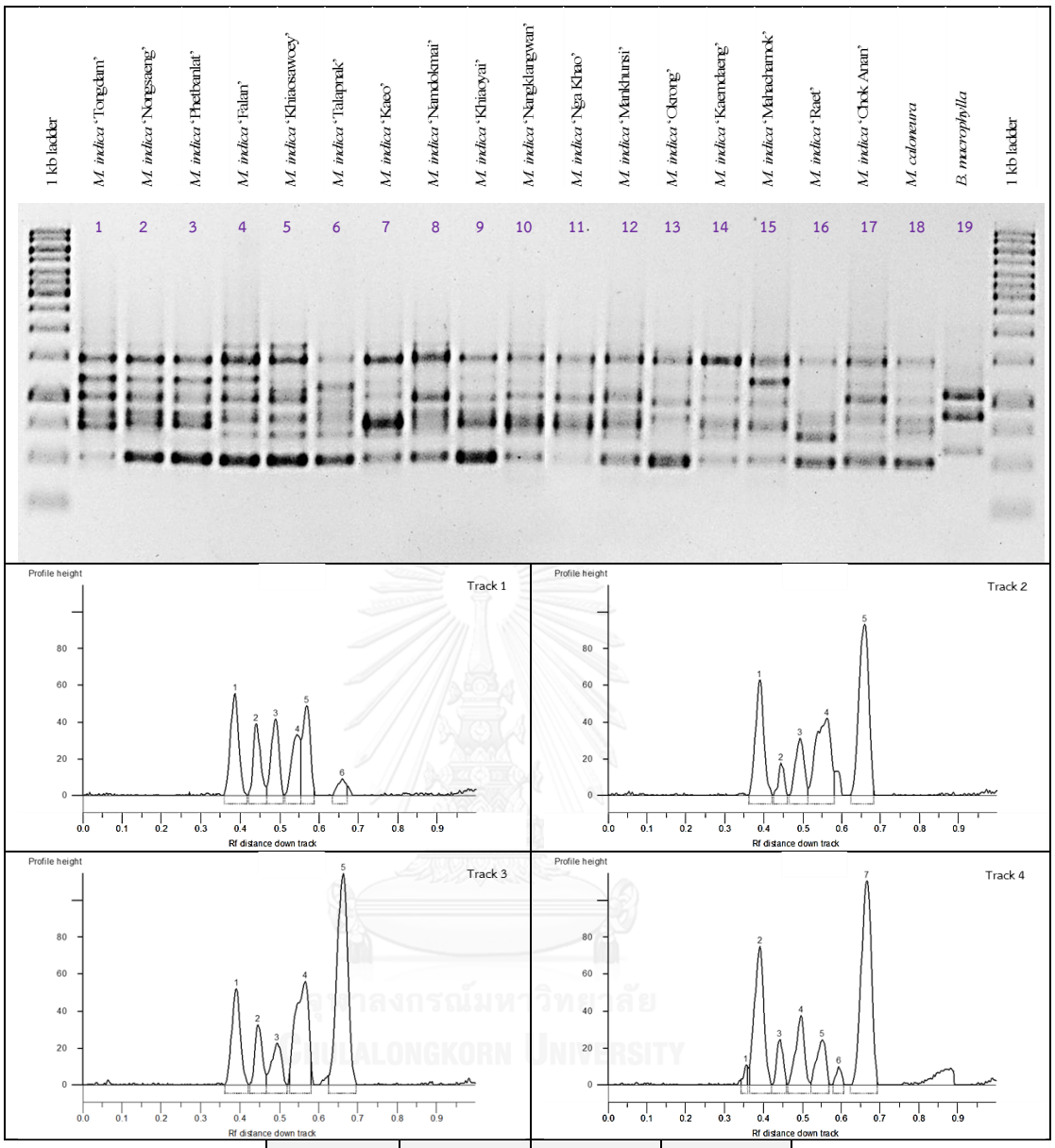
Table 42 Fingerprint and molecular weight plots of ISSR 03 (AGAGAGAGAGAGAGC), an annealing temperature 46 °C, fragment sizes range from 640 to 2560 bps, 92.30 % polymorphic



Tract 5		Tract 6		Tract 7		Tract 8		Tract 9		Tract 10		Tract 11		Tract 12	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1500	1	1940	1	2560	1	1990	1	1560	1	1570	1	1570	1	1560
2	1210	2	1480	2	1540	2	1540	2	1270	2	1280	2	1280	2	1220
3	860	3	1190	3	1220	3	1260	3	1040	3	910	3	1140	3	900
4	780	4	1070	4	700	4	1030	4	840	4	720	4	900	4	700
5	660	5	870			5	890	5	710			5	720		
		6	800			6	700								
		7	680												

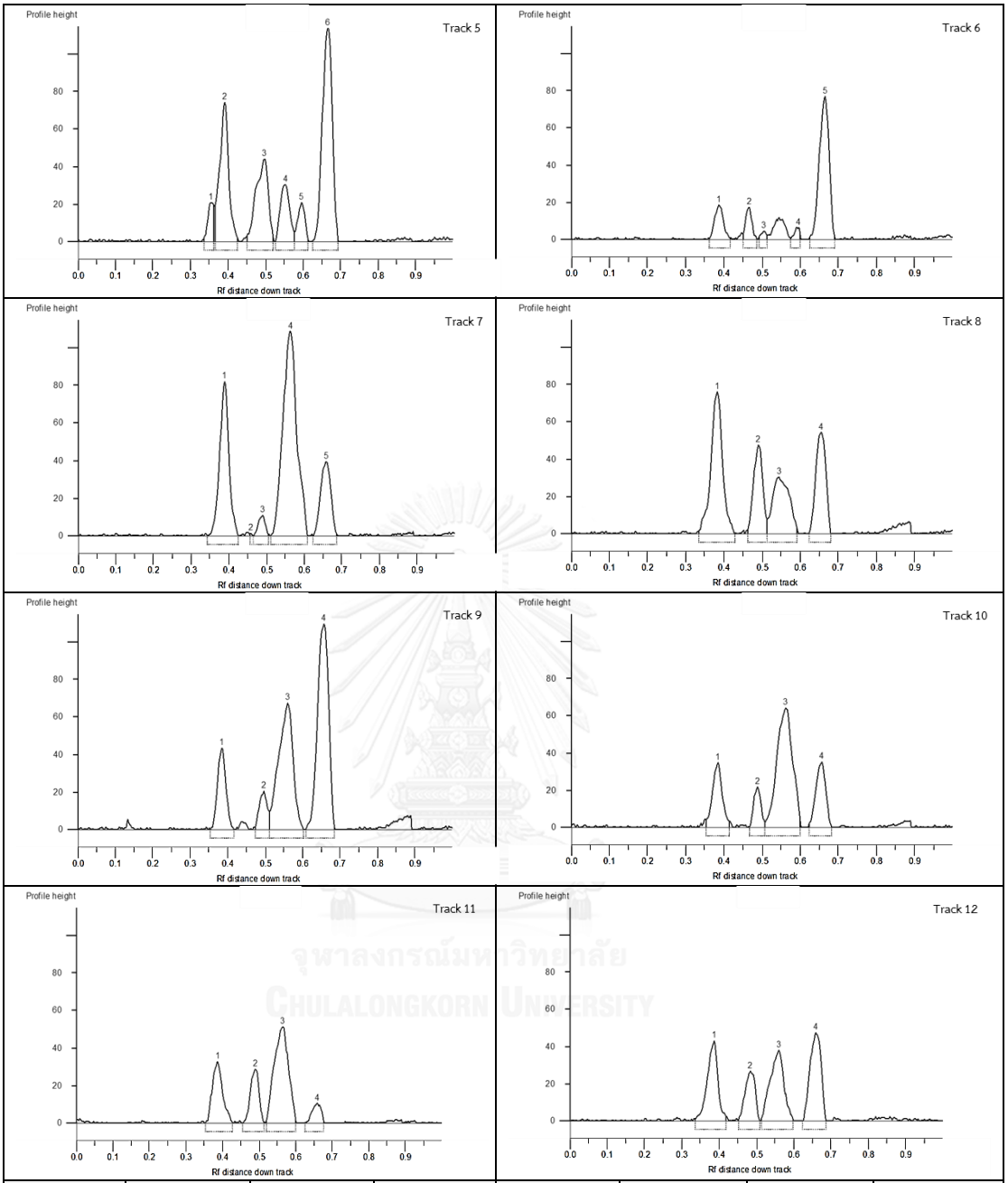


Tract 13		Tract 14		Tract 15		Tract 16		Tract 17		Tract 18		Tract 19	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	2030	1	1540	1	2020	1	1530	1	1540	1	1530	1	1680
2	1500	2	1240	2	1540	2	1220	2	1220	2	1250	2	1550
3	1450	3	1100	3	1220	3	900	3	900	3	900	3	1350
4	1210	4	900	4	1100	4	820	4	700	4	710	4	870
5	1100	5	700	5	900	5	700					5	590
6	900			6	700								
7	820												
8	700												

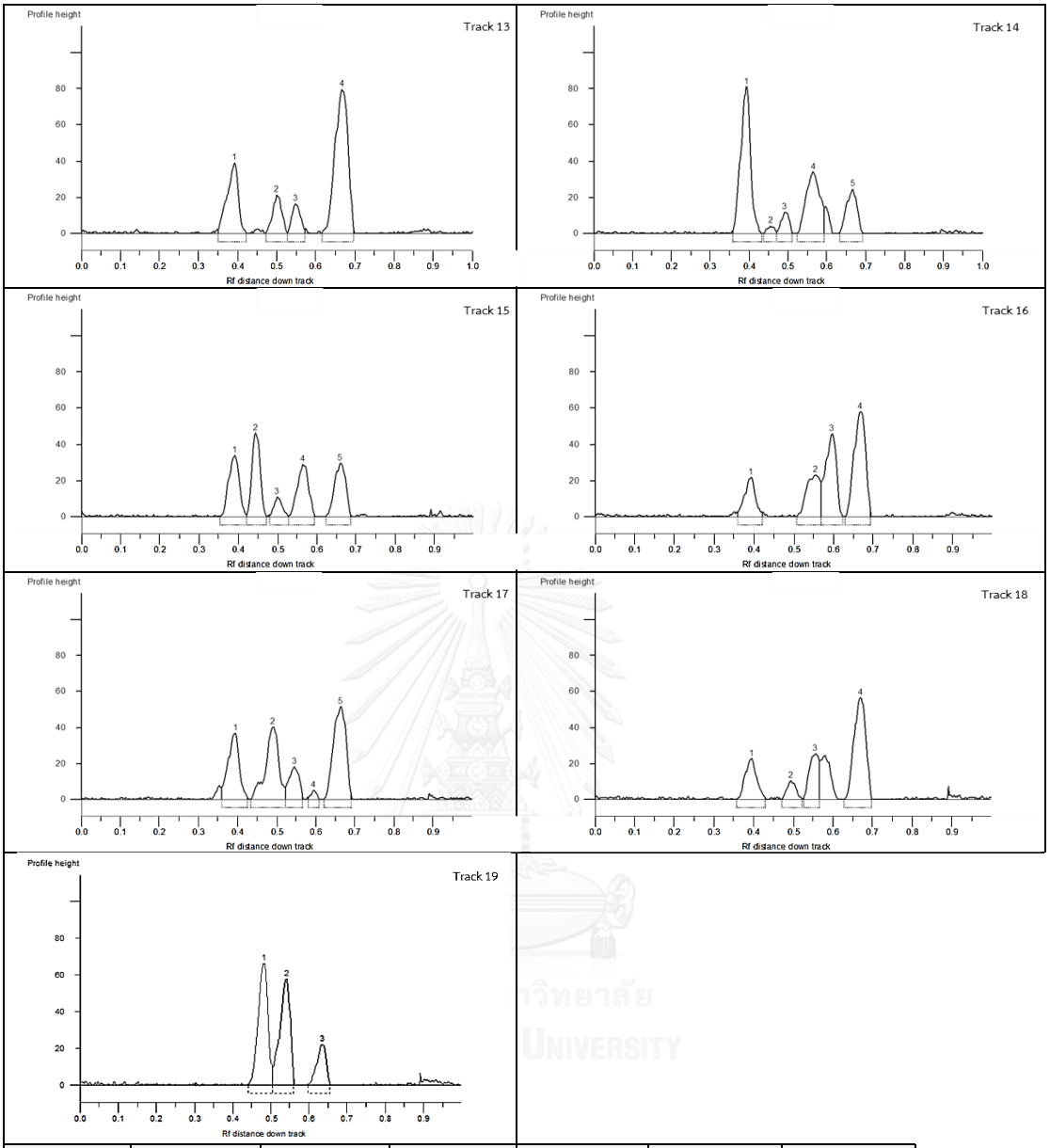


Tract 1		Tract 2		Tract 3		Tract 4	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1470	1	1450	1	1440	1	1760
2	1190	2	1170	2	1160	2	1440
3	980	3	980	3	970	3	1180
4	790	4	740	4	730	4	960
5	720	5	500	5	490	5	780
6	500					6	650
						7	480

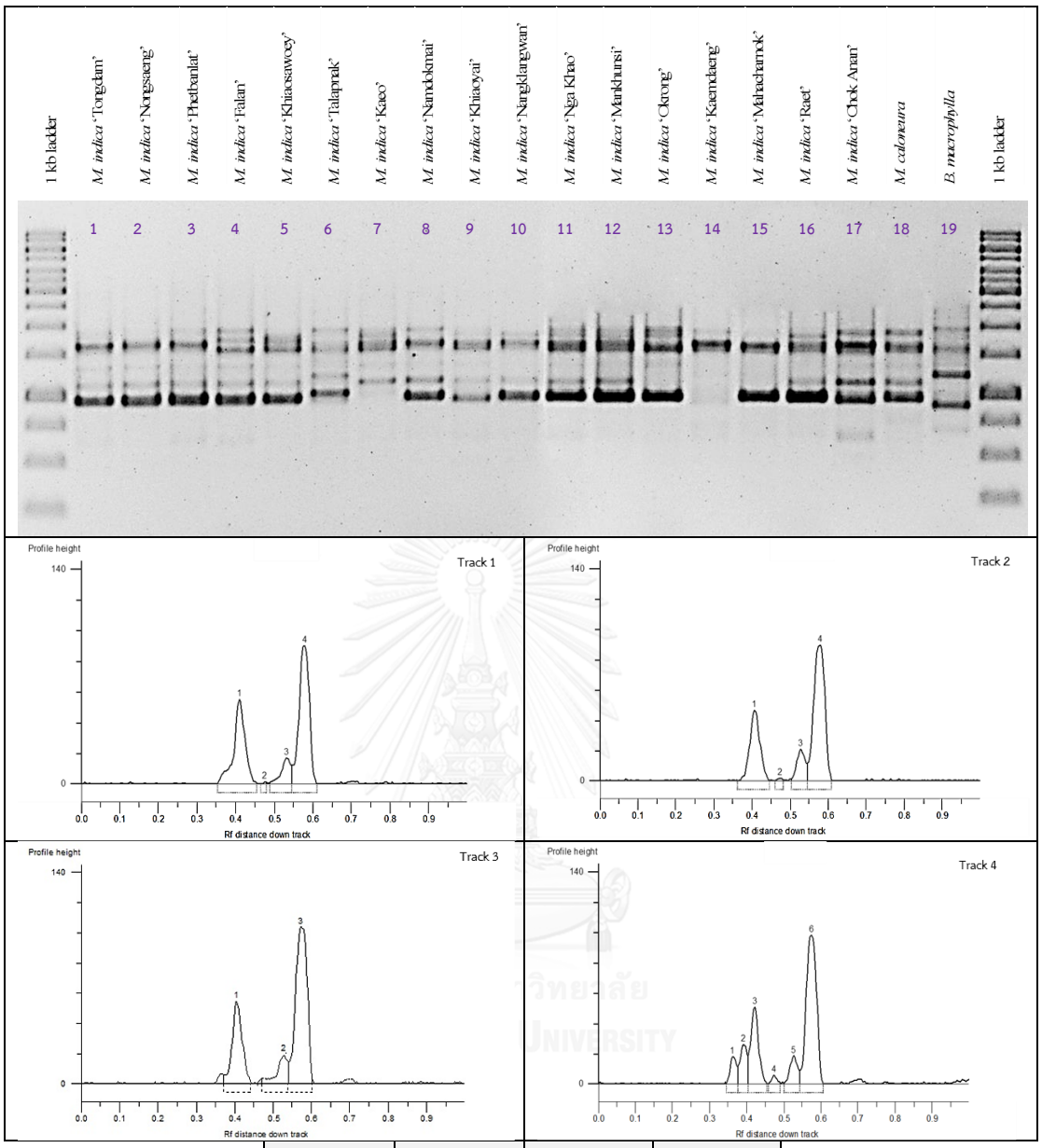
Table 43 Fingerprint and molecular weight plots of ISSR13 (AGAGAGAGAGAGAGAGYA), an annealing temperature 50 °C, fragment sizes range from 480 to 1760 bps, 77.78 % polymorphic



Tract 5		Tract 6		Tract 7		Tract 8		Tract 9		Tract 10		Tract 11		Tract 12	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1760	1	1460	1	1450	1	1490	1	1470	1	1470	1	1460	1	1460
2	1440	2	1070	2	1110	2	980	2	960	2	1000	2	980	2	1010
3	960	3	930	3	980	3	790	3	750	3	740	3	740	3	750
4	770	4	650	4	740	4	510	4	500	4	500	4	500	4	500
5	650	5	480	5	500										
6	480														

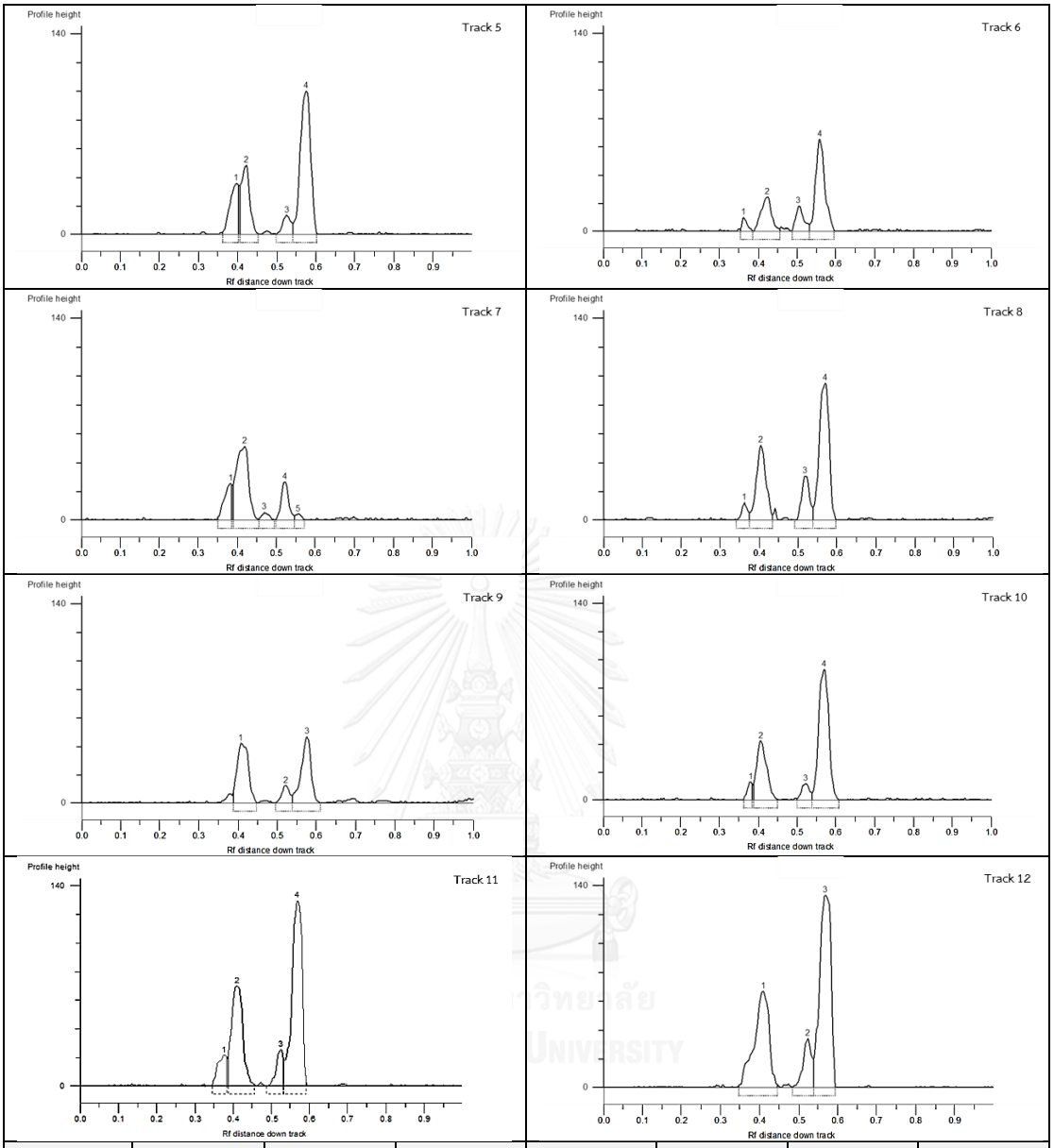


Tract 13		Tract 14		Tract 15		Tract 16		Tract 17		Tract 18		Tract 19	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1440	1	1420	1	1440	1	1420	1	1410	1	1410	1	1010
2	950	2	1130	2	1170	2	760	2	980	2	970	2	800
3	780	3	980	3	950	3	650	3	790	3	760	3	560
4	480	4	740	4	740	4	480	4	650	4	480		
		5	480	5	500			5	490				

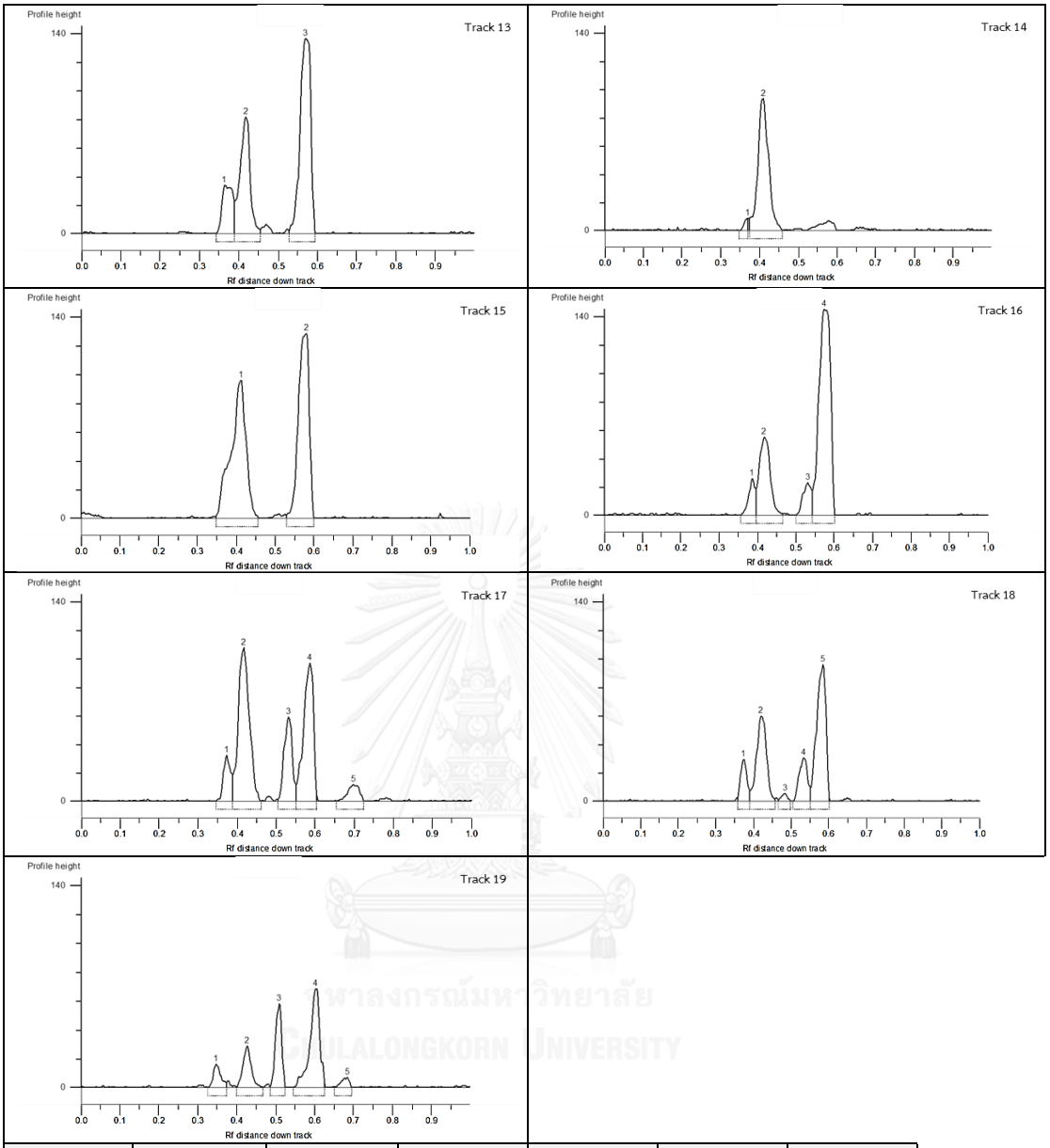


Tract 1		Tract 2		Tract 3		Tract 4	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1640	1	1650	1	1640	1	1910
2	1310	2	1320	2	1340	2	1740
3	1080	3	1100	3	950	3	1570
4	940	4	940			4	1320
						5	1110
						6	950

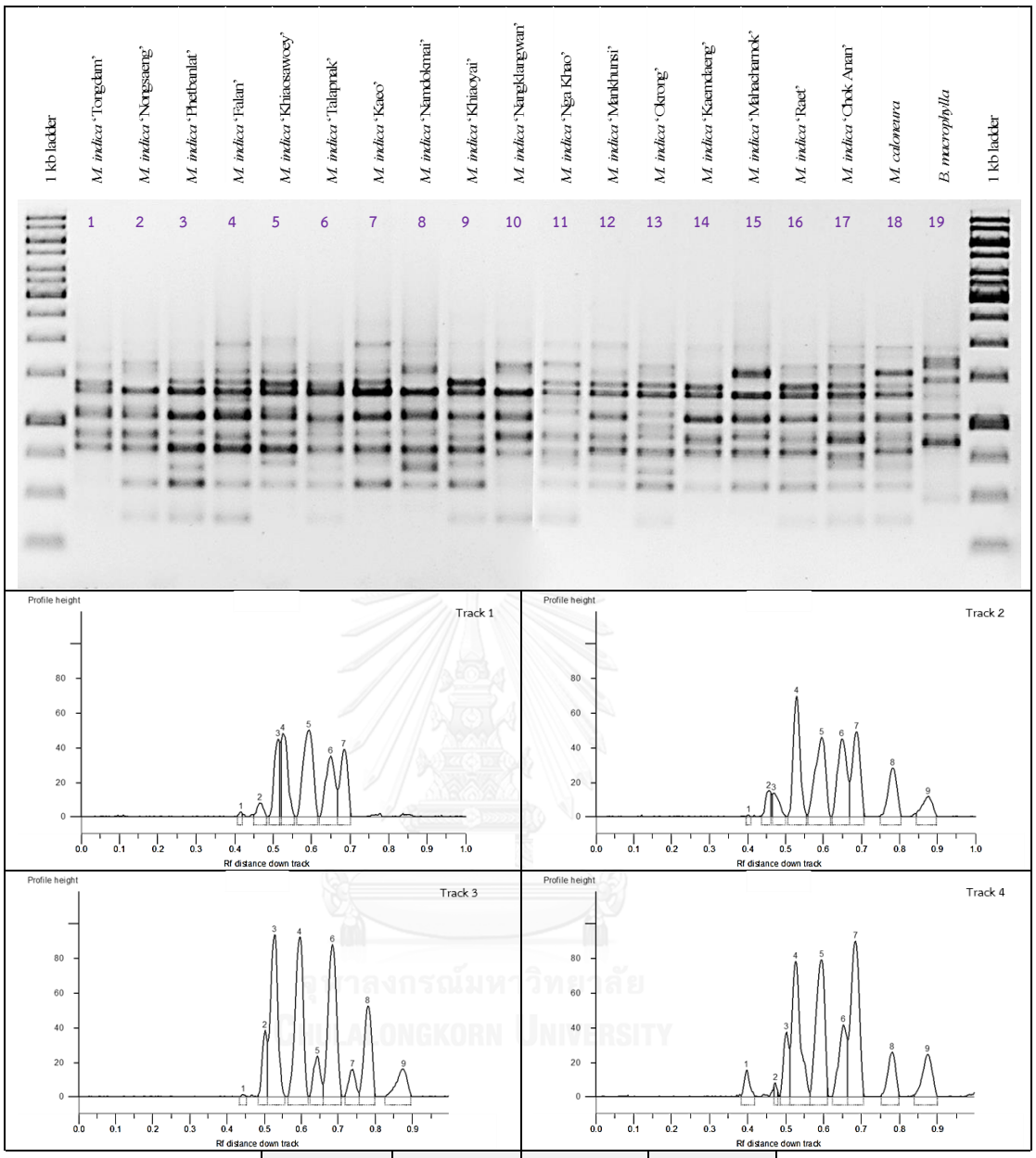
Table 44 Fingerprint and molecular weight plots of ISSR 19 (ACACACACACACACYT), an annealing temperature 54 °C, fragment sizes range from 650 to 1910 bps, 87.50 % polymorphic



Tract 5		Tract 6		Tract 7		Tract 8		Tract 9		Tract 10		Tract 11		Tract 12	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1700	1	1910	1	1790	1	1910	1	1650	1	1800	1	1800	1	1640
2	1570	2	1570	2	1590	2	1670	2	1130	2	1650	2	1640	2	1120
3	1110	3	1200	3	1340	3	1140	3	950	3	1130	3	1120	3	970
4	950	4	990	4	1130	4	970			4	970	4	970		
				5	1000										

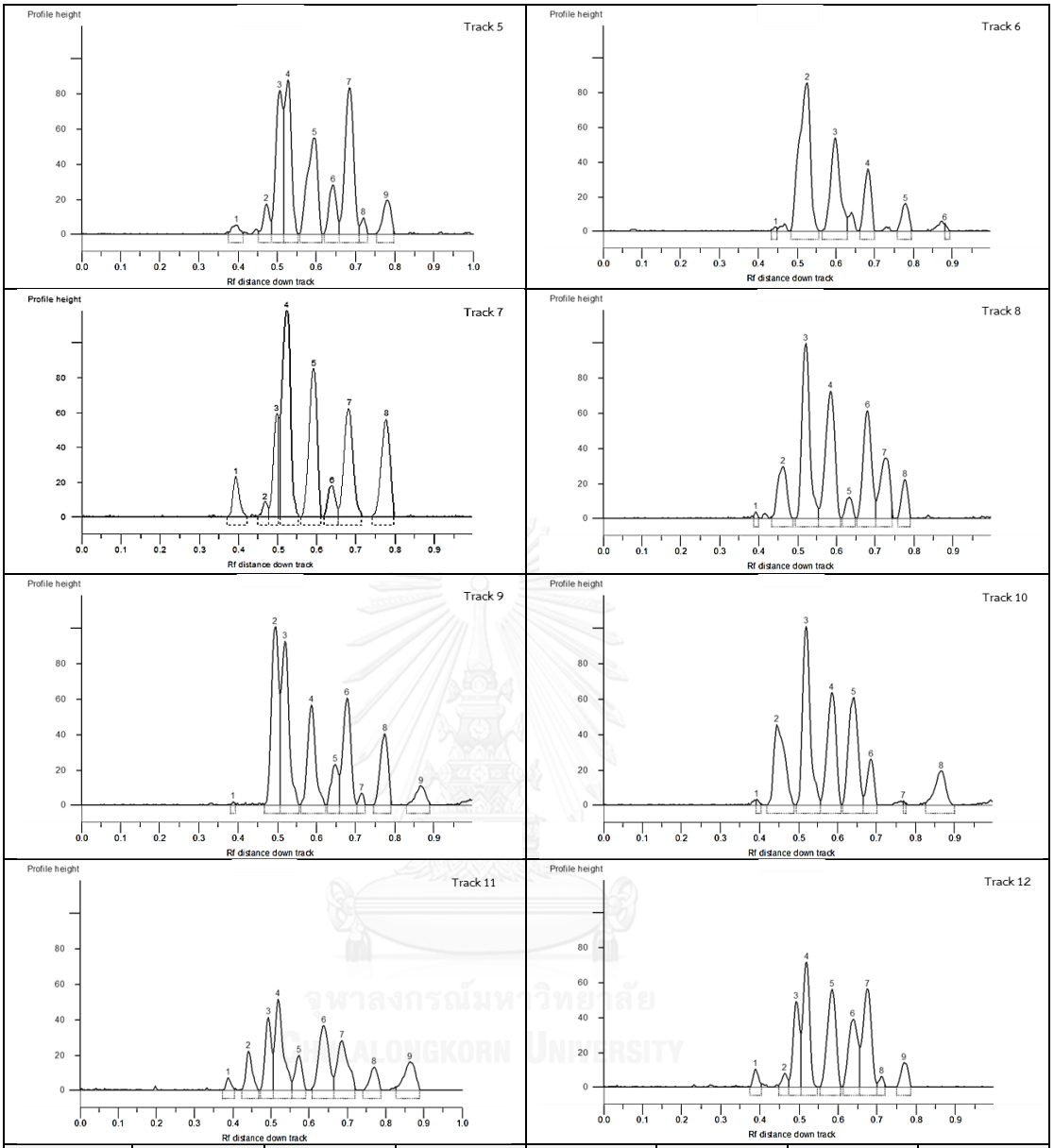


Tract 13		Tract 14		Tract 15		Tract 16		Tract 17		Tract 18		Tract 19	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1890	1	1860	1	1620	1	1780	1	1850	1	1850	1	1910
2	1590	2	1640	2	940	2	1600	2	1600	2	1590	2	1550
3	960					3	1090	3	1090	3	1280	3	1180
						4	960	4	920	4	1080	4	880
								5	640	5	930	5	680

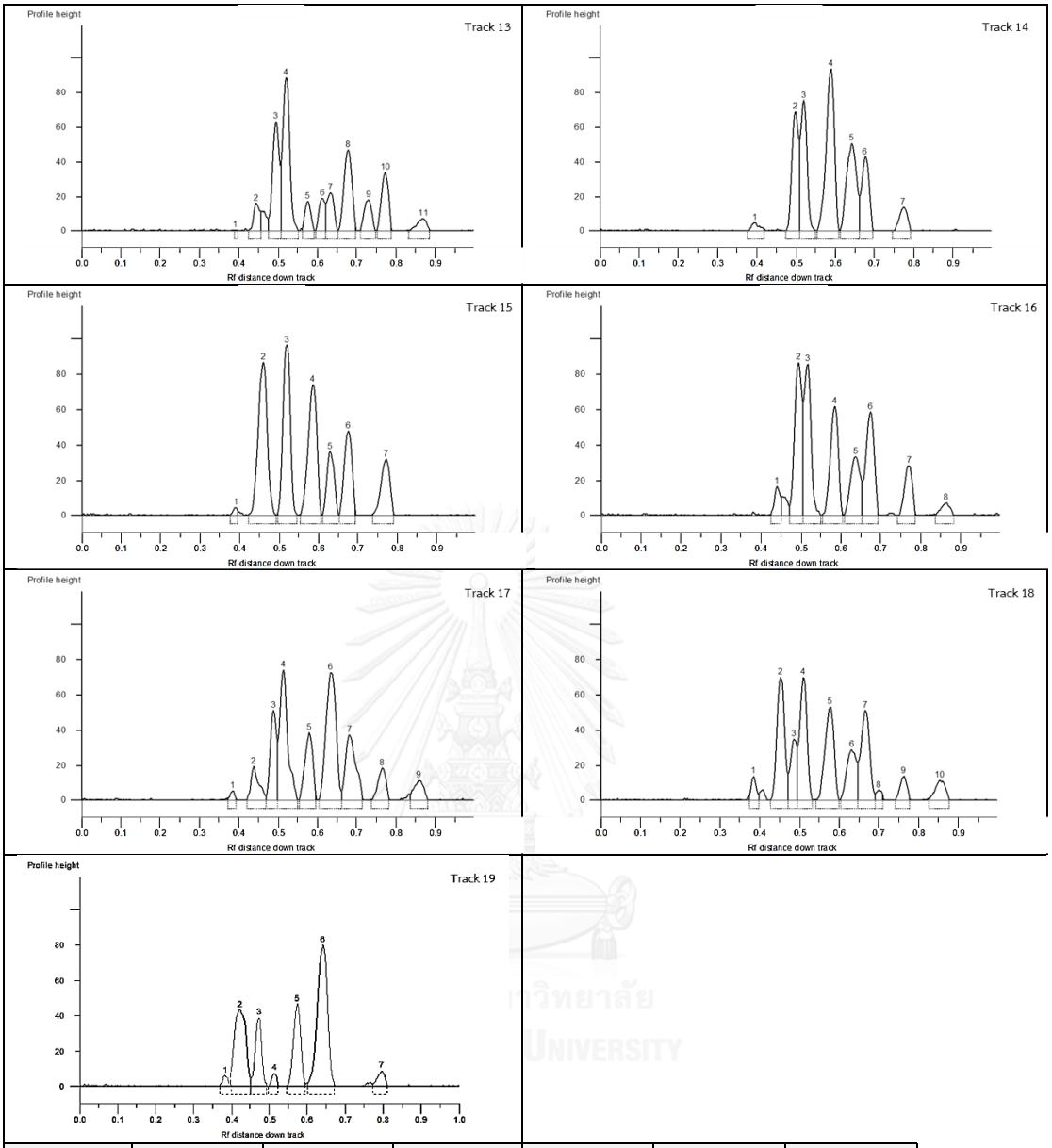


Tract 1		Tract 2		Tract 3		Tract 4	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1850	1	1940	1	1700	1	1960
2	1600	2	1650	2	1420	2	1550
3	1380	3	1570	3	1310	3	1420
4	1330	4	1310	4	1070	4	1320
5	1090	5	1080	5	920	5	1080
6	910	6	920	6	800	6	900
7	800	7	800	7	660	7	800
		8	560	8	560	8	560
		9	360	9	360	9	360

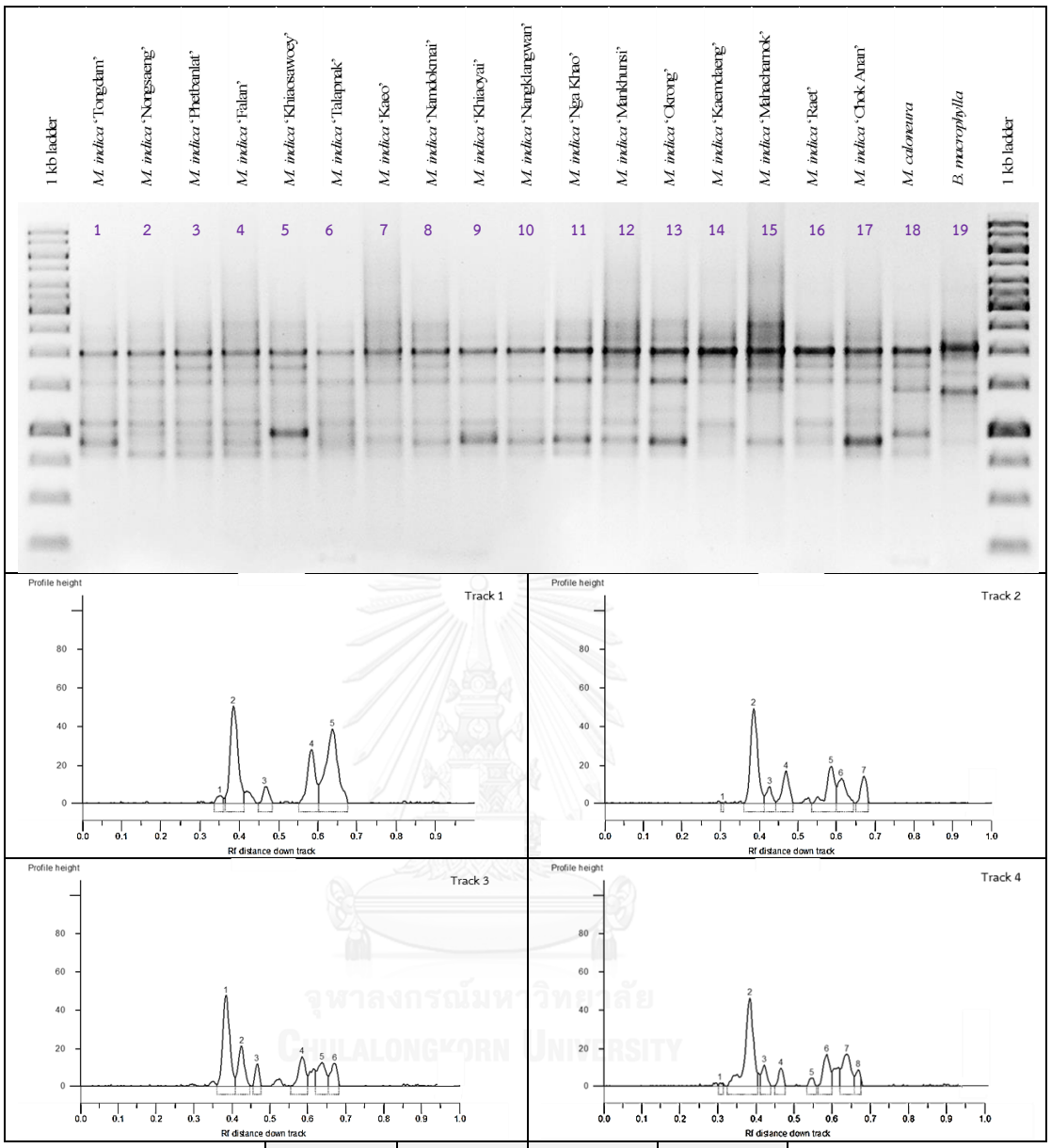
Table 45 Fingerprint and molecular weight plots of ISSR 22 (TGTGTGTGTGTGTGTGRC), an annealing temperature 54 °C, fragment sizes range from 360 to 2070 bps, 84.62 % polymorphic



Tract 5		Tract 6		Tract 7		Tract 8		Tract 9		Tract 10		Tract 11		Tract 12	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1980	1	1680	1	1990	1	1990	1	2030	1	1990	1	2070	1	2070
2	1570	2	1320	2	1590	2	1600	2	1450	2	1700	2	1710	2	1600
3	1410	3	1070	3	1430	3	1340	3	1340	3	1340	3	1460	3	1460
4	1320	4	800	4	1330	4	1100	4	1100	4	1110	4	1350	4	1340
5	1080	5	560	5	1090	5	950	5	910	5	930	5	1150	5	1110
6	930	6	350	6	940	6	800	6	820	6	790	6	940	6	940
7	800			7	810	7	690	7	710	7	590	7	800	7	820
8	700			8	570	8	570	8	580	8	380	8	590	8	720
9	570							9	370			9	390	9	590

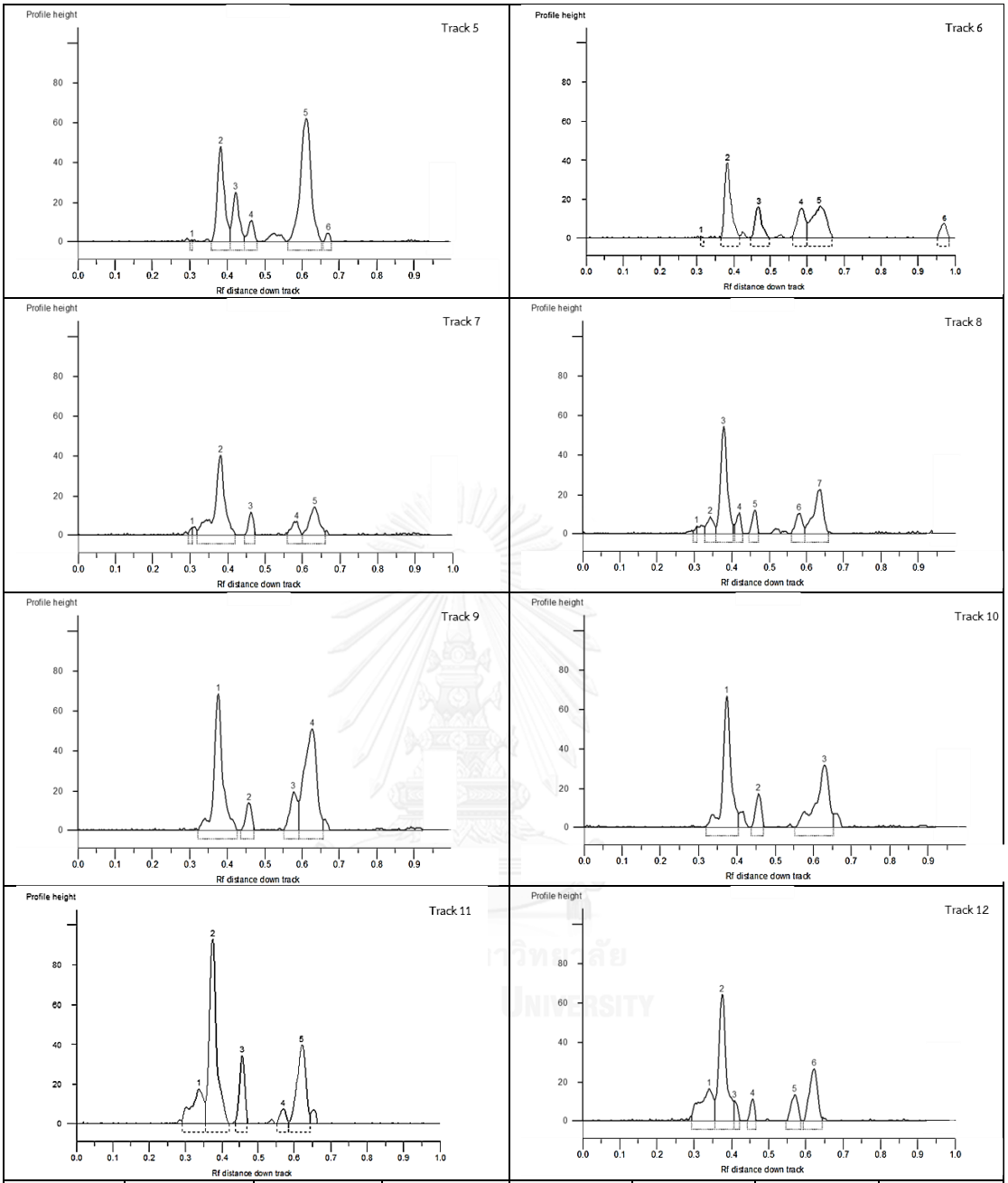


Tract 13		Tract 14		Tract 15		Tract 16		Tract 17		Tract 18		Tract 19	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	2000	1	1970	1	2000	1	1710	1	2000	1	2000	1	1990
2	1690	2	1430	2	1610	2	1450	2	1730	2	1650	2	1820
3	1450	3	1340	3	1340	3	1350	3	1480	3	1490	3	1560
4	1340	4	1100	4	1110	4	1110	4	1370	4	1390	4	1380
5	1140	5	930	5	960	5	940	5	1130	5	1140	5	1150
6	1020	6	820	6	820	6	830	6	950	6	960	6	930
7	950	7	580	7	580	7	580	7	800	7	850	7	540
8	820					8	380	8	600	8	760		
9	680							9	400	9	600		
10	580									10	410		
11	370												

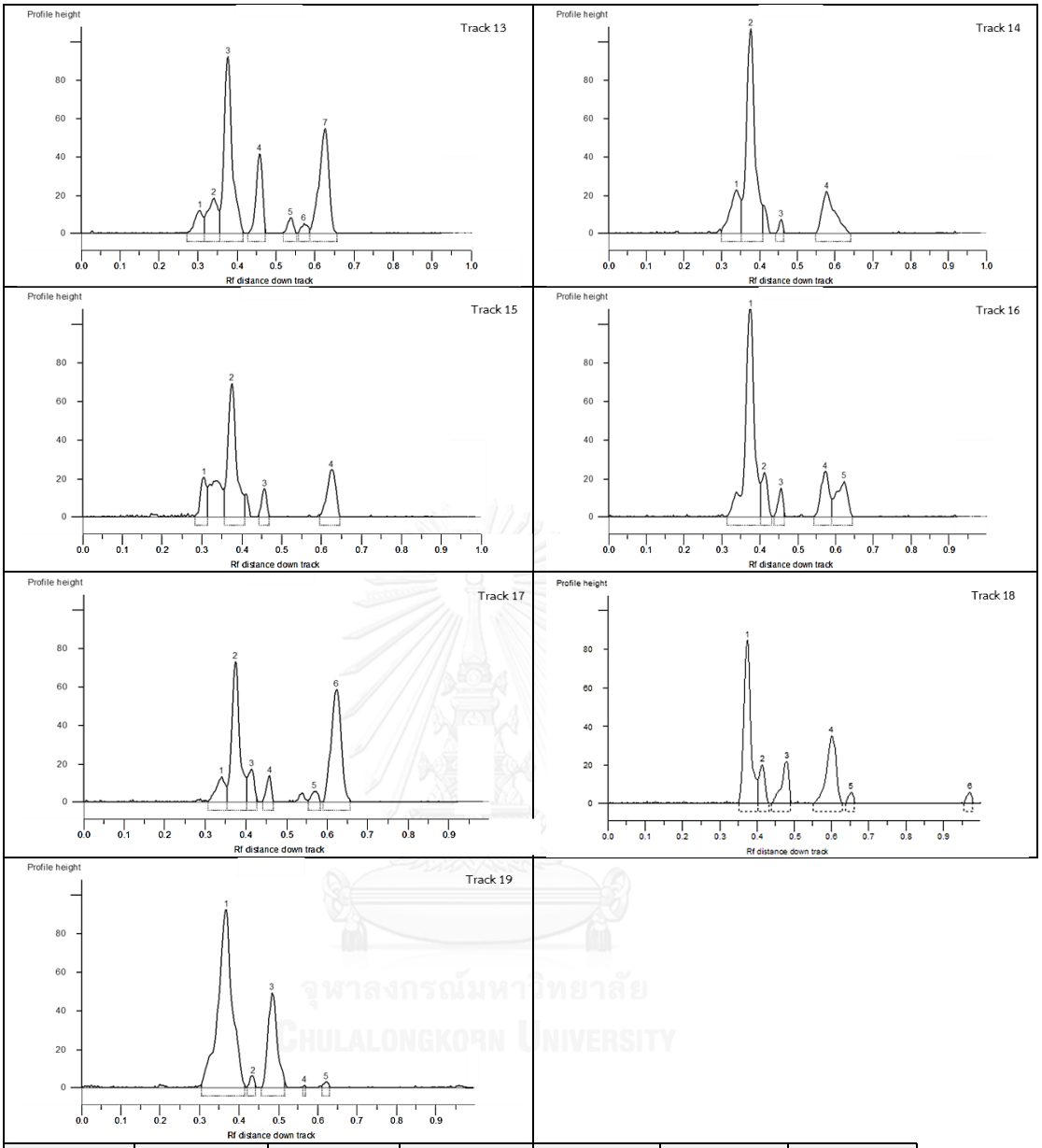


Tract 1		Tract 2		Tract 3		Tract 4	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	2260	1	2660	1	2030	1	2660
2	2020	2	2010	2	1780	2	2030
3	1570	3	1770	3	1580	3	1800
4	1090	4	1550	4	1100	4	1570
5	900	5	1090	5	910	5	1230
		6	1010	6	800	6	1090
		7	800			7	900
						8	810

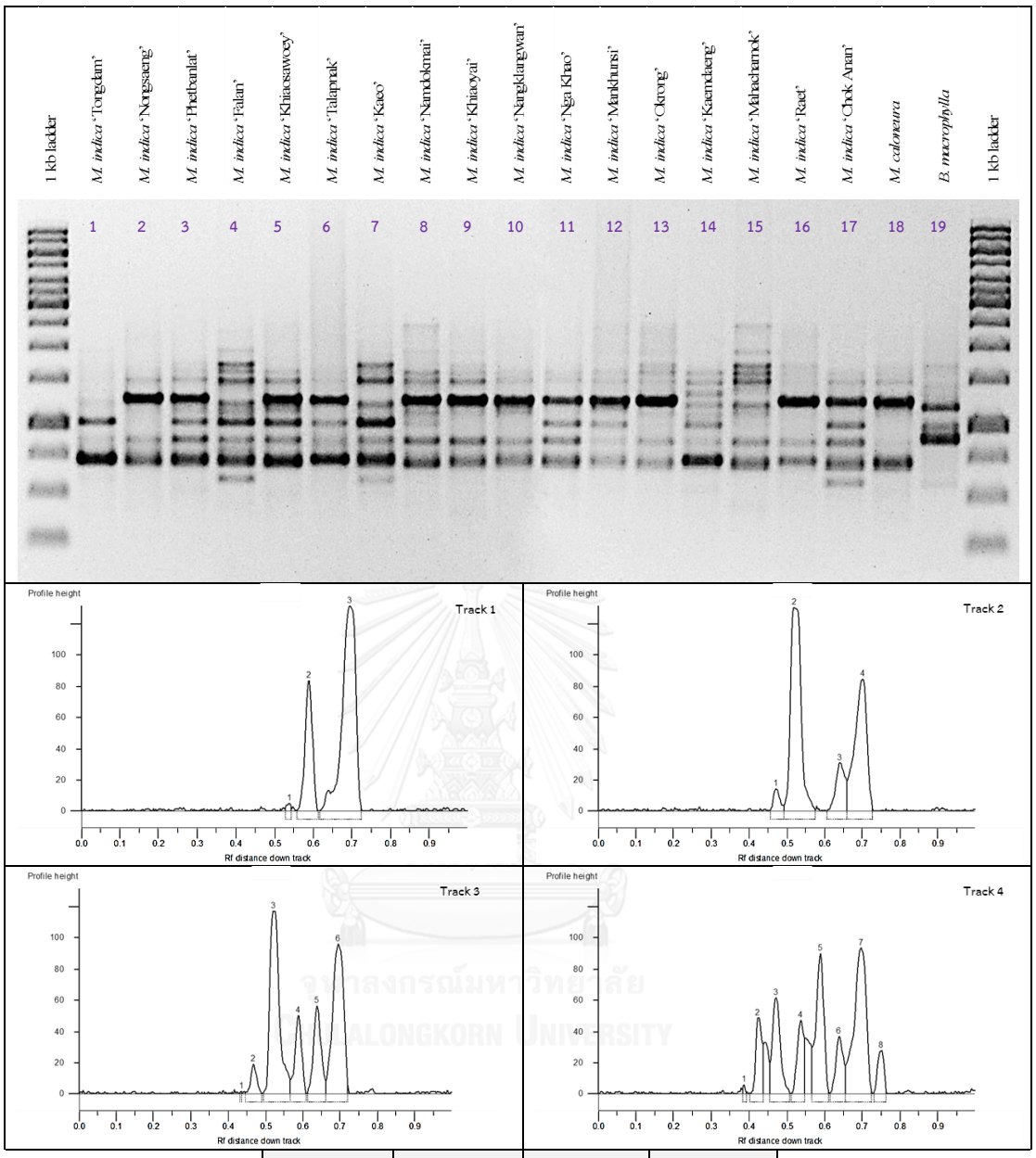
Table 46 Fingerprint and molecular weight plots of ISSR 27 (GGATGGATGGATGGAT), an annealing temperature 48 °C, fragment sizes range from 190 to 2660 bps, 81.82 % polymorphic



Tract 5		Tract 6		Tract 7		Tract 8		Tract 9		Tract 10		Tract 11		Tract 12	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	2660	1	2580	1	2660	1	2660	1	2070	1	2090	1	2360	1	2330
2	2030	2	2030	2	2040	2	2310	2	1610	2	1620	2	2090	2	2088
3	1800	3	1560	3	1600	3	2060	3	1120	3	930	3	1630	3	1880
4	1580	4	1090	4	1100	4	1800	4	940			4	1150	4	1620
5	1010	5	930	5	930	5	1580					5	970	5	1150
6	800	6	190			6	1100							6	970
						7	910								

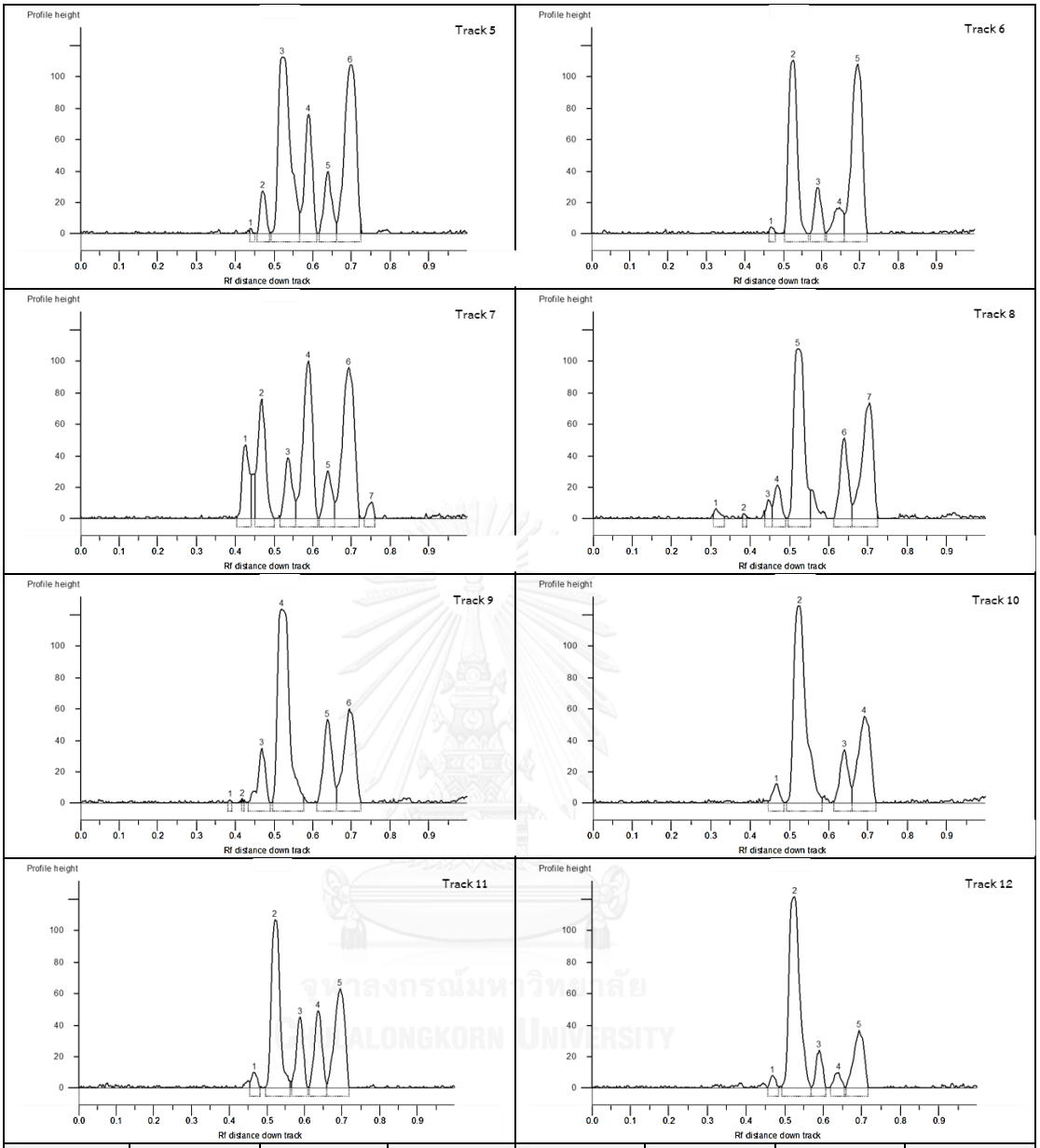


Tract 13		Tract 14		Tract 15		Tract 16		Tract 17		Tract 18		Tract 19	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	2660	1	2340	1	2660	1	2080	1	2330	1	2090	1	2130
2	2330	2	2070	2	2090	2	1860	2	2090	2	1850	2	1730
3	2070	3	1620	3	1620	3	1620	3	1850	3	1510	3	1490
4	1620	4	1120	4	960	4	1130	4	1610	4	1040	4	1160
5	1260					5	960	5	1150	5	860	5	970
6	1140							6	960	6	190		
7	950												

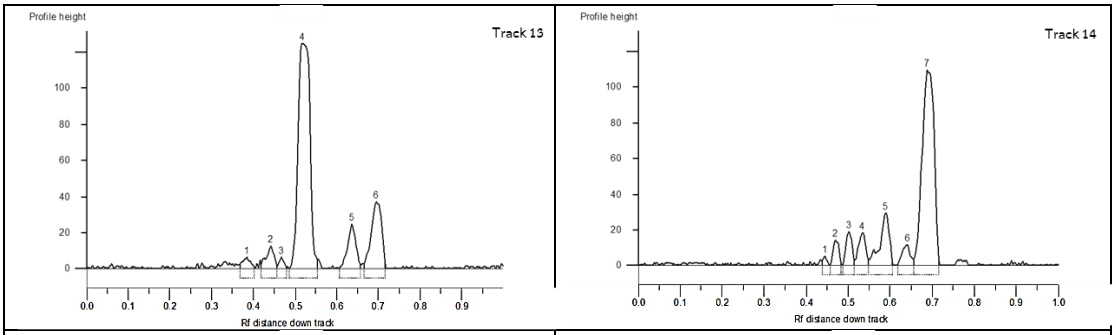


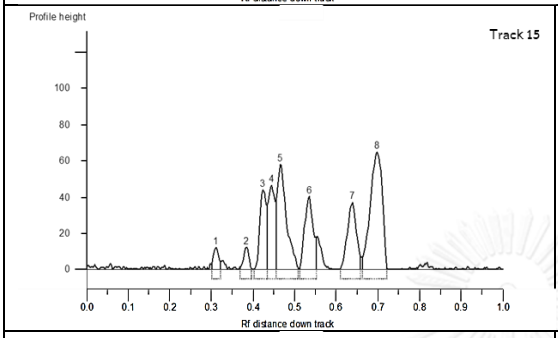
Tract 1		Tract 2		Tract 3		Tract 4	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1180	1	1480	1	1660	1	1940
2	1000	2	1260	2	1500	2	1730
3	700	3	850	3	1250	3	1480
		4	690	4	1000	4	1200
				5	850	5	1000
				6	700	6	850
						7	700
						8	570

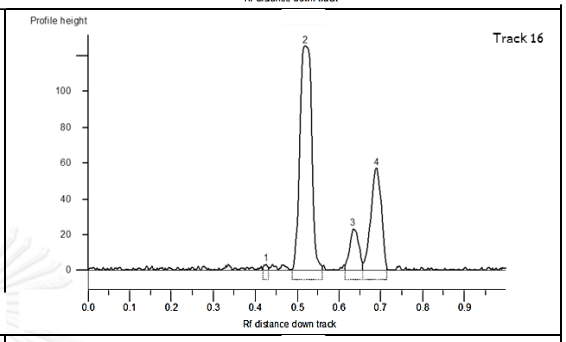
Table 47 Fingerprint and molecular weight plots of ISSR 31 (AGAGAGAGAGAGAGT), an annealing temperature 44 °C, fragment sizes range from 570 to 2520 bps, 75.00 % polymorphic

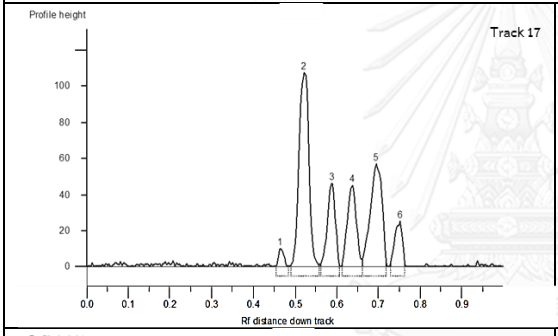


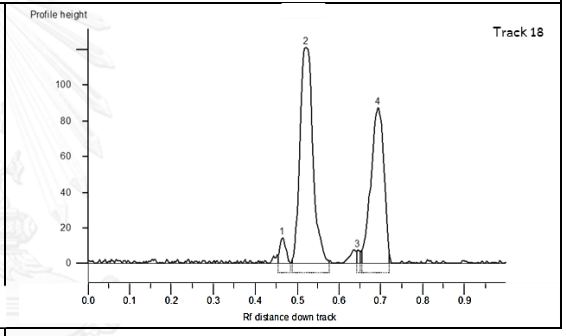
Tract 5		Tract 6		Tract 7		Tract 8		Tract 9		Tract 10		Tract 11		Tract 12	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1640	1	1490	1	1710	1	2480	1	1940	1	1500	1	1500	1	1490
2	1480	2	1240	2	1490	2	1960	2	1750	2	1240	2	1250	2	1240
3	1250	3	1000	3	1200	3	1600	3	1490	3	850	3	1000	3	1000
4	1000	4	830	4	1000	4	1490	4	1260	4	720	4	860	4	850
5	850	5	700	5	850	5	1250	5	850			5	700	5	710
6	690			6	710	6	850	6	700						
				7	570	7	680								

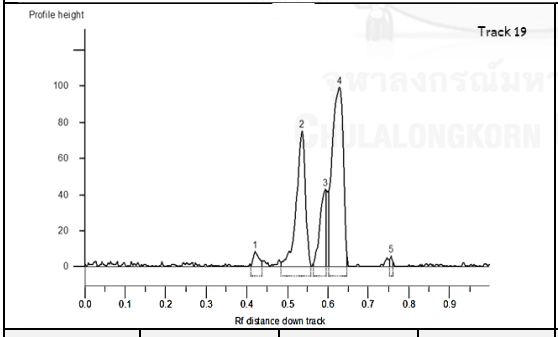












Tract 13		Tract 14		Tract 15		Tract 16		Tract 17		Tract 18		Tract 19	
No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)	No.	Mol. Weight (bps)
1	1960	1	1610	1	2520	1	2520	1	1510	1	1510	1	1740
2	1630	2	1490	2	1970	2	1970	2	1250	2	1250	2	1200
3	1500	3	1350	3	1730	3	1730	3	1000	3	830	3	980
4	1270	4	1210	4	1610	4	1610	4	850	4	710	4	880
5	860	5	1010	5	1510			5	700			5	570
6	700	6	850	6	1200			6	570				
		7	720	7	850								
				8	690								

APPENDIX C

Mangiferin quantitative analysis

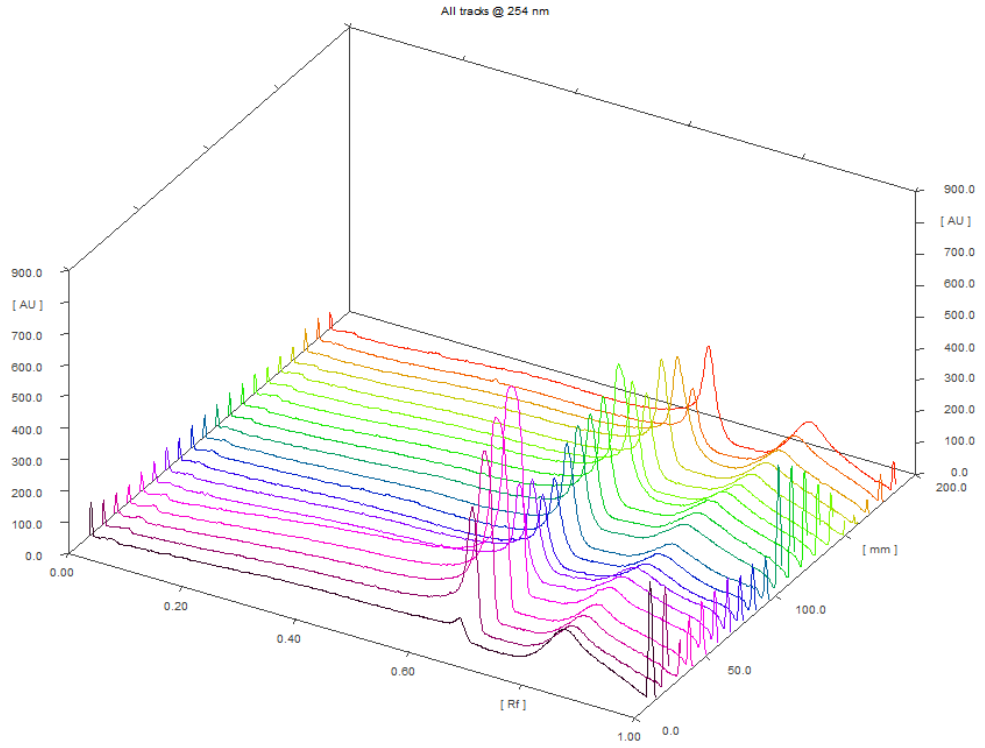
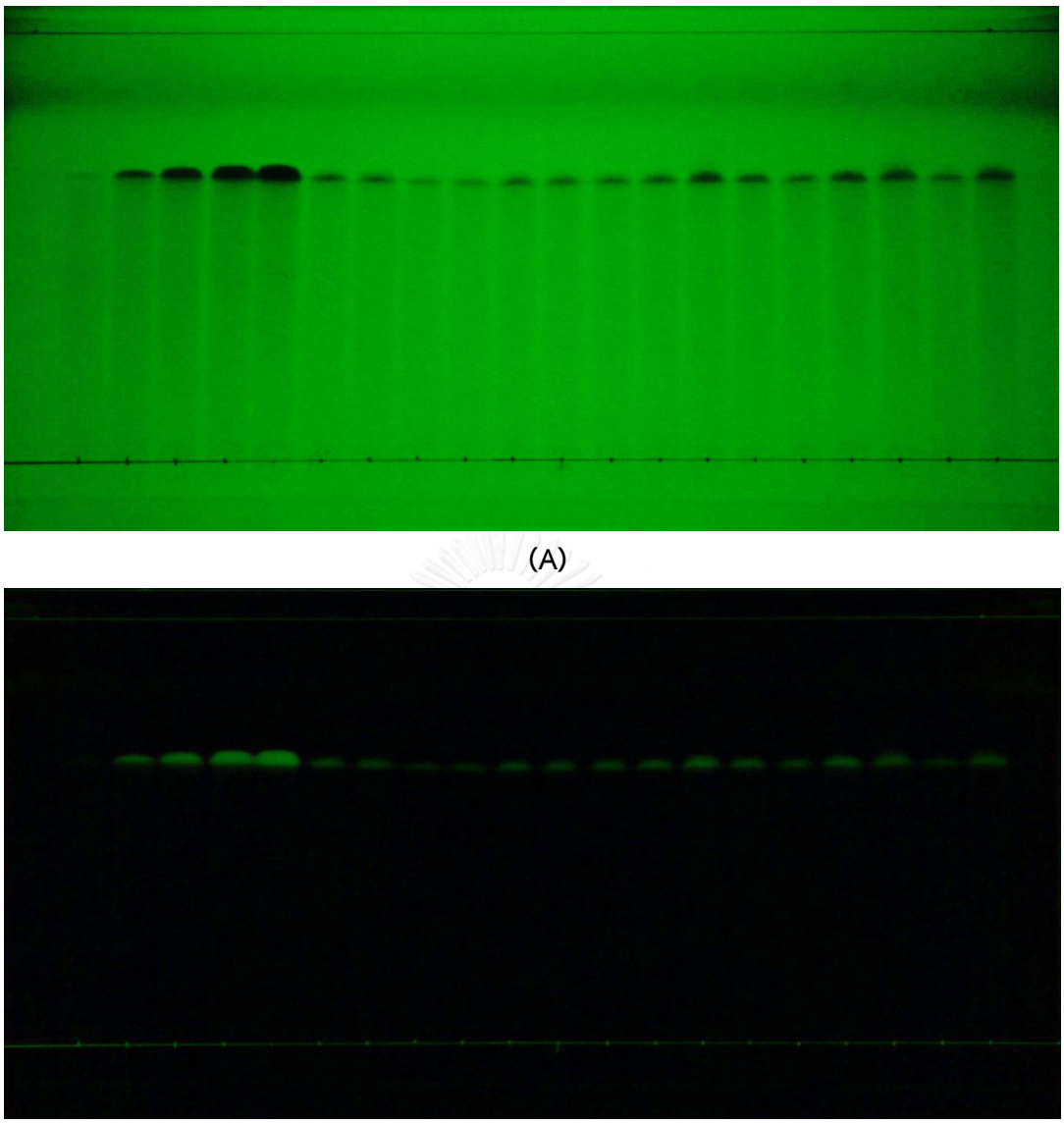


Figure 46 3D TLC densitometry chromatogram of mangiferin standard and the extracts



(A)

(B)

Figure 47 (A) TLC chromatogram developed by a mobile phase; ethyl acetate: methanol: formic acid (3.9: 6: 0.1) visual under 254 nm; mangiferin standard (tract 1-5) and *Mangifera indica* leaf extracts from 15 different locations (tract 6-20); (B) TLC image subtract background using image J software; mangiferin standard (tract 1-5) and *Mangifera indica* leaf extracts from 15 different locations (tract 6-20)

APPENDIX D

Antidiabetic activities

Table 48 Yeast alpha-glucosidase inhibition of mango leaf extract, mangiferin and acarbose

Conc.	OD_{405}			% inhibition of mango leaf extract			
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Average
Control	0.309	0.294	0.308				
0.156	0.043	0.035	0.044	86.084	88.095	85.714	86.631±1.28
0.078	0.086	0.076	0.150	72.168	74.150	51.299	65.872±12.66
0.039	0.199	0.165	0.178	35.599	43.878	42.208	40.561±4.38
0.020	0.262	0.204	0.162	15.210	30.612	47.403	31.075±16.10
0.005	0.270	0.245	0.205	12.621	16.667	33.442	20.910±11.04

Conc.	OD_{405}			% inhibition of mangiferin			
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Average
Control	0.346	0.312	0.319				
2.5	0.026	0.02	0.02	92.486	93.590	93.730	93.269±0.68
1.3	0.093	0.045	0.106	73.121	85.577	66.771	75.156±9.57
0.6	0.118	0.148	0.153	65.896	52.564	52.038	56.833±7.85
0.31	0.242	0.177	0.253	30.058	43.269	20.690	31.339±11.34
0.156	0.254	0.262	0.195	26.590	16.026	38.871	27.162±11.43

Conc.	OD_{405}			% inhibition of acarbose			
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Average
Control	0.348	0.322	0.326				
40	0.074	0.088	0.097	78.736	72.671	70.245	73.884±4.37
20	0.124	0.133	0.111	64.368	58.696	65.951	63.005±3.81
10	0.198	0.191	0.148	43.103	40.683	54.601	46.129±7.44
5	0.200	0.237	0.195	42.529	26.398	40.184	36.370±8.72
2.5	0.258	0.245	0.254	25.862	23.913	22.086	23.954±1.89

Table 49 Rat alpha-glucosidase inhibition of mango leaf extract, mangiferin and acarbose

Conc.	OD $_{405}$			% inhibition of mango leaf extract			
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Average
Control	1.048	0.989	0.986				
2.500	0.158	0.129	0.138	84.924	86.957	86.004	85.961±1.02
1.250	0.595	0.586	0.645	43.225	40.748	34.584	39.519±4.45
0.625	0.764	0.668	0.760	27.099	32.457	22.921	27.492±4.78
0.313	0.902	0.869	0.918	13.931	12.133	6.897	10.987±3.65
0.156	0.972	0.977	0.970	7.252	1.213	1.623	3.363±3.37

Conc.	OD $_{405}$			% inhibition of mangiferin			
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Average
Control	0.949	0.915	0.911				
1.25	0.073	0.093	0.041	92.308	89.836	95.499	92.548±2.84
0.625	0.246	0.26	0.223	74.078	71.585	75.521	73.728±1.99
0.313	0.688	0.626	0.638	27.503	31.585	29.967	29.685±2.06
0.156	0.867	0.879	0.82	8.641	3.934	9.989	7.521±3.18
0.078	0.937	0.864	0.901	1.264	5.574	1.098	2.645±2.54

Conc.	OD $_{405}$			% inhibition of acarbose			
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Average
Control	0.907	0.923	0.893				
1.25	0.391	0.379	0.356	56.891	58.938	60.134	58.654±1.64
0.625	0.427	0.416	0.392	52.922	54.930	56.103	54.651±1.61
0.313	0.494	0.506	0.507	45.535	45.179	43.225	44.646±1.24
0.156	0.587	0.578	0.560	35.281	37.378	37.290	36.650±1.19
0.039	0.660	0.700	0.688	27.233	24.160	22.956	24.783±2.21

Table 50 Pancreatic alpha-amylase inhibition of mango leaf extract, mangiferin and acarbose

Conc.	OD $_{405}$			% inhibition of mango leaf extract			
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Average
Control	1.280	1.386	1.396				
5.000	0.239	0.208	0.245	81.328	84.993	82.450	97.241±3.18
2.500	0.653	0.687	0.729	48.984	50.433	47.779	82.924±1.88
1.250	0.916	0.923	0.956	28.438	33.405	31.519	49.066±1.33
0.625	0.797	0.992	1.055	37.734	28.427	24.427	31.121±2.51
10.000	0.082	0.018	0.008	93.594	98.701	99.427	30.196±6.83

Conc.	OD $_{405}$			% inhibition of mangiferin			
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Average
Control	0.784	0.912	0.874				
5.000	0.032	0.040	0.041	95.918	95.614	95.309	95.614±0.30
2.500	0.090	0.095	0.098	88.520	89.583	88.787	88.964±0.55
1.250	0.333	0.302	0.331	57.526	66.886	62.128	62.180±4.68
0.625	0.551	0.572	0.658	29.719	37.281	24.714	30.571±6.33
0.313	0.717	0.719	0.681	8.546	21.162	22.082	17.264±7.56

Conc.	OD $_{405}$			% inhibition of acarbose			
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Average
Control	1.003	0.924	0.850				
0.156	0.265	0.222	0.238	73.579	75.974	72.000	73.851±2.00
0.078	0.431	0.313	0.401	57.029	66.126	52.824	58.659±6.80
0.039	0.597	0.426	0.479	40.479	53.896	43.647	46.007±7.01
0.020	0.572	0.631	0.549	42.971	31.710	35.412	36.698±5.74
0.010	0.666	0.546	0.673	33.599	40.909	20.824	31.777±10.17

APPENDIX E

Anticancer activity

Table 51 Cytotoxic activities of mango leaf extract, mangiferin and doxorubicin

Conc.	BT474 (OD_{540})				% Survival of mango leaf extract				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.12	1.04	1.06	1.11					
	1.08	1.00	1.06	1.06					
0.02	0.85	0.74	0.78	0.79	116.48	101.69	107.03	108.54	108.43 ± 6.11
0.2	0.89	0.98	0.73	0.94	121.40	133.99	99.64	128.38	120.86 ± 15.05
2	0.75	0.70	0.68	0.69	102.38	95.26	93.07	94.30	96.25 ± 4.18
20	0.95	0.87	0.78	0.87	129.48	118.53	106.62	118.39	118.25 ± 9.33
200	0.32	0.51	0.51	0.51	44.35	70.08	70.08	70.21	63.68 ± 12.89

Conc.	Chago-K1 (OD540)				% Survival of mango leaf extract				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	0.89	0.89	0.88	0.99					
	0.91	0.90	0.92	0.98					
0.02	0.56	0.49	0.62	0.64	100.40	87.54	110.05	114.69	103.17 ± 12.00
0.2	0.52	0.50	0.60	0.62	92.90	88.79	107.55	111.48	100.18 ± 11.03
2	0.56	0.70	0.64	0.59	99.33	124.88	114.52	105.40	111.03 ± 11.14
20	0.42	0.54	0.57	0.65	75.75	95.94	101.12	115.59	97.10 ± 16.48
200	0.31	0.35	0.25	0.26	54.85	61.81	44.66	46.27	51.90 ± 7.98

Conc.	Hep-G2 (OD540)				% Survival of mango leaf extract				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.07	0.95	0.93	1.02					
	1.05	1.04	1.05	1.00					
0.02	0.60	0.62	0.74	0.73	94.12	96.61	114.53	113.28	104.64 ± 10.77
0.2	0.69	0.67	0.54	0.69	107.99	103.78	84.61	107.21	100.90 ± 11.01
2	0.55	0.53	0.57	0.57	85.24	83.21	88.51	88.35	86.33 ± 2.57
20	0.49	0.48	0.62	0.47	76.20	74.80	96.30	73.39	80.17 ± 10.81
200	0.30	0.49	0.52	0.35	47.37	76.98	81.03	54.85	65.06 ± 16.47

Table 51 (Cont.) Cytotoxic activities of mango leaf extract, mangiferin and doxorubicin

Conc.	Kato-III (OD540)				% Survival of mango leaf extract				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.22	1.28	1.39	1.44					
	1.31	1.37	1.39	1.37					
0.02	0.77	0.92	0.72	0.79	109.03	129.68	102.10	111.58	113.10 ± 11.76
0.2	0.76	0.64	0.72	0.85	107.62	90.37	101.40	120.63	105.00 ± 12.63
2	0.73	0.61	0.53	0.51	103.52	85.70	74.81	72.26	84.07 ± 14.21
20	0.56	0.56	0.60	0.49	79.05	79.76	84.28	69.58	78.17 ± 6.18
200	0.35	0.48	0.31	0.44	49.50	68.45	43.13	61.80	55.72 ± 11.49

Conc.	SW620 (OD540)				% Survival of mango leaf extract				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.12	1.29	1.17	1.31					
	1.31	1.23	1.25	1.32					
0.02	1.14	1.16	0.87	1.24	100.75	102.08	77.07	109.41	97.33 ± 14.03
0.2	1.13	1.17	0.86	1.20	99.43	103.76	75.74	105.61	96.13 ± 13.84
2	1.25	1.26	1.09	1.29	110.56	110.91	96.42	113.57	107.87 ± 7.75
20	1.15	1.17	1.14	1.24	101.99	103.40	100.49	109.68	103.89 ± 4.04
200	0.53	0.63	0.65	0.75	46.84	55.86	57.36	66.46	56.63 ± 8.03

Conc.	CCD (OD540)				% Survival of mango leaf extract				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	0.40	0.46	0.41	0.51					
	0.34	0.39	0.36	0.46					
0.02	0.30	0.36	0.30	0.30	84.73	100.49	85.01	83.60	88.46 ± 8.05
0.2	0.32	0.33	0.29	0.29	89.51	92.33	82.76	82.48	86.77 ± 4.93
2	0.38	0.37	0.29	0.34	107.25	103.87	81.07	96.27	97.11 ± 11.64
20	0.35	0.46	0.33	0.34	99.65	128.64	92.33	96.27	104.22 ± 16.55
200	0.57	0.57	0.59	0.67	159.32	161.29	167.21	187.19	168.75 ± 12.74

Table 51 (Cont.) Cytotoxic activities of mango leaf extract, mangiferin and doxorubicin

Conc.	Wi-38 (OD540)				% Survival of mango leaf extract				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	0.86	0.86	0.84	0.97					
	0.85	0.83	0.85	0.99					
0.02	0.55	0.60	0.58	0.55	113.96	123.85	120.14	112.52	117.62 ± 5.31
0.2	0.58	0.54	0.55	0.59	119.94	111.90	113.76	120.56	116.54 ± 4.36
2	0.47	0.55	0.61	0.48	95.83	112.73	125.91	99.74	108.55 ± 13.64
20	0.53	0.49	0.49	0.49	109.84	101.60	101.60	100.15	103.30 ± 4.41
200	0.30	0.28	0.25	0.24	61.41	57.50	51.73	50.28	55.23 ± 5.17

Conc.	BT474 (OD_{540})				% Survival of mangiferin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.12	1.04	1.06	1.11					
	1.08	1	1.06	1.06					
0.02	0.64	0.74	0.65	0.61	87.19	101.28	89.24	84.04	90.44 ± 7.54
0.2	0.58	0.56	0.87	0.81	79.38	77.06	118.53	111.14	96.53 ± 21.37
2	0.51	0.6	0.71	0.75	70.08	81.44	96.9	102.51	87.73 ± 14.76
20	0.89	0.89	0.66	0.68	122.09	122.09	89.65	92.93	106.69 ± 17.83
200	0.65	0.67	0.83	0.76	88.55	91.43	112.92	103.61	99.13 ± 11.27

Conc.	Chago-K1 (OD540)				% Survival of mangiferin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	0.89	0.89	0.88	0.99					
	0.91	0.90	0.92	0.98					
0.02	0.60	0.55	0.42	0.46	106.48	97.72	75.21	82.36	90.44 ± 14.23
0.2	0.59	0.64	0.48	0.47	106.12	113.98	86.29	84.50	97.72 ± 14.61
2	0.53	0.65	0.49	0.56	94.33	116.12	86.82	100.58	99.46 ± 12.45
20	0.65	0.46	0.53	0.40	116.66	82.36	93.97	72.18	91.29 ± 19.11
200	0.77	0.55	0.60	0.41	138.10	97.36	106.30	73.43	103.80 ± 26.75

Table 51 (Cont.) Cytotoxic activities of mango leaf extract, mangiferin and doxorubicin

Conc.	Hep-G2 (OD540)				% Survival of mangiferin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.07	0.95	0.93	1.02					
	1.05	1.04	1.05	1.00					
0.02	0.72	0.62	0.60	0.68	111.88	96.30	93.96	106.58	102.18 ± 8.48
0.2	0.47	0.67	0.57	0.47	72.61	104.56	88.35	73.55	84.77 ± 15.03
2	0.44	0.62	0.65	0.42	67.94	96.77	100.82	65.45	82.74 ± 18.63
20	0.69	0.85	0.75	0.58	108.14	131.83	116.87	91.00	111.96 ± 17.06
200	0.87	0.78	0.83	0.62	135.88	121.70	129.02	95.99	120.65 ± 17.43

Conc.	Kato-III (OD540)				% Survival of mangiferin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.22	1.28	1.39	1.44					
	1.31	1.37	1.39	1.37					
0.02	0.67	0.69	0.61	0.54	94.89	97.72	85.84	75.66	88.53 ± 9.96
0.2	0.75	0.71	0.72	0.66	106.63	99.98	102.25	93.62	100.62 ± 5.42
2	0.59	0.70	0.74	0.64	83.30	98.85	104.08	90.08	94.08 ± 9.22
20	0.86	0.77	0.69	0.58	121.62	108.18	97.58	81.46	102.21 ± 16.98
200	0.65	0.64	0.80	0.64	92.20	89.94	112.57	90.65	96.34 ± 10.86

Conc.	SW620 (OD540)				% Survival of mangiferin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.12	1.29	1.17	1.31					
	1.31	1.23	1.25	1.32					
0.02	1.14	1.13	1.02	1.23	101.02	99.96	90.15	108.97	100.02 ±7.72
0.2	1.05	1.19	1.08	1.04	93.15	104.99	95.54	92.27	96.49 ± 5.84
2	1.26	1.25	1.07	1.17	111.27	110.03	94.39	103.23	104.73 ± 7.75
20	1.31	1.21	1.10	1.03	115.78	107.29	97.22	91.12	102.85 ± 10.90
200	1.01	1.17	1.17	1.06	89.44	103.58	103.31	93.24	97.39 ± 7.16

Table 51 (Cont.) Cytotoxic activities of mango leaf extract, mangiferin and doxorubicin

Conc.	CCD (OD540)				% Survival of mangiferin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	0.40	0.46	0.41	0.51					
	0.34	0.39	0.36	0.46					
0.02	0.29	0.35	0.37	0.28	80.79	98.24	103.03	77.41	89.87 ± 12.66
0.2	0.30	0.36	0.32	0.32	85.57	102.46	90.64	90.08	92.19 ± 7.22
2	0.31	0.36	0.30	0.35	88.11	102.18	85.57	97.68	93.38 ± 7.85
20	0.50	0.42	0.38	0.41	140.46	117.95	107.53	116.54	120.62 ± 14.01
200	0.59	0.60	0.49	0.50	166.64	168.90	138.21	139.62	153.34 ± 16.69

Conc.	Wi-38 (OD540)				% Survival of mangiferin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	0.86	0.86	0.84	0.97					
	0.85	0.83	0.85	0.99					
0.02	0.4	0.45	0.57	0.58	81.61	92.94	117.88	119.32	102.94 ± 18.68
0.2	0.51	0.51	0.59	0.49	105.51	105.31	120.56	100.77	108.04 ± 8.63
2	0.53	0.51	0.62	0.62	109.84	104.07	128.59	126.94	117.36 ± 12.26
20	0.61	0.61	0.66	0.59	124.68	126.12	136.01	122.41	127.31 ± 6.00
200	0.77	0.77	0.83	0.67	158.48	158.68	170.63	138.07	156.47 ± 13.51

Conc.	BT474 (OD_{540})				% Survival of doxorubicin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.12	1.04	1.06	1.11					
	1.08	1.00	1.06	1.06					
0.001	1.04	1.02	1.02	1.01	97.17	95.30	96.05	94.64	95.79 ± 1.09
0.01	1.07	0.96	0.90	0.98	100.27	89.86	84.14	91.45	91.43 ± 6.68
0.1	0.94	0.94	0.89	0.94	88.54	88.54	83.57	87.98	87.16 ± 2.41
1	0.45	0.53	0.48	0.51	42.21	49.81	44.74	47.56	46.08 ± 3.31
10	0.33	0.37	0.41	0.32	30.77	34.71	38.18	30.30	33.49 ± 3.70

Table 51 (Cont.) Cytotoxic activities of mango leaf extract, mangiferin and doxorubicin

Conc.	Chago-K1 (OD540)				% Survival of doxorubicin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	0.89	0.89	0.88	0.99					
	0.91	0.90	0.92	0.98					
0.001	1.01	0.90	0.88	0.82	109.89	97.50	95.43	89.46	98.07 ± 8.59
0.01	1.00	0.95	0.94	0.84	108.70	102.83	102.17	91.52	101.30 ± 7.15
0.1	0.82	0.83	0.73	0.70	88.80	89.67	79.78	75.76	83.51 ± 6.83
1	0.38	0.39	0.39	0.37	41.09	42.39	42.83	39.67	41.49 ± 1.42
10	0.12	0.11	0.12	0.11	13.48	11.63	12.72	12.07	12.47 ± 0.81

Conc.	Hep-G2 (OD540)				% Survival of doxorubicin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.07	0.95	0.93	1.02					
	1.05	1.04	1.05	1.00					
0.001	0.95	1.02	0.98	0.95	93.35	100.25	96.60	93.74	95.98 ± 3.19
0.01	0.88	0.93	0.98	0.92	87.04	91.77	96.80	90.39	91.50 ± 4.05
0.1	0.56	0.58	0.57	0.57	55.59	57.37	56.58	55.79	56.33 ± 0.81
1	0.14	0.12	0.14	0.14	14.10	12.12	13.90	13.60	13.43 ± 0.89
10	0.27	0.32	0.34	0.31	26.81	31.35	33.42	30.75	30.58 ± 2.76

Conc.	Kato-III (OD540)				% Survival of doxorubicin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.22	1.28	1.39	1.44					
	1.31	1.37	1.39	1.37					
0.001	1.41	1.34	1.21	1.24	104.77	99.28	89.84	92.36	96.56 ± 6.77
0.01	1.28	1.40	1.22	1.12	95.34	103.66	90.43	83.00	93.11 ± 8.67
0.1	1.13	1.15	1.01	1.12	84.12	85.68	75.27	83.52	82.15 ± 4.67
1	0.63	0.49	0.61	0.49	46.52	36.71	45.40	36.26	41.22 ± 5.49
10	0.65	0.62	0.63	0.65	48.52	46.15	46.44	48.52	47.41 ± 1.29

Table 51 (Cont.) Cytotoxic activities of mango leaf extract, mangiferin and doxorubicin

Conc.	SW620 (OD540)				% Survival of doxorubicin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	1.12	1.29	1.17	1.31					
	1.31	1.23	1.25	1.32					
0.001	1.36	1.27	1.20	1.11	109.12	101.60	95.60	89.12	98.86 ± 8.53
0.01	1.35	1.29	1.37	0.81	107.76	102.80	109.68	65.12	96.34 ± 21.01
0.1	1.19	1.15	1.14	1.21	94.88	92.32	91.52	96.64	93.84 ± 2.35
1	0.86	1.02	0.96	0.93	68.56	81.36	76.88	74.16	75.24 ± 5.35
10	0.28	0.31	0.30	0.30	22.72	24.48	24.16	24.32	23.92 ± 0.81

Conc.	CCD (OD540)				% Survival of doxorubicin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	0.40	0.46	0.41	0.51					
	0.34	0.39	0.36	0.46					
0.001	0.39	0.35	0.35	0.33	92.79	84.38	83.65	78.85	84.92 ± 5.79
0.01	0.40	0.33	0.33	0.33	94.95	79.09	79.09	79.81	83.23 ± 7.82
0.1	0.40	0.34	0.37	0.38	95.43	82.69	87.98	91.59	89.42 ± 5.42
1	0.28	0.28	0.31	0.30	67.55	66.83	74.04	72.12	70.13 ± 3.50
10	0.26	0.23	0.24	0.19	63.46	54.81	58.17	45.43	55.47 ± 7.58

Conc.	Wi-38 (OD540)				% Survival of doxorubicin				
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 1	Exp 2	Exp 3	Exp 4	Average
Control	0.86	0.86	0.84	0.97					
	0.85	0.83	0.85	0.99					
0.001	1.07	0.78	0.79	0.77	121.24	88.94	90.08	87.12	96.84 ± 16.31
0.01	0.89	0.93	0.90	0.82	101.00	105.77	102.59	93.26	100.65 ± 5.31
0.1	0.51	0.51	0.61	0.56	57.55	58.46	69.04	63.92	62.24 ± 5.33
1	0.23	0.20	0.25	0.23	26.39	22.75	28.43	25.59	25.79 ± 2.36
10	0.27	0.29	0.33	0.29	30.59	32.87	37.76	33.32	33.64 ± 3.00

VITA

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Publications

1. Ganogpichayagrai A, Palanuvej C, Ruangrungsi N. Evaluation of antimicrobial, antioxidant and cytotoxic potentials of Tree Karl Phit remedy. Bulletin of Health, Science and Technology. 2014; 12(1): 27-32.

2. Ganogpichayagrai, A, Rungsihirunrat K, Palanuvej C, Ruangrungsi N. Characterization of Mangifera indica cultivars in Thailand based on macroscopic, microscopic, and genetic characters. Journal of advanced pharmaceutical technology and research. 2016; 7(4): 127-133.

3. Ganogpichayagrai A, Palanuvej C, Ruangrungsi N. Antidiabetic and anticancer activities of Mangifera indica cv. Okrong leaves. Journal of advanced pharmaceutical technology and research. 2017; 8(1): (In press)

Oral presentations

1. Ganogpichayagrai A, Palanuvej C, Ruangrungsi N. "Evaluation of antimicrobial, antioxidant and cytotoxic potentials of Tree Karl Phit remedy" The 1st International conference on herbal medicines: Herbal remedies: The art of sciences, November 2, 2012, Pathum Thani, Thailand.

2. Ganogpichayagrai A, Palanuvej C, Ruangrungsi N. "Microscopic evaluation of selected seventeen Mangifera indica cultivars leaves in Thailand" The 2nd International conference on advanced pharmaceutical research: Strategies and innovation in pharmaceutical research, safety, efficacy and quality, March 12, 2015, Pathum Thani, Thailand.

3. Ganogpichayagrai A, Rungsihirunrat K, Palanuvej C, Ruangrungsi N. "Characterization of genetic relationships among Mangifera indica cultivars in Thailand" Mae Fah Luang University international conference 2016 on advance in medical and health sciences and a Kaleidoscope of traditional and complementary medicines international conference on fostering traditional and complementary medicine through research, November 23-25, 2016, Chiang Rai, Thailand.

4. Ganogpichayagrai A, Palanuvej C, Ruangrungsi N. "Antidiabetic and anticancer activities of Mangifera indica cv. Okrong leaves" 4th International conference on food and agricultural sciences, December 25-27, 2016, Kyoto, Japan.

Honor

1. Bronze prize in oral presentation in the 1st International conference on herbal medicines: Herbal remedies: The art of sciences, November 2, 2012.