PHARMACOGNOSTIC SPECIFICATION AND CHRYSIN CONTENT OF *OROXYLUM INDICUM* SEED

Miss Kirana Praserdmek

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
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ข้อกำหนดทางเภสัชเวทและปริมาณวิเคราะห์สารคริซินในเมล็ดเพกา

นางสาวกิรณา ประเสริฐเมฆ

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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By	Miss Kirana Praserdmek
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Thesis Advisor	Assistant Professor Chanida Palanuvej, Ph.D.
Thesis Co-Advisor	Associate Professor Nijsiri Ruangrungsi, Ph.D.

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.....Dean of the College of Public Health Sciences

(Professor Sathirakorn Pongpanich, Ph.D.)

THESIS COMMITTEE

.....Chairman

(Assistant Professor Naowarat Kanchanakhan, Ph.D.)

.....Thesis Advisor

(Assistant Professor Chanida Palanuvej, Ph.D.)

.....Thesis Co-Advisor

(Associate Professor Nijsiri Ruangrungsi, Ph.D.)

.....External Examiner

(Professor Leena Suntornsuk, Ph.D.)

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Chulalongkorn University

กิรณา ประเสริฐเมฆ : ข้อกำหนดทางเภสัชเวทและปริมาณวิเคราะห์สารคริซินในเมล็ดเพกา (PHARMACOGNOSTIC SPECIFICATION AND CHRYSIN CONTENT OF *OROXYLUM INDICUM* SEED) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. ชนิดา พลานุเวช, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. นิจศิริ เรืองรังษี, 127 หน้า.

เพกาหรือลิ้นฟ้ามีชื่อวิทยาศาสตร์คือ *Oroxylum indicum* (L.) Kurz ซึ่งอยู่ในวงศ์ Bignoniaceae ในประเทศไทยมีการใช้ในการรักษาอาการไอและใช้เป็นยาระบาย อย่างไรก็ตาม ข้อกำหนดทางเภสัชเวทและการวิเคราะห์ปริมาณสารคริซินในเมล็ดเพกายังไม่เคยมีการจัดทำในประเทศไทยมาก่อน ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาข้อกำหนดทางเภสัชเวท วิเคราะห์ปริมาณสารคริซินและองค์ประกอบทางเคมีของน้ำมันเมล็ดเพกา โดยเก็บรวบรวมเมล็ดเพกาจาก 15 พื้นที่ในประเทศไทย ผลการทดลองพบว่าน้ำหนักที่หายไปเมื่อทำให้แห้ง ปริมาณความชื้น ปริมาณเถ้ารวม เถ้าที่ไม่ละลายในกรด ปริมาณสารสกัดด้วยเอธานอลและปริมาณสกัดด้วยน้ำ มีค่าเป็นร้อยละ 3.32 ± 0.12, 6.89 ± 0.80, 4.40 ± 0.08, 0.47 ± 0.05, 9.74 ± 0.68 และ 12.11 ± 0.80 โดยน้ำหนักแห้ง ตามลำดับ ลักษณะทางมหทรรศน์และจุลทรรศน์ของเมล็ดเพกาได้แสดงรายละเอียดทางพฤกษศาสตร์โดยภาพลายเส้น การจัดทำลายพิมพ์องค์ประกอบทางเคมีโดยเทคนิคทางทินเลเยอร์โครมาโทกราฟี สกัดเมล็ดเพกาด้วยเอทานอลเข้มข้นร้อยละ 95 โดยใช้เครื่องซอกห์เลต วิเคราะห์สารคริซินในสิ่งสกัดจากเอทานอลด้วยเทคนิคทินเลเยอร์โครมาโทกราฟี โดยใช้ตัวทำละลายโทลูอีนต่อคลอโรฟอร์มต่ออะซีโตนต่อกรดฟอร์มิก (5:4:1:0.2) เป็นวัฏภาคเคลื่อนที่ ตรวจวัดภายใต้แสงขาว แสงอัลตราไวโอเลตความยาวคลื่น 254 นาโนเมตร ความยาวคลื่น 365 นาโนเมตร และทำปฏิกิริยาเกิดสีกับ 10% ของกรดซัลฟิวริก วิเคราะห์ปริมาณสารคริซินโดยวิธีเดนซิโทเมทรีภายใต้แสงอัลตราไวโอเลตที่ความยาวคลื่น 269 นาโนเมตรและวิธีภาพถ่ายวิเคราะห์ภายใต้แสงอัลตราไวโอเลตที่ความยาวคลื่น 254 นาโนเมตรโดยใช้โปรแกรมอิมเมจเจ พบสารคริซินปริมาณร้อยละ 0.17 ± 0.05 และ 0.20 ± 0.07 โดยน้ำหนัก การทดสอบความเที่ยงตรงของวิธีทินเลเยอร์โครมาโทกราฟี-เด็นซิโตเมทรีและวิธีทินเลเยอร์โครมาโทกราฟีโดยวิเคราะห์ภาพถ่ายโดยใช้โปรแกรมอิมเมจเจ พบว่ามีช่วงวิเคราะห์แบบโพลีโนเมียล 0.3 - 1.2 ไมโครกรัมต่อจุด โดยมีค่าสัมประสิทธิ์การตัดสินใจเท่ากับ 0.9998 และ 0.9998 ค่าเฉลี่ยการคืนกลับร้อยละ 109.2 - 109.7 และ 96.7 - 116.0 ค่าความสามารถในการวัดซ้ำ มีค่าระหว่างร้อยละ 1.98 ± 0.78% RSD และ 3.00 ± 2.23% RSD ค่าความแม่นยำมีค่าระหว่างร้อยละ 4.30 ± 2.24% RSD และ 5.12 ± 3.71% RSD ขีดจำกัดของการตรวจพบมีค่า 0.015 และ 0.016 ไมโครกรัมต่อจุด และขีดจำกัดของการหาปริมาณ มีค่า 0.046 และ 0.048 ไมโครกรัมต่อจุด ค่าความคงที่มีค่าสัมประสิทธิ์ของการกระจายร้อยละ 2.05% RSD และ 4.07% RSD ตามลำดับ นอกจากนี้ สกัดน้ำมันเมล็ดเพกาด้วยปิโตรเลียมอีเทอร์โดยใช้เครื่องซอกห์เลต วิเคราะห์กรดไขมันในน้ำมันเมล็ดเพกาโดยวิธีแกสโครมาโทกราฟี-แมสสเปคโทเมทรี และพบว่าประกอบด้วยกรดไขมัน 9 ชนิด ได้แก่ กรดโอเลอิก (ร้อยละ 67.99 ± 5.98), กรดปามมิติก (ร้อยละ 10.22 ± 0.88), กรดบีฮีนิค (ร้อยละ 7.28 ± 5.60), กรดกอนโดอิค (ร้อยละ 5.60 ± 1.28) กรดลิกโนซิลิค (ร้อยละ 3.11 ± 2.83), กรดไลโนเลอิก (ร้อยละ 2.69 ± 0.77), กรดสเตียริก (ร้อยละ 1.86 ± 0.42), กรดอะราชิดิก (ร้อยละ 1.02 ± 0.31) และกรด 9, 10 ไดไฮดรอกซี่สเตียริก (ร้อยละ 0.41 ± 0.20) ตามลำดับ

สาขาวิชา วิทยาศาสตร์สาธารณสุข ลายมือชื่อนิสิต .....

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ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KIRANA PRASERDMEK: PHARMACOGNOSTIC SPECIFICATION AND CHRYSIN CONTENT OF *OROXYLUM INDICUM* SEED. ADVISOR: ASST. PROF. CHANIDA PALANUVEJ, Ph.D., CO-ADVISOR: ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., 127 pp.

Oroxylum indicum (L.) Kurz, belonging to Bignoniaceae family, is known in Thai common name as Phe-Kaa. In Thailand, it has been used in the treatment of a cough and as a purgative. However, the pharmacognostic specification of this crude drug and its chrysin content have never been established in Thailand. Consequently, this study aimed to exhibit the pharmacognostic specification, analyses of the content of chrysin and fixed oil chemical constituents of *O. indicum* seeds. *O. indicum* were collected from 15 different locations in Thailand. The results indicated that the contents of loss on drying, moisture, total ash, acid-insoluble ash, ethanol soluble extractive value and water soluble extractive value were found to be 3.32 ± 0.12, 6.89 ± 0.80, 4.40 ± 0.08, 0.47 ± 0.05, 9.74 ± 0.68 and 12.11 ± 0.80 % by dry weight, respectively. The macroscopic and microscopic characteristics were illustrated in detail. Thin layer chromatographic fingerprints were also established. *O. indicum* seeds were extracted in 95% ethanol using Soxhlet apparatus. Chrysin of the ethanolic extract was analyzed by thin layer chromatography (TLC) using silica gel 60 GF_{254} as stationary phase, toluene: chloroform: acetone: formic acid (5:4:1:0.2) as mobile phase, visualization under daylight, UV 254 nm, UV 365 nm and staining with 10 % sulfuric acid reagent. For quantitative analysis of chrysin, the contents in 15 ethanolic extracts were evaluated by TLC-densitometry under UV 269 nm and TLC image analysis under UV 254 nm using image J software which were respectively found to be 0.17 ± 0.05 and 0.20 ± 0.07 % by dry weight. The method validity of TLC-densitometry and TLC image analysis were shown that the calibration range was polynomial with 0.3 – 1.2 µg/spot (R^2=0.9998 and R^2=0.9998). The accuracy was 109.2 – 109.7 %recovery and 96.7 – 116.0 % recovery. The repeatability was 1.98 ± 0.78%RSD and 3.00 ± 2.23%RSD. The intermediate precision was 4.30 ± 2.24%RSD and 5.12 ± 3.71%RSD. LOD were 0.015 and 0.016 and LOQ were 0.046 and 0.048 µg/spot. The robustness was 2.05%RSD and 4.07%RSD, respectively. Additionally, the fixed oil of *O. indicum* was extracted in petroleum ether using Soxhlet apparatus. Fatty acid composition of seed oil was analyzed by GC/MS after methylation and 9 components were identified. It was indicated that the main compositions of the fatty acids in *O. indicum* seed oil are oleic acid (67.99 ± 5.98), palmitic acid (10.22 ± 0.88), behenic acid (7.28 ± 5.60), gondoic acid (5.60 ± 1.28), lignoceric acid (3.11 ± 2.83), linoleic acid (2,69 ± 0.77), stearic acid (1.86 ± 0.42), arachidic acid (1.02 ± 0.31) and 9, 10-dihydroxystearic acid (0.41 ± 0.20) % respectively.

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Advisor's Signature .....
Co-Advisor's Signature .....

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LIST OF ABBREVIATION

%	=	Percent
°C	=	Degree Celsius
µg/ml	=	Microgram per milliliter
µl	=	Microliter
µm	=	Micrometer
EI	=	Electron impact or electron ionization
eV	=	Electron volt
F_{254}	=	Fluorescence under 254 nanometers
F. A. M. E.	=	Fatty acid methyl ester
g	=	Gram
g/l	=	Gram per liter
GC/MS	=	Gas chromatography/mass spectrometry
HPLC	=	High performance liquid chromatography
ICH	=	International Conference on Harmonisation
kg	=	Kilogram
l	=	Liter
LOD	=	Limit of detection
LOQ	=	Limit of quantification
m	=	Meter

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min	=	Minute
mm	=	Millimeter
mg	=	Milligram
mg/ml	=	Milligram per milliliter
ml	=	Milliliter
nm	=	Nanometer
R_f	=	Retention factor
rpm	=	Revolutions per minute
RSD	=	Relative standard deviation
S	=	Slope of regression line
SD	=	Standard deviation
TLC	=	Thin layer chromatography
UV	=	Ultraviolet
WHO	=	World Health Organization

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CHAPTER I
INTRODUCTION

Background and significance of the study

For centuries, folklore healing practices and herbal medicines have been promoted as the basic health projects for prevention and treatment of diseases by World Health Organization [1]. People living in the rural areas are usually poor and away from the health service; thereby, using herbs is an alternative way for medication with low cost. However, the herbal medicines are not always safe just because it is natural. Herbal remedies need a quality control of ingredients. Medicinal plant materials can be adulterated or contaminated with other plant parts or other materials which lead to low quality. Therefore, it is necessary to ensure the quality control of the herb using modern techniques for standardization of the herbal remedies.

The Bignoniaceae is a family of about 100 genera and 800 species, having mainly climbing plants. *Oroxylum indicum* (L.) Kurz is a plant belonging to the Bignoniaceae family with a medium-sized structure growing in Southern China, South and South East Asia [2]. *O. indicum* is recognizable due to its distinctive feature which is the flat, kidney-shaped, yellow-green seeds surrounded by a light-brown papery wing with a diameter of about five to eight centimeters. *O. indicum* is a medicinal plant locally known in Thai as Phe Kaa, its seed has been used for the treatment of cough, throat infections, hypertension and purgative. In Chinese medicinal system, *O. indicum*

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seed or Mu Hu Die has been extensively used to treat cough, acute or chronic bronchitis, pharyngitis, pertussis and other respiratory disorders [3]. In Nepal, the seed is used as a digestive while a seed paste is applied to treat abscess and wound [4]. Four flavonoids found in the seeds of *O. indicum* are chrysin, baicalein, oroxin A and oroxin B [5]. Chrysin is one of interesting subgroup of flavonoids called flavone)Figure.1(. Along with other polyphenolic compounds, chrysin is known to have potent biological activities including anti-inflammation [6], anti-cancer [7, 8] and anti-oxidation [9].

The previous studies investigated the pharmacognostic parameters on *O. indicum* stem bark in India [10] and *O. indicum* root in Thailand [11]. Although *O. indicum* seed is widely used in many countries, there is no pharmacognostic specification available for the standardization of this crude drug in Thailand. Additionally, plant seed is a source of natural fixed oil which does not evaporate on warming. It is composed of glycerides of fatty acid. Fixed oil, unlike volatile oil, is permanent, leaving a stain on an absorbent surface. It cannot be obtained by distillation, instead it is gained by expression or extraction. Fatty acid composition of fixed oil is one of chemical characteristics of each plant seed oil and affects the physical property as liquid, semi-solid or solid at ambient temperature.

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Therefore, this study aims to establish the pharmacognostic specification of *O. indicum* seed with the special reference to the chrysin content and fixed oil constituents of *O. indicum* seed crude drug in Thailand.

Research gap

The pharmacognostic specification parameters, chrysin content and the fatty acid constituents of fixed oil in *Oroxylum indicum* seed in Thailand have never been determined.

Objectives

1. To determine the standardization parameters of *Oroxylum indicum* seed in Thailand.
2. To investigate the chrysin content in *Oroxylum indicum* seed by TLC image analysis using Image J free software compared to TLC densitometry.
3. To investigate the fatty acid constituents of fixed oil in *Oroxylum indicum* seed by gas chromatography – mass spectrometry.

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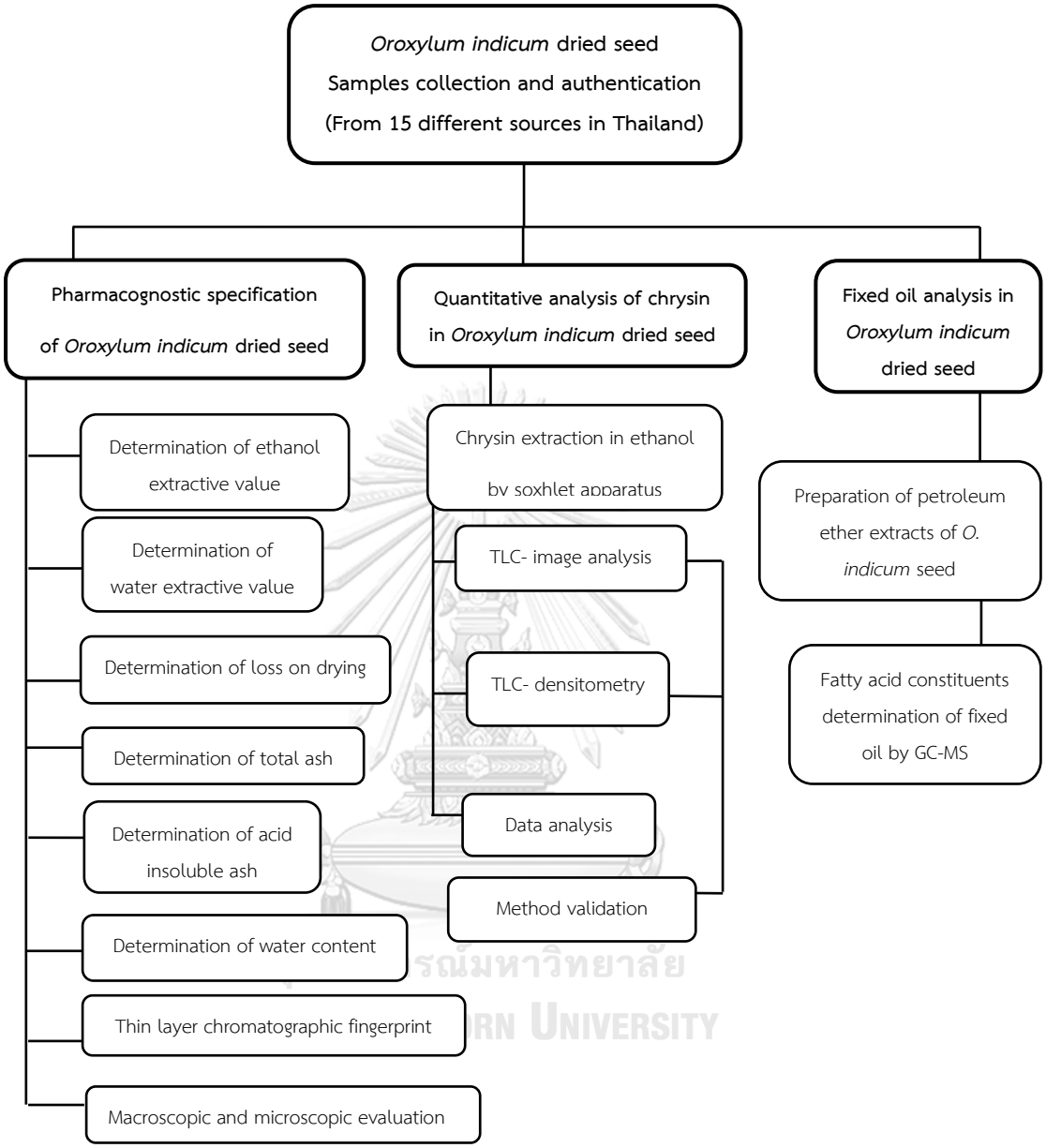
Conceptual framework



CHAPTER II

REVIEW OF LITERATURE

Taxonomy of the genus *Oroxylum* [12]

The taxonomic hierarchy of the genus *Oroxylum* can be classified as follow:

Kingdom Plantae

Class Magnoliophyta

Order Lamiales

Family Bignoniaceae

Genus *Oroxylum*

Species *Oroxylum indicum* Kurz

Common Names: Phe-Kaa, Kaa-do-dong (Karen-Kanchanaburi), Do-ka, Dok-ka, Du-kae (Karen-Mae Hong Son), Be-ko (Malay-Narathiwat), Ma-lit-mai, Ma-lin-mai, Lit-mai (Northern), Lin-faa (Loei), Maak-lin-kaang, Maak-lin-saang (Shan-Northern), Mu-Hu-Die (China), Indian Trumpet Flower (India), Broken Bones Tree, and Midnight horror

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Synonyms [13]

Bignonia indica L.

Calosanthes indica (L.) Blume.

Hippoxylon indica (L.) Raf.

Spathodea indica (L.) Pers.

Plant description of *Oroxylum indicum*

"The botanical characteristics of *O. indicum* are a medium tree, 4-12 m tall, branched at top; bark light-brown. Leaves are 3-7 cm long, 2-3 pinnate with opposite pinnae, rachis very stout, cylindrical, leaflets 2-4 pairs, 6-12 cm long and 4-10 cm broad, ovate or elliptic, acuminate, glabrous or base rounded; petioles of the lateral leaflets 6-15 mm long. Flowers numerous, foetid, in large erect racemes, 0.3-0.6 meter-long or even more pedicels 6-30 mm long. Corolla usually lurid-purple, reaching 10 cm long, fleshy lobes about 4 cm long with crisped margins. Stamens 5, slightly exerted beyond the corolla tube, two long, two short and the last one little shorter than the 4, filaments cottony at the base. Fruits that hang down from bare branches. Those long fruits curve downward 10-20 cm and resemble the wings of a large bird or dangling sickles or swords in the night. Seeds numerous, 6-8 cm long, winged all round except at the base" [13].

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Figure 1 *Oroxylum indicum* (L) Kurz (A) Leaves, (B) Seeds, (C) Fruits and (D) Inflorescence

Traditional uses of *Oroxylum indicum*

Roots are sweet, astringent, bitter, acrid, refrigerant [14]. Roots has been widely used in expectorate, febrifuges, digestive, carminative, constipating and diuretic. Tonic is useful in cough, hiccough, asthma, bronchitis, vomiting, fever, gout, rheumatoid arthritis, flatulence, dyspepsia, diarrhea and wound. Root bark is used to treat tuberculosis, stomatitis and nasopharyngeal cancer [14]. Bark decoction is taken for curing gastric ulcer and the bark powder is used to treat for scabies, mouth cancer and other skin diseases.

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Leaves are used as carminative, stomachic and antiflatulence. Leaf decoction is used for treating rheumatic pain, ulcer, cough, and bronchitis [14].

Mature fruits have to acrid and sweet. It is useful in treating gastropathy, dyspepsia, cardiac disorders, jaundice, cough, bronchitis and haemarrhoids [15].

In traditional Chinese medicine, the seed of this plant named as Mu Hu Die, has been used in the treatment of cough, acute or chronic bronchitis, pharyngitis, pertussis and other respiratory disorders [3]. Seeds are used as purgative. The seeds are ground with fire soot and the paste is applied to the neck for relief of tonsil pain. This plants are used in traditional Indian Ayurvedic medicine, included in famous tonic formulations [15, 16].

Biological activity of *Oroxylum indicum*

Antioxidant activities

Yan *et al*. was studied for the antioxidant property by DPPH and ORAC assays in *O. indicum* seed. Scutellarein-7-O-gentiobioside and scutellarein-7-O-glucoside, which were detected as the main compounds in the methanolic extract of *O. indicum* seeds showed highest antioxidant activity compared to other isolated flavonoids and the positive control quercetin. A comparative study of antioxidant activities revealed that the hydroxyl group at the C-6 position and glycosylation of 7-hydroxide resulted in an increased and decreased antioxidant activities, respectively [17].

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The previous study evaluated the *in vitro* antioxidant potential of different parts of *Oroxylum indicum*. 2, 2-Diphelyl 1-picrylhydrazyl (DPPH), nitric oxide, superoxide anion and hydroxyl radical scavenging potential and reductive ability assay of methanol extract of different parts i.e. root, root bark, stem, stem bark, leaves and fruits were performed. Leaf and root bark extracts exhibited highest free radical scavenging activity than stem bark, stem and fruit extracts. The extract of leaf shown maximum reductive ability and found to contain the maximum amount of polyphenolic compounds. The highest free radical activity may be due to presence of polyphenolic compounds [18].

Another study on the antioxidant activity of ethanol and water extract of *O. indicum* leaves was performed in two *in vitro* models viz. radical scavenging activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) reduction and nitric oxide radical scavenging activity in Griess reagent system. Ethanol extract possessed significant antioxidant activity in both models. The result proved that ethanol extract of *O. indicum* leaves expressed free radical scavenging activity [19].

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Anti-gout activities

For the *in vitro* xanthine oxidase inhibition assay using column-switching liquid chromatography with a diode array detection system, the methanolic extract of *O. indicum* seeds displayed potent anti-gout activity. Allopurinol was used as a primary xanthine oxidase inhibitor. Among the compounds of the methanolic extract, baicalein

showed higher anti-gout activity compared to allopurinol. On the other hand, baicalin, oroxin A and oroxin B exhibited lower activities [20].

Anti-inflammatory activities

Different amounts of aqueous extract of *O. indicum* leaves (150 mg/kg and 300 mg/kg) were given to the experimental rats with carrageenan induced rat paw edema. Compared to a positive control diclofenac sodium, the extract at a dose of 300 mg/kg expressed highest anti-inflammatory activity [21].

With respect to the inhibition of tumor necrosis factor-**α**-induced nuclear factor *kappa B* activation in Human embryonic kidney 293 cells (HEK-293), *O. indicum* stem bark extracted with dichloromethane exhibited potent anti-inflammatory activity. In the midst of the isolated compounds extracted with dichloromethane and ethyl acetate, oroxylin A, hispidulin, chrysin and baicalein revealed strong inhibitory activities [22].

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Anticancer and anti-tumor activities

According to the in vitro proliferation of Human promyelocytic leukemia *cells* (HL-60), baicalein, one of the active constituents isolated from *O. indicum* fruit in methanolic extracts. Therefore, the tumor cells have apoptosis at a higher dose, because the methanolic extracts of *O. indicum* fruit possessed strong cytotoxicity against HL-60 cells [23].

For the anticancer activity, the baicalein of flavonoid was found to be the active compound in the methanolic extract of *O. indicum* fruit. The results shown that baicalein caused a 50% inhibition of Human promyelocytic leukemia cells (HL-60) at concentrations of 25-30 μM. The inhibition of proliferation of HL-60 cells due to 36-48 hours exposure to 10 or 20 μM baicalein was associated with the accumulation of cells at S or G2M phases. It showed that the significant cytotoxicity against HL-60 cells and induced the apoptosis of tumor cells at a higher dose [23].

The previous study, the petroleum ether and chloromethylene extracts of the *O. indicum* stem bark shown cytotoxicity against human caucasian breast adenocarcinoma (MDA-MB-231) and hepatic human cell line (WRL-68) in the cell proliferation kit II assay (XTT). All of the extracts, particularly the petroleum ether hot extract, exhibited significantly ($P<0.05$) higher cytotoxicity in MDA-MB-231 cells. The hot extract of petroleum ether-induced apoptosis in estrogen receptor (ER)-negative MDA-MB-231 cells, and also exhibited antimetastatic potential in the tumor cell migration inhibition assay [24].

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Antidiabetic activities

In 2011, Temboli reported that the aqueous and ethanolic extracts of *O. indicum* roots had high potential in reducing the elevated serum glucose levels in alloxan-induced diabetic rats. A dose of 500 mg/kg of both aqueous and ethanolic extracts decreased serum glucose by 50.92 and 49.59%, respectively. Moreover, these

two extracts were also effective in lowering the levels of glucose, triglycerides and total cholesterol in dexamethasone-induced insulin resistance in rats [25].

The fifty percentage aqueous and ethanolic extract of the stem bark shown a significant, strong *in vitro* antidiabetic effect by inhibiting the activities of α-glucosidase, bovine serum albumin (BSA) glycation, and insulin-sensitive potential in glucose transporter-4 translocation studies in mature 3T3-L1 adipocyte cells. The *in vitro* anti-diabetic finding was further confirmed by the *in vivo* effect observed in STZ-induced diabetic rat by administration of the extract for 28 days, analysis of its glucose-lowering capacity, normalized antioxidant status, reduction of liver biomarker enzymes, total cholesterol and *High-density lipoprotein* levels. Moreover, the restoration of glycated hemoglobin protein as well as enhanced insulin sensitivity ($P<0.01$) in response to glucose transporter-4 translocation in skeletal muscles [26].

Anthelmintic activities

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In 2000, Jessica et al. reported the evaluation of anthelmintic activity of *O. indicum* against equine strongyle eggs in vitro and with respect to ivermectin, one of the most powerful deworming agents. *O. indicum* extract at 2×10^{-5} g/mL and greater was able to delay the hatching of the strongyle eggs, and a dose of 2×10^{-1} g/mL resulted in 0% hatching. No viability (0%) of the strongyle eggs and larvae were found at a dose of 2×10^{-4} g/mL and greater. This indicated that *O. indicum* had high potential as an anthelmintic agent to defend equine strongyles [27].

Anti-hepatotoxic activities

In 2009, Tenpe *et al.* evaluated the anti-hepatotoxic activity of *O. indicum* leaves extract with four different solvents (petroleum ether, chloroform, ethanol and aqueous) against Carbon tetrachloride (CCl_4) induced hepatotoxicity. Four various extracts were given to induced rats at a dose of 300 mg/kg body weight to measure the serum enzymes levels. All the test groups showed a significant reduction in serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), *alkaline phosphatase* (ALP), total bilirubin content and a significant increase in the level of total protein. The ethanolic extract was found to be most effective compared to the rest of extracts [28].

Antimicrobial activities

In 2003, Kawsar *et al.* investigated the anti-microbial activity of various extracts of *O. indicum* against fourteen pathogenic bacteria (five gram-positive and nine gram-negative) and seven pathogenic fungi using disk diffusion method. The crude ethyl acetate extract exhibited mild to moderate activity against all bacteria and fungi; on the other hand, the methanolic extract expressed little activity against bacteria but moderate activity against fungi. The minimum inhibitory concentration (MIC) of two isolated flavonoid compounds from *O. indicum* were determined against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Shigella dysenteriae*. The results

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were between 64–128 µg/ml. A study by Thatoi *et al.* further confirmed the activity by using different strains [29, 30].

Antifungal activities

In 1998, Ali *et al*. studied the effect of dichloromethane extract of *O. indicum* against dermatophytes and wood rot fungi and suggested a strong antifungal activity in dichloromethane extract of *O. indicum* [31].

Toxicity activities

In 2011, Joshi *et al*. examined the acute oral toxicity of *O. indicum* root bark aqueous extract in overnight-fasted Wistar rats. The results shown no mortality at doses ranging from 175-5000 mg/kg [32].

In 2011, Tamboli *et al*. reported that a dose of 5 g/kg of both aqueous and ethanolic extracts of *O. indicum* roots in the overnight fasted rats displayed, no effect on any behavioral changes [25].

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In 2012, Joshi *et al.* revealed that the butanol extract of *O. indicum* root bark (175–2000 mg/kg) tested orally in rats for 14 days shown no mortality or behavioral changes [33].

In 2011, Tripathy *et al*. suggested that the water and ethanolic extracts of *O. indicum* stem bark (5–3000 mg/kg) treated orally in rats for 72 hours expressed no behavioral changes or mortality [34].

In 2011, Kaldate *et al*. experimented the long term toxicity test by orally giving normal and diabetic rats methanolic and water extracts of *O. indicum* leaves (300 mg/kg) for 28 days. No signs of toxicity were found in the biochemical blood test or in hematological parameters [35].

In 2013, Singh and Kakkar tested the 50% ethanolic extract of *O. indicum* stem bark (250 mg/kg) in normal and diabetic rats orally for 28 days. No observation was found on the hematological and clinical parameters [26].

In 2014, Thokchom *et al*. reported that the 60% ethanolic extract of *O. indicum* fruits was given intra-peritoneally to Albino rats at a dose of 2.5, 3.0, 3.5, 4.0 and 4.5 g/kg for 30 days. Mortality was recorded every 24 hours. The LD_{50} in 72 hours was 4.02 g/kg, and the maximum tolerated dose was 2.25 g/kg [36].

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Flavonoids

Flavonoids are a group of natural compounds with variable polyphenolic structures and are found in plants. The flavonoids are categorized, according to chemical structure, into flavones, flavonols, flavanones, flavanonol, isoflavones and flavanols. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have

antioxidant, anti-inflammatory, antitumor, anti-allergic, antiviral, and antiplatelet activities.

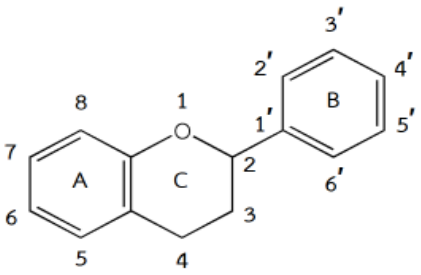

Figure 2 Basic flavonoid structure

Chemically flavonoids are based into a fifteen-carbon structure including of two benzene rings (A and B) linked *via* a heterocyclic pyrane ring (C) as shown in Figure 2. “They can be classified into a variety of classes such as flavones (e.g., chrysin, apigenin, and baicalein), flavonols (e.g., kaempferol, quercetin, and galangin), flavanones (e.g., pinocembrin, hesperetin and butin), flavanonols (e.g., taxifolin), isoflavones (e.g., genistein and daidzein), and flavanols (e.g., catechin)”. Their general structures are shown in Figure 3. The various classes of flavonoids differ in the level of oxidation and pattern of superseding of the C ring, while individual compounds within a class differ in the pattern of superseding of the A and B rings [37].

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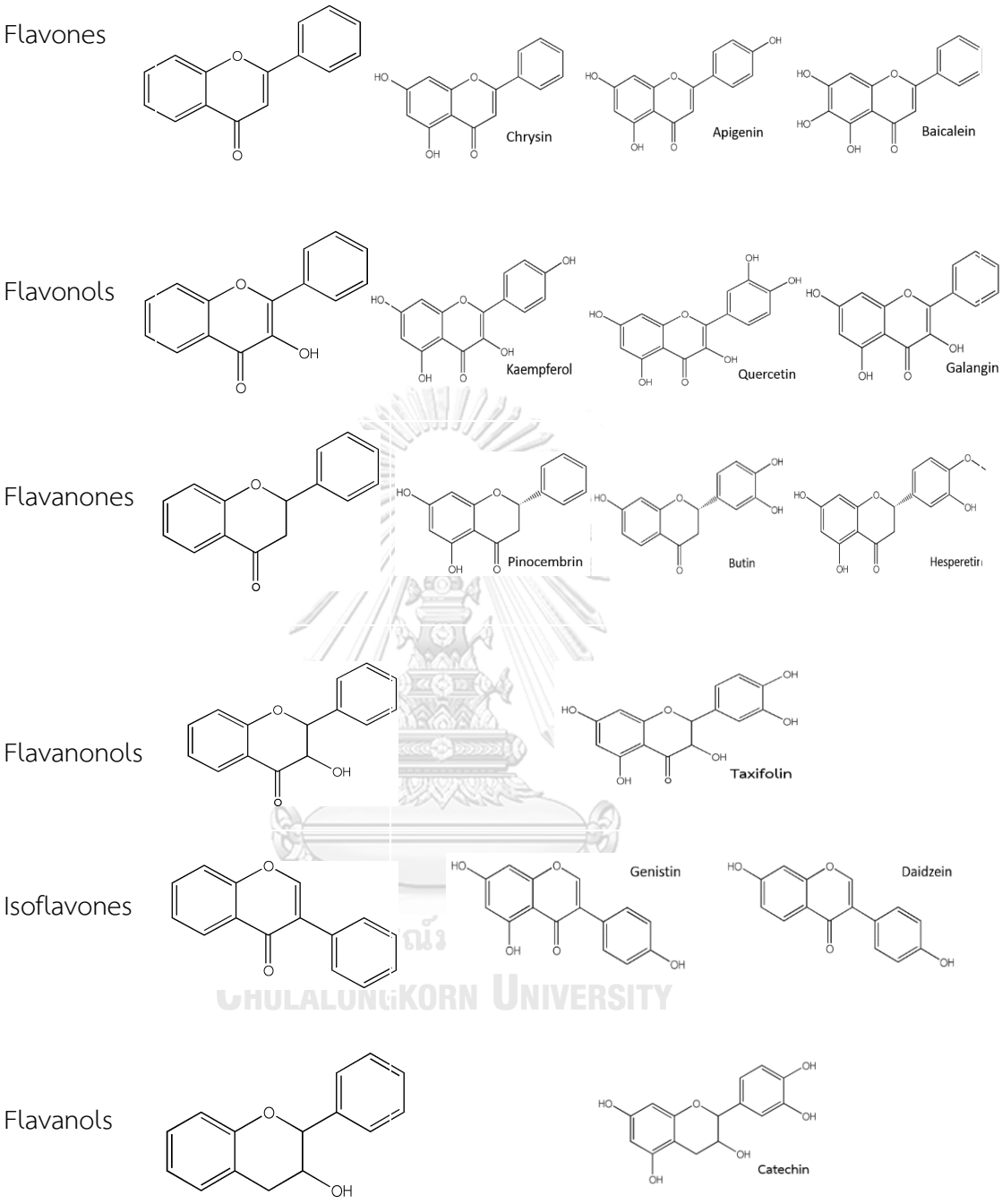

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Figure 3 Structure of flavonoids

Chemical constituents of flavonoids in *Oroxylum indicum*

In 2003, Chen studied the leaves of *O. indicum* and found flavones, baicalein (5, 6, 7-trihydroxy flavone), chrysin and their glycosides as the active compounds [5]. In 2006, Ali and Khandhar reported that oroxylin A and baicalein were the major components of the root bark of *O. indicum* [31, 38]. Later, in 2007, Roy *et al*. reported that the major constituent of the fruits was baicalein [23]. After that, in 2011, Yan *et al*. found that baicalein, chrysin, oroxin A, oroxin B and chrysin7-O-diglucoside were the major chemical constituents of seeds [17]. The other studies, also reported baicalein, chrysin and oroxylin A as the major flavones in the extract from the stem bark of *O. indicum* [39, 40].

Chrysin (5, 7-Dihydroxyflavone)

Chrysin (Figure 4) is a naturally occurring flavone which is one of the active compounds which isolated from the seed of *O. indicum*.

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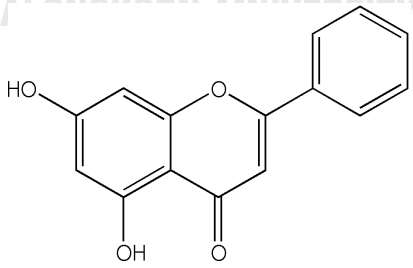

Figure 4 Chrysin structure

Table 1 The data of compound in chrysin

Chemical name	Chrysin; 5, 7-Dihydroxyflavone; 480-40-0; Chrysine; 5, 7-Dihydroxy-2-phenyl-4H-chromen-4-one; Chrysin
IUPAC name	5, 7-dihydroxy-2-phenylchromen-4-one
Molecular formula	$C_{15}H_{10}O_4$
Molecular weight	254.241 g/mol
Physical description	Yellow-tan powder
Melting Point	284-286 °C
Classification	Flavonoid, Flavone

Pharmacological activities of chrysin

Antioxidant

The antioxidant activities of chrysin were demonstrated on D-galactosamine hepatotoxic rats. Chrysin was found to increase the activities of free scavenging enzymes such as of superoxide dismutase, catalase, glutathione peroxidase and decrease lipid peroxidation products in the erythrocyte and tissues of hepatotoxic rats and these values improved towards normalcy on treatment with chrysin [41].

Chrysin administration to rats with ethanol-induced liver injury significantly decreased the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes, and significantly elevated the activity of superoxide dismutase,

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catalase, glutathione-related enzymes and the levels of reduced glutathione, vitamin C and vitamin E in the tissues and circulation compared with those of the unsupplemented ethanol-treated rats [42].

Hepatoprotective

The effect of chrysin was done on hepatic marker enzymes such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase in D-galactosamine hepatotoxic rats. Among various dose of chrysin (5, 10, and 20 mg/kg/body weight), a dose of 20 mg was most effective for reducing the hepatic marker enzyme activities [41].

The previous studies of the metabolism of the dietary flavonoid chrysin by human intestinal and hepatic cell lines as well as rat hepatocytes demonstrated glucuronidation and sulfation to be the rat-limiting metabolic reactions. The results corroborated the protective action of chrysin in D-galactosamine intoxication of rats, particularly noticeable with the lower dose used (25 mg/kg body weight). Supplementation with this flavonoid ameliorated the hepatoprotective and antioxidant activity in D-galactosamine-induced hepatitis in rats [43].

Anticancer

Chrysin has suppressed the growth of 1, 2-dimethylhydrazine-induced colorectal cancer in female Wistar rats by recovering antioxidant mineral levels in the intestinal mucosa, reducing nitrosative stress and cell proliferation [44].

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Khan *et al*., reported that the chrysin a dose of 250 mg/kg could abrogate N-nitrosodiethylamine induced early hepatocarcinogenesis and induce apoptosis in preneoplastic nodules in male Wistar rats [45].

In addition to its antitumor activity in a variety of cancer cell lines, chrysin also suppressed tumor growth *in vivo* in a number of animal studies. In an animal model of N-nitrosodiethylamine induced and ferric nitrilotriacetate promoted renal carcinogenesis, chrysin (20 and 40 mg/kg) was shown to exhibit chemopreventive activity by ameliorating oxidative stress and inflammation *via* NF-kB pathway [46].

Anti-inflammation

Ahad et al., reported the nephroprotective effects of chrysin (5, 7-dihydroxyflavone) in a high fat diet/streptozotocin (HFD/STZ)-induced type 2 diabetic Wistar albino rat model. Chrysin treatment for 16 weeks post induction of diabetes significantly abrogated renal dysfunction and oxidative stress. Chrysin treatment considerably reduced renal TNF-α expression and inhibited the nuclear transcription factor-kappa B (NF-kB) activation. Furthermore, chrysin treatment improved renal pathology and suppressed transforming growth factor-beta (TGF-β), fibronectin and collagen-IV protein expressions in renal tissues. Moreover, chrysin also significantly reduced the serum level of pro-inflammatory cytokine, interleukin-1beta (IL-1β) and IL-6 [47].

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Kaidama and Gacche, reported the anti-inflammatory activity of chrysin on acute inflammation (carrageenan-induced paw edema) and chronic inflammation (cotton pellet granuloma), tested in guinea pigs. Group one (control) received. The results were indicated that chrysin at a dose of 40 mg/kg, body weight exhibited significant inhibition in acute and chronic inflammation models, which was comparable with the standard drug, indomethacin a dose of 10 mg/kg [48].

Ha et al., reported that the treatment with chrysin significantly inhibited the release of nitric oxide (NO) and pro-inflammatory cytokines such as tumor necrosis factor-a (TNF-a) and interleukin-1b (IL-1b) in lipopolysaccharide (LPS)-stimulated microglia. The expressions of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) were also significantly inhibited by chrysin. Importantly, chrysin was also shown to inhibit the activations of c-Jun N-terminal kinase (JNK) and nuclear factor-kB (NF-kB), which were key mediators of neuroinflammation [49].

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Lipids

Lipids (fixed oil, fat and waxes) are esters of long-chain fatty acids and alcohols. The common feature of these lipids is that they are all esters of moderate to long chain fatty acids. Acid or base-catalyzed hydrolysis yields the component fatty acid, together with the alcohol component of the lipid. Natural fatty acids may be saturated or unsaturated, the saturated acids have higher melting points than unsaturated acids of corresponding size. In fixed oil and fat, the triesters of fatty acids with glycerol compose the class of lipids known as fats and oils. These triglycerides are found in

both plants and animals, and compose one of the major food groups of our diet. Triglycerides that are solid or semisolid at room temperature are classified as fats, and occur predominantly in animals. Those triglycerides that are liquid are called oils and originate chiefly in plants. Waxes are esters of fatty acids with long chain monohydric alcohols. Natural waxes are often mixtures of such esters, and may also contain hydrocarbons. Waxes are widely distributed in nature. The leaves and fruits of many plants have waxy coatings, which may protect them from dehydration and small predators. The feathers of birds and the fur of some animals have similar coatings which serve as a water repellent.

Fixed oil and fat are obtained from either plants (olive oil, peanut oil) or animals (lard). Their primary function is food (energy) storage. The fixed oil and fat are important products used pharmaceutically, industrially and nutritionally. Waxes may also be of plant or animal origin. Many drugs contain fixed oil and fat as their principal constituents; the fixed oil and fat are often separated from the crude vegetable drugs (by expression) or the crude animal drugs (by rendering or extraction) and are employed as drugs in the refined state. Their physicochemical characteristics depend on the types of fatty acid which are saturated fatty acids and unsaturated fatty acids. Fixed oil differs from fat only as to melting point; those that are liquid at normal temperatures are known as fatty or fixed oil, whereas those that are semisolid or solid at ordinary temperature are known as fats. Fixed oil and fats of vegetable and animals

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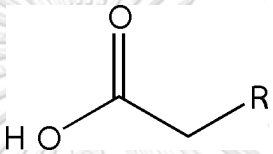
origin are obtained by expression in hydraulic presses. If the expression is carried out in the cold, the oil is known as a "cold pressed oil". In contrast, if the expression is carried out in heat, the oil is known as a "hot pressed oil". Non-polar organic solvents can be used for the extraction of oils [50].

Chemically, the fixed oil and fats are glycerides of fatty acids that have the general formula:



Usually, the glycerides of unsaturated fatty acids are liquid, whereas the glycerides of saturated fatty acids of chain length are solid. The eminence of either type in an oil determines whether the mixture is liquid or solid. Some of the more common fatty acids are:

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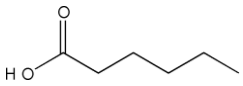
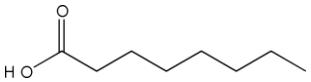
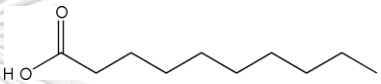
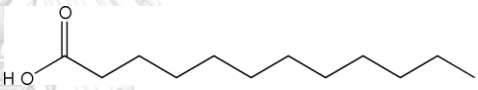
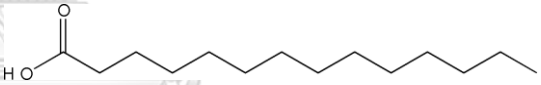
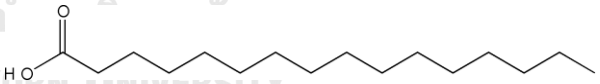
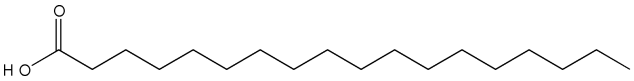
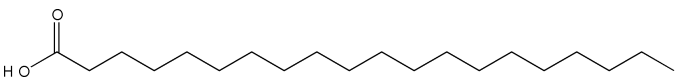
Table 2 Chemical structure of fatty acids

Fatty acids	Molecular Formula	Chemical structure
Caproic	$C_6H_{12}O_2$	
Caprylic	$C_8H_{16}O_2$	
Capric	$C_{10}H_{20}O_2$	
Lauric	$C_{12}H_{24}O_2$	
Myristic	$C_{14}H_{28}O_2$	
Palmitic	$C_{16}H_{32}O_2$	
Stearic	$C_{18}H_{36}O_2$	
Arachidic	$C_{20}H_{40}O_2$	

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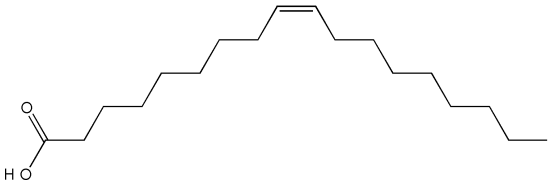
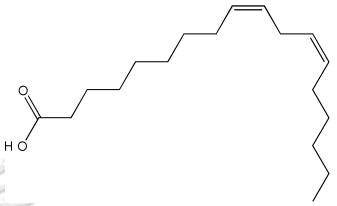
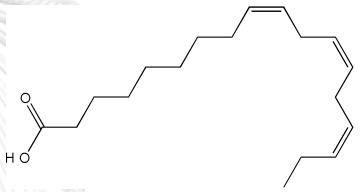
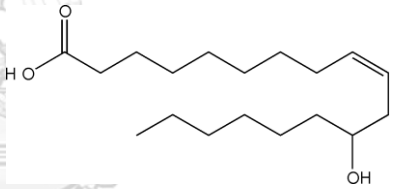
Fatty acids	Molecular Formula	Chemical structure
Oleic	$C_{18}H_{34}O_2$	
Linoleic	$C_{18}H_{32}O_2$	
Linolenic	$C_{18}H_{30}O_2$	
Ricinoleic	$C_{18}H_{34}O_3$	

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Plant material quality control [1]

Macroscopic and microscopic examination

Macroscopic identity of medicinal plant materials is based on shape, size, color, surface characteristics, texture, fracture characteristics and appearance of the cut surface. This method can be observed using visual observation or hand lens and stereomicroscope. In addition, microscopic inspection of plants materials is a necessary method for identification of powdered materials [51].

Determination of foreign matter

Foreign matter referred to any matter which does not from a needed part of the plant sample. Foreign matter consists of any of the matter coming from the source plant but not defined as plant sample. It may be not coming from the source plant such as any organism, and it may include matter of mineral origin such as sand, stone, soil, dust, etc. Medicinal plant materials should be free from any contamination [1].

Determination of water content

This constant parameter is important for plant material specification. An excess of water in herbal materials will be encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Azeotropic method is specifically used for measurement of the water content in crude drug. The crude drug is distilled with a water-immiscible solvent such as toluene or xylene. However, the organic solvents can absorb a small amount of water. Therefore, the solvent should be saturated with water before use for accurate result [1].

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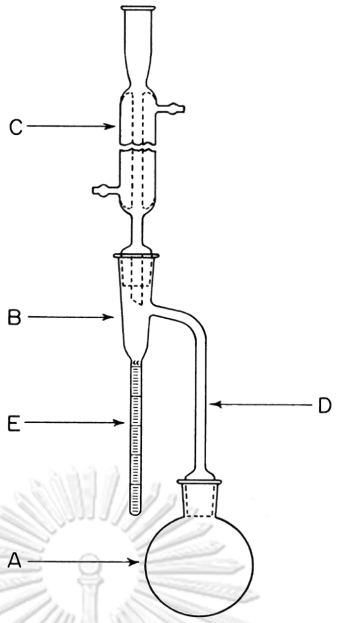

Figure 5 Apparatus used to determine water content by the azeotropic method [52]

Determination of extractable matter

Determination of extractable matter is used for determination of constituent from plant materials. The plant materials are extracted with water and ethanol. Water is uses for the polar substances while ethanol is uses for the slightly non-polar substances [1].

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Determination of loss on drying

Loss on drying is a method which used for determination of water and volatile matter in suspect plant material. Drying method can be performed in several ways. The easiest way to determined water and volatile matter in plant materials was heating method. The heating method can be done by heating in oven at the temperature

around 100-105 °C, keeping in desiccator under atmospheric or reducing of pressure at room temperature for a specified period of time [1].

Determination of ash

Total ash is the inorganic substances in plant materials remaining after incineration.

Acid-insoluble ash is the residue obtained after boiling the total ash with hydrochloric acid (70 g/L) and incineration of the remaining insoluble matters. This measures the amount of some inorganic matters which are not solubilized in hydrochloric solution [1].

Soxhlet extraction

Extraction using Soxhlet apparatus is a continuous maceration which uses a Soxhlet apparatus to macerate plant sample with fresh distilled solvent to prepare crude plant extracts. The advantages of this method are automatic, continuous method that does not required further manipulation other than concentration of the extractive, saves solvent and not time consuming [53].

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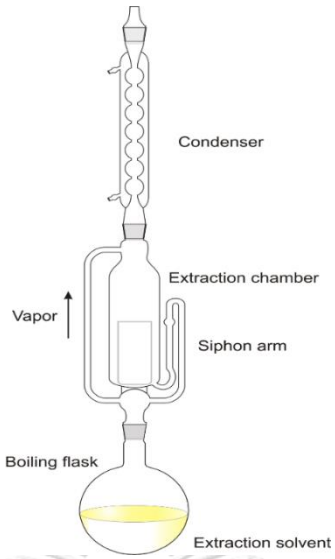

Figure 6 Soxhlet apparatus

Thin Layer Chromatography

Thin layer chromatography (TLC) is a form of liquid chromatographic method used to separate the extract using a thin stationary phase. It can be used to check purity of compounds, to identify the compounds in the extract and to achieve a quantitative analysis of the compounds [54]. TLC is a popular chromatographic technique because it is simple and inexpensive. It used for qualitative and quantitative results base on visual. TLC system is composed of stationary phase and mobile phase. The stationary phase in TLC is common adsorbent such as silica gel, aluminium oxide, cellulose and derivatives which coated on a glass, plastic or aluminium plate. Mobile phase is the mixture of solvents with specified ratio for separation of the components in extract. The result of TLC can be detected when separated compound absorb UV light or illuminate fluorescence [55]. Moreover, the developed TLC plates will be sprayed with or dipped into the staining reagent for visualization.

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Retention factor

The retention factor (R_f) is a calculated value for the distance of the spots of compound appear of origin in TLC plates and the distance move of the solvent from origin. The R_f value can be used for identify the compounds under the same conditions. The R_f values can be calculated using the formula below [55].

$$R_f = \frac{\text{Distance of compound from origin}}{\text{Distance of solvent front from origin}}$$

TLC quantitative analysis

TLC quantitative analysis can be executed by scanning densitometry or image analysis. There techniques transform the substance amount on a TLC plate into digital computer data that allow quantitative measurements [56].

TLC densitometry

Densitometry can be used to measure the amount of a substance that is on the TLC plate based on the intensity of the light absorption or fluorescent emission which is the characteristics of each substance and then converts the signal into densitogram or peak chromatogram. It can scan wavelength ranging 190-800 nm. The analysis is accurate and available for quantitative analysis [57].

TLC image analysis

The image J software is one of image analysis programs which is a public domain Java image processing available for online downloadable application. The data

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processing functions of image J program are used to calculate pixel intensity and a given area of the image and represented as peak area.

Image J software is adapted for assessment of quantitative TLC analysis. The TLC plate is photographed by digital camera and bring into the program. Then, it supports standard image processing functions such as contrast manipulation, sharpening, smoothing, edge detection and median filtering. It does geometric transformations such as rotation, scaling and flips. The pixel of sample bands and background on developed TLC plate photographed under ultra violet light at 254 nm or 356 nm can be transformed to peak chromatogram by Image J software. The image should be saved as TIFF file. Image J can be downloaded from http://imagej.nih.gov/ij/download.html [58]

Gas Chromatography / Mass Spectrometry (GC/MS)

The gas chromatography (GC) is a technique can be used for the separation and analysis of the fatty acids in fixed oil. Compounds with a lower molecular weight will elute out earlier than compounds with higher molecular weight due to differences in boiling points. Derivatization may be performed to modify the compound structure for increasing the ability of vaporization. For fatty acid analysis, methyl ester derivatives are suitable. Fatty acid methyl esters are formed through reactions between an acid and a methanol with the elimination of water (Figure 7).

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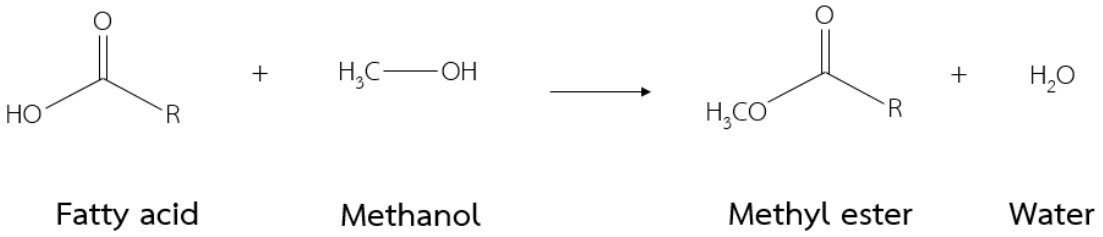

Figure 7 Methylation reaction

The mobile phase is inert carrier gas such as helium, nitrogen, hydrogen and argon. The stationary phase is a usually chemical that can selectively interact with the components in a sample mixture. Mass Spectrometer (MS) is a kind of detector machine which uses electron or chemical to ionize the chemical compound and measures the mass-to charge ratio of ions based on the details of motion of the ions as they transit through electromagnetic fields [59].

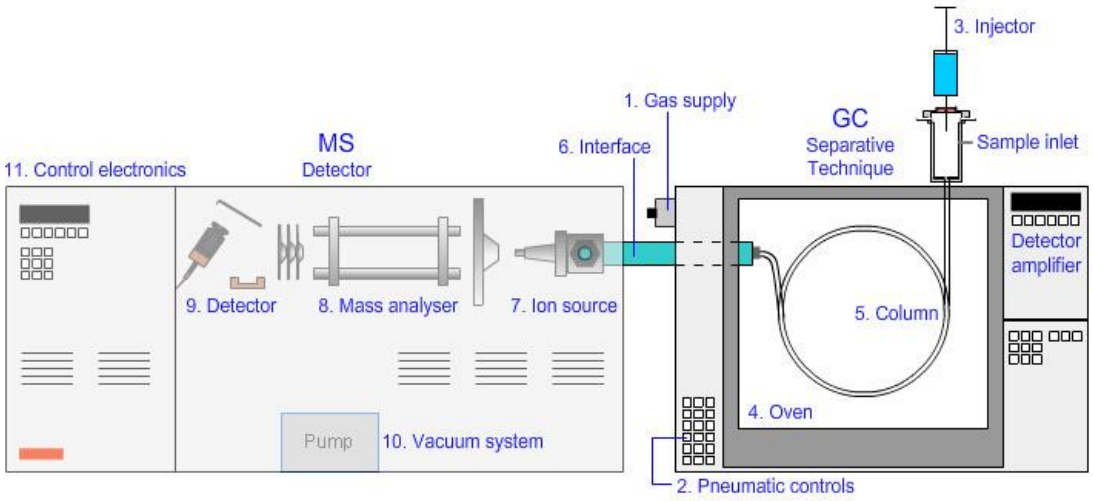

Figure 8 Gas chromatography / Mass spectrometry (1) gas supply, (2) Pneumatic controls, (3) Injector, (4) Oven, (5) Column, (6) Interface, (7) Ion source, (8) Mass analyser, (9) Detector, (10) Vacuum system and (11) Control electronics

CHAPTER III

MATERIALS AND METHODS

Chemicals and reagents

1. Acetone (E. Merck, Darmstadt, Germany)
2. Chloroform, AR grade (E. Merck, Darmstadt, Germany)
3. Chrysin (Sigma-Aldrich, St. Louis, USA)
4. Ethanol, AR grade (RCI Labscan, Bangkok, Thailand)
5. Formic acid (Fisher Scientific, UK)
6. Fatty acid methyl ester (Mix GLC-20) (SUPELCO, Bellefonte, USA)
7. Hexane, AR grade (RCI Labscan, Bangkok, Thailand)
8. Hydrochloric acid 37 %, AR grade (RCI Labscan, Bangkok, Thailand)
9. Methanol, AR grade (RCI Labscan, Bangkok, Thailand)
10. Petroleum ether, AR grade (RCI Labscan, Bangkok, Thailand)
11. Potassium hydroxide (KOH) (Mallinckrodt, Sweden)
12. Sodium chloride (NaCl) (QRëC, New Zealand)
13. Sulfuric acid (H_2SO_4) (BDH Limited Poole, England)
14. Toluene (RCI Labscan, Bangkok, Thailand)

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Materials

1. Filter paper No.4	(Whatman™ Paper, UK)
2. Filter paper No.40 ashless	(Whatman™ Paper, UK)
3. TLC silica gel 60 GF254	(Merck, LTD, USA)

Instrument and equipment

1. Aqua-shaker	(Adolf Kuhner AG, Switzerland)
2. Ashing furnaces	(Carbolite, Scientific Promotion, Bangkok, Thailand)
3. CAMAG TLC plate Heater III	(CAMAG, Switzerland)
4. CAMAG TLC chamber	(CAMAG, Switzerland)
5. CAMAG TLC scanner 3	(CAMAG, Switzerland)
6. Digital balance (Model: SI-234)	(Denver Instrument, New York, USA)
7. Digital camera (Canon Power Shot A650)	(Canon Inc., Japan)
8. Digital orbital shaker (Model: SHO-2D)	(Daihan Scientific, Korea)
9. Gas chromatography / mass spectrometry	(Thermo Finnigan model Trace GC Ultra equipped with Finnigan DSQ MS detector, USA)

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10. Hot air oven	(WTC Binder, Germany)
11. Image J software	(National Instutes of Health, USA)
12. Incinerator	(Carbilite, UK)
13. Microscope	(Zeiss Axioskop, Germany)
14. Rotary vacuum evaporator	(Buchi, Switzerland)
15. Ultraviolet viewing cabinet	(Spectronic corp., USA)
16. Vortex mixer	(Scientific Industries, USA)
17. Water bath (Model: SC/48 R)	(Brinkmann, USA)
18. WinCATS software	(CAMAG, Switzerland)
19. Ultra-pure water system (NW20VF)	(Heal Force, China)
20. Ultrasonic bath	(Analytical Lab Science, Thailand)

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Plant materials

The seeds of *Oroxylum indicum* were collected from 15 different sources throughout Thailand and were authenticated by Associate Professor Nijsiri Ruangrungsi. The voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. After removal of any foreign matters, the seeds were crushed into powders.

Pharmacognostic specification of *Oroxylum indicum*

Macroscopic examination

Macroscopic examinations of *O. indicum* were estimated by shape, size, color, surface characteristics, texture, fracture characteristics and appearance of the cut surface. Drawing of the whole plant of *O. indicum* in Thailand was done.

Microscopic examination

Microscopic appearances of the *O. indicum* were examined in cross section and in powdered form. The tissue section and powders were mounted with water in the glass slide for observation and was examined under the microscope with magnification of 4x, 10x and 40x.

Determination of water content (Azeotropic method)

Thirty grams of *O. indicum* dried seed powders were added with 400 ml of water saturated toluene and boiled by azeotropic distillation. The volume of water was measured and calculated in percentage.

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Determination of loss on drying

Three grams of *O. indicum* dried seed powders were weighed in previously weighed crucible and dried at 105 °C for 6 hours. After that, the crucible was left to cool at room temperature. Calculate the loss of weight in a percentage of dried material.

Determination of total ash and acid-insoluble ash

Three grams of *O. indicum* dried seed powders were added into a pre-weighed crucible. The sample was incinerated at 500 °C for 5 hours until it is become white, indicating the absence of carbon. The crucible was left to cool in desiccator and weighed without delay. The content of total ash was calculated in a percentage of air-dried material.

Twenty-five milliliters of hydrochloric acid (70 g/L) were added to the crucible, which contains ash from the total ash then covered with the watch-glass and boiled for 5 minutes. The insoluble matters were collected on an ashless filter-paper, transferred to the original crucible, dried on a hotplate and incinerated to ashes again. The crucible was left to cool in desiccator and weighed without delay. The content of acid-insoluble ash was calculated in a percentage of air-dried material.

Determination of ethanol soluble extractive value

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Five grams of *O. indicum* dried seed powders were macerated with 70 ml of ethanol in conical flask under shaking for 6 hours and standing for 18 hours. The marc was filtered and washed. The filtrate was adjusted to 100 ml with ethanol. After that, twenty milliliters of the filtrate were transferred to a pre-weighed beaker and evaporate to dryness. The extract was dried at 105 °C for 6 hours, cooled in desiccator for 30 minutes and weighed without delay. The content of extractable matter was calculated in a percentage of air-dried material.

Determination of water soluble extractive value

Five grams of *O. indicum* dried seed powders were macerated with 70 ml of water in conical flask under shaking for 6 hours and standing for 18 hours. The marc was filtered and washed. The filtrate was adjusted to 100 ml with water. After that, twenty milliliters of the filtrate were transferred to a pre-weighed beaker and evaporate to dryness. The extract was dried at 105 ℃ for 6 hours, cooled in desiccator for 30 minutes and weighed without delay. The content of extractable matter was calculated in a percentage of air-dried material.

Thin layer chromatographic fingerprint

One gram of *O. indicum* dried seed powdered was macerated with 20 ml of 95% ethanol in conical flask for 6 hours under shaking and 18 hours understanding, filtered, evaporated to dryness and dissolved with 1 ml of 95 % ethanol. The extract was spotted on TLC silica gel 60 GF_{254} plate and developed in toluene: chloroform: acetone: formic acid (5:4:1:0.2). After that, the plate was examined under ultraviolet light (254, 365 nm) and stained with 10 % sulfuric acid in ethanol then heated at 100 ℃ for 15 minutes.

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Fixed oil analysis of *Oroxylum indicum* seed

Preparation of petroleum ether extracts of *Oroxylum indicum* seed

The accurate 5 g of *O. indicum* dried seed powders were exhaustively extracted with 250 ml of petroleum ether in a Soxhlet apparatus. The petroleum ether extract was filtered and evaporated under reduced pressure in a rotary evaporator. The seed oil was stored at 5 ℃ for methylation procedure.

Methylation of *Oroxylum indicum* seed fixed oil

Five milligrams of *O. indicum* seed oil were added with 2 ml of 0.3 M potassium hydroxide in methanol. The sample was incubated at 80 ℃ for 1 hour. Then, it was left to cool, added with 0.5 ml of 10% sulfuric acid in methanol and incubated at 80 ℃ for 20 minutes. After cooling, it was added with 0.5 ml of saturated sodium chloride and 1 ml of hexane, mixed and hexane layer were separated for GC-MS analysis [60], [61].

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Gas chromatography – mass spectrometry (GC/MS) analysis

The fixed oil was analyzed by a Finnigan Trace GC ultra with DSQ Quadrupole detector. Zabron ZB-5 MS fuse silica column (30m x 0.25mm, 0.25μm film thicknesses) was used as stationary phase. The oven temperature started from 160 ℃ then heated up to 170 ℃ with the rate of 0.5 ℃/min hold time 15 minutes, and heated up to 210 ℃ with the rate of 1 ℃/min. The carrier gas with helium with the flow rate of 1 ml/min. One microliter of the oil solution (1:10 in AR grade hexane) was injected by Finnigan Autoinjector AI3000 with split ratio of 10:1. MS was performed by EI positive mode at

70 eV ionization. The fatty acid constituents of the oil were identified by matching mass spectra and retention indices with NIST05 Mass Spectral libraries and certified reference material F.A.M.E. Mix GLC-20. The content of each composition was determined as peak area ratio in percentage.

Quantitative analysis of chrysin in *Oroxylum indicum* seed

Preparation of chrysin solution

One milligram of standard chrysin was dissolved in 1 ml of 95% ethanol. The stock solutions were diluted to obtain the series of standard solutions range from 0.1, 0.15, 0.2, 0.3 and 0.4 mg/ml. These solutions were stored in refrigerator at 5 °C.

Preparation of ethanol extracts of *Oroxylum indicum* seed

The accurate 5 g of *O. indicum* dried seed powders were exhaustively extracted with 250 ml of 95% ethanol in a soxhlet apparatus. The ethanolic extract was filtered and evaporated under reduced pressure in a rotary evaporator till dryness. The extracted sample was stored at 5 °C for TLC-densitometry and TLC image analyses.

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TLC-densitometry

Three microliters of *O. indicum* extract and standard chrysin solution in ethanol were applied on the silica gel 60 F254 TLC plate. The plate was developed in a TLC chamber that contained a mixture of toluene: chloroform: acetone: formic acid (5: 4: 1: 0.2), then the plate was removed and allowed to dry at room temperature. The chrysin spots on the developed TLC plates were quantitatively analyzed by

scanning with TLC densitometer under UV 254 nm. The calibration curve of chrysin was examined by plotting peak areas and concentrations of chrysin in µg/spot.

TLC image analysis by Image J software

The chrysin spots on the developed TLC plates were photographed under short wave ultraviolet light (254 nm) by a digital camera. Peak area of each spot was quantitated using Image J software. The content of chrysin was determined by comparing peak area to the calibration curve obtained from the same TLC plate.

Method validation

According to the ICH guidelines, the method validation including calibration range, accuracy, precision, specificity, LOD, LOQ and robustness was performed.

Calibration range

The calibration range was calculated by plotting peak areas and concentrations of standard chrysin applied.

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Accuracy

The accuracy was tested by recovery method. Standard chrysin solution was spiked into the extract to have three different levels of chrysin (low, medium, high). The spiked and un-spiked sample were analyzed under the same conditions in triplicate. The accuracy was determined by using following formula. The acceptable range of recovery is during 80-120 % of the test concentration [62].

% Recovery = (A / B+C) x 100

Where: A = the actual calculated amount in recovery sample

B = the amount un-spiked into the sample

C = the amount standard added to the sample

Precision

The precision was examined by repeatability (intra-day) and intermediate (inter-day) precision. The method was performed by analyzing sample solution of three concentrations in three replicates on the same day and three different days respectively. The content calculated by measurement of peak area was determined for %relative standard deviation (% RSD) by following formula. The criteria of repeatability and intermediate precision were not more than 15 %RSD [63].

% RSD = (SD / Mean) x 100

Where: SD = the standard deviation of each measurement

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Specificity

The TLC plates after development were scanned for absorption spectra under the range of 200-700 nm by TLC scanner. The specificity was evaluated by comparison of the absorption of the standard chrysin and each sample as well as comparison of the absorption spectra at up-slope, apex and down-slope of the peak.

Limit of detection

The limit of detection (LOD) was determined from the calibration range using this formula.

$$\mathbf{LOD = 3.3(SD) / S}$$

Where: SD = the residual standard deviation of regression line

S = the slope of regression line

Residual standard deviation: the standard deviation of the residuals (residuals = differences between observed and predicted values). It is calculated as follows:

$$SD_{res} = \sqrt{\frac{\sum (y - y_{est})^2}{n - 2}}$$

Where: $\sum$ = the means "sum of"

y = the observed values for the dependent variable

y_{est} = the predicted values for the dependent variable (the predicted values are calculated using the regression equation)

n = the number of data points

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Limit of quantitation

The limit of quantitation (LOQ) was determined from the calibration range using this formula.

$$\mathbf{LOQ = 10(SD) / S}$$

Where: SD = the residual standard deviation of regression line

S = the slope of regression line

Robustness

The robustness was examined by changing the mobile phase. The selected mobile phase ratio of toluene : chloroform : acetone : formic acid at the ratio of 5.5 : 4.5 : 1.5 : 0.3, 5 : 4 : 1 : 0.2 and 4.5 : 3.5 : 0.5 : 0.1 were examined and calculated for % RSD of peak area.

Data analysis

The parameters due to standardization were expressed as grand mean ± pooled standard deviation. The chrysin contents between TLC image analysis and TLC-densitometry were compared by paired t-test statistical analysis.

$$\text{Grand mean} = \frac{\sum n_i x_i}{N}$$

$$\text{Pooled SD} = \sqrt{\frac{\sum (n_i - 1)\, S_i^2}{(\sum n_i) - N}}$$

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Where: n_i = the numbers of sample replicate from each source

x_i = the mean of sample replicate from each source

N = the number of sample from various sources

S_i^2 = the variance of each replicate from each source

CHAPTER IV

RESULTS

Pharmacognostic specification

Common Name	PHE KAA
Scientific Name	*Oroxylum indicum* (L.) Kurz
Family	BIGNONIACEAE
Distribution	Asian tropical and subtropical low-altitude open forests
Used Part	Seed
Ethnomedical Use	Cough, Throat infections, Hypertension and Purgative

Macroscopic evaluation

The dried seed of *O. indicum* was yellow and brown color, 3-4 cm in length and 5-7 cm in width (Figure 9). The botanical drawing of *O. indicum* was shown in Figure 10.

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Crude drugs

Figure 9 Dried seed of *Oroxylum indicum* (L.) Kurz

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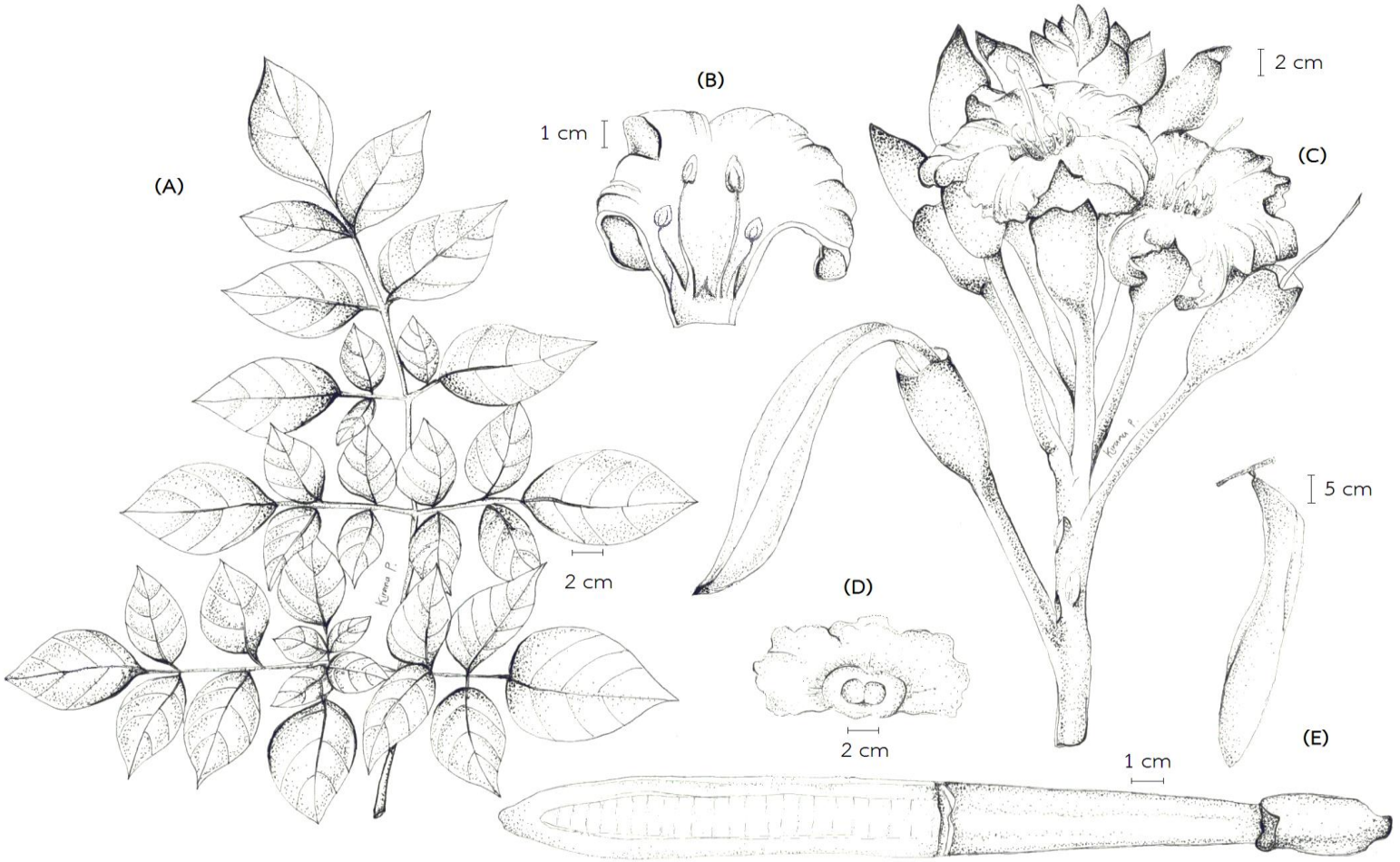

Figure 10 Whole plant of *Oroxylum indicum* (L.) Kurz

(A) Tripinnately compound leaf, (B) Petal and stamen, (C) Inflorescence, (D) Seed and (E) Pod

Microscopic evaluation

The transverse section of *O. indicum* seed showed the anatomical characteristics of endodermis, endosperm, cortical parenchyma, cortical fiber and epidermis (Figure 11). The histological characteristics of powder of *O. indicum* seed demonstrated parenchyma in transverse view, aleurone grains, parenchyma in longitudinal view, septate fibre, stone cells and fibre (Figure 12).

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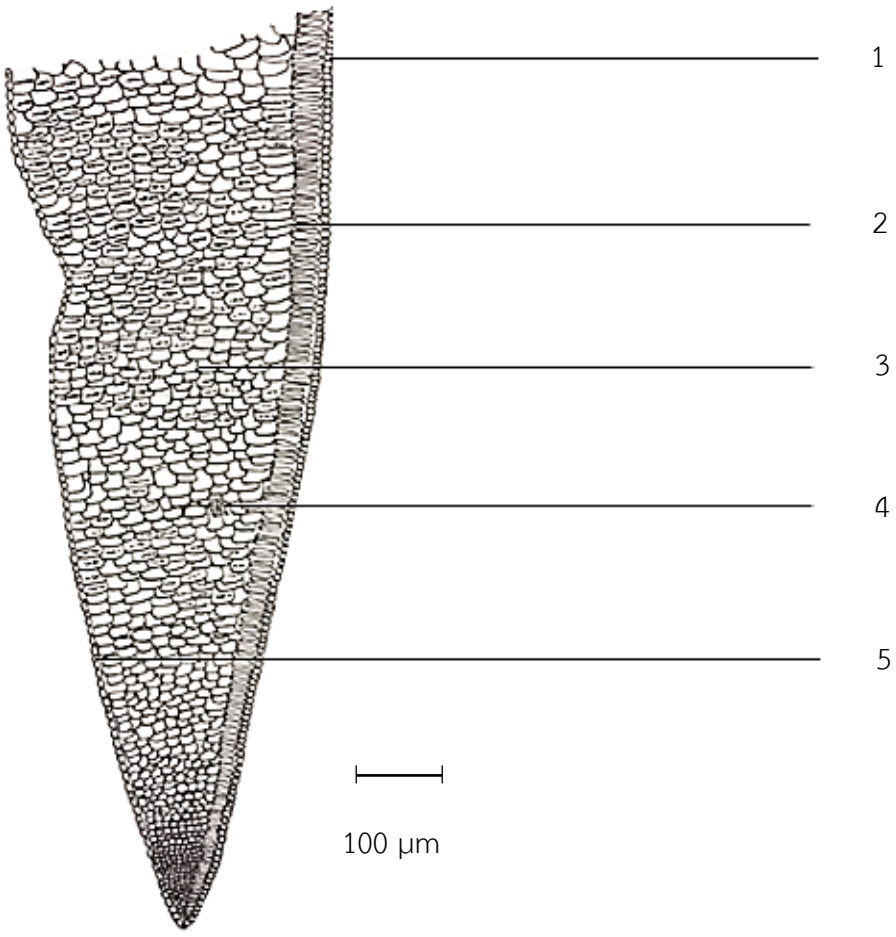

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Figure 11 The sum part anatomical character of *Oroxylum indicum* seed in transverse section view

1. Endodermis
2. Endosperm
3. Cortical parenchyma
4. Cortical fiber
5. Epidermis

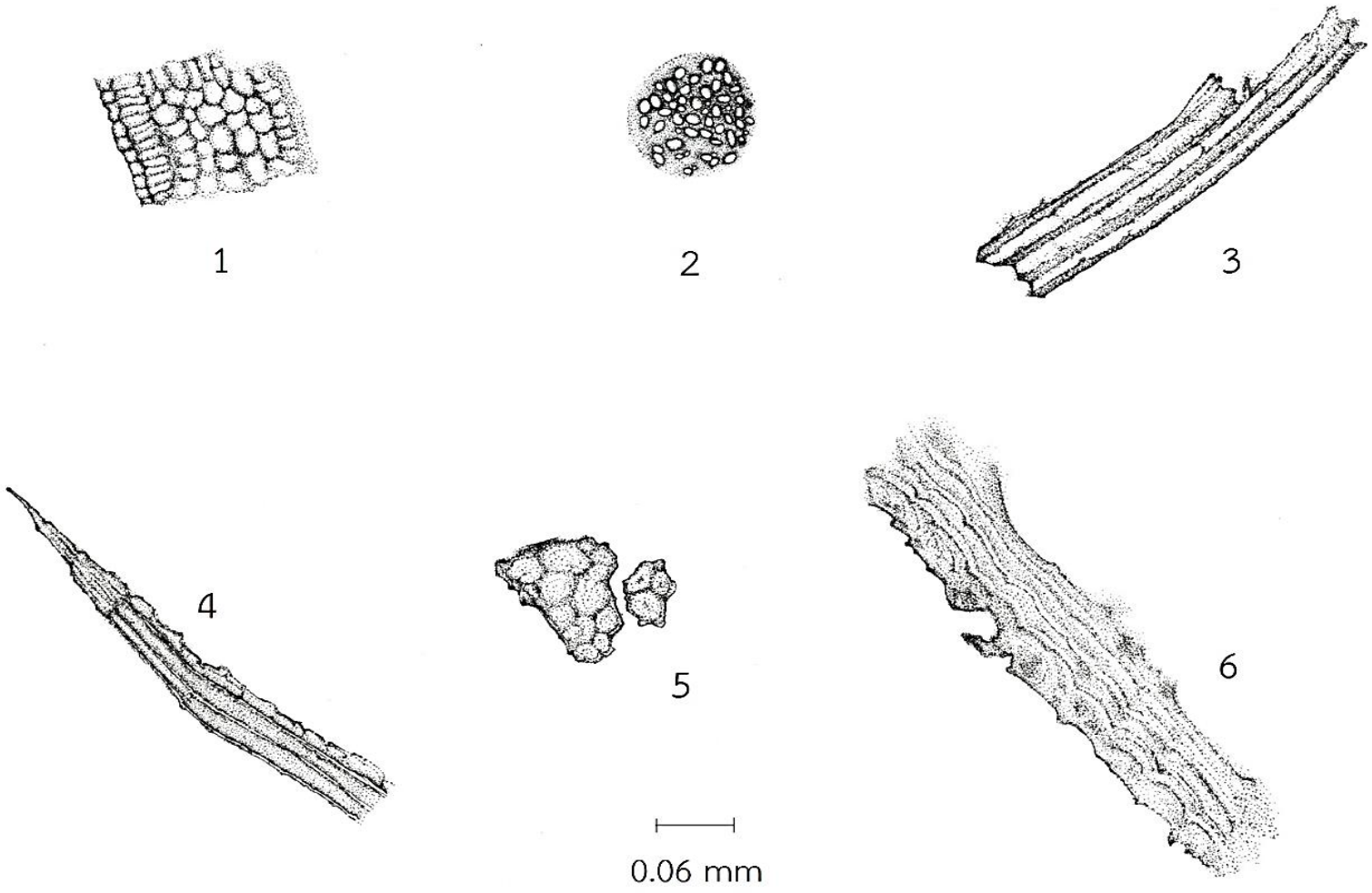

Figure 12 Histological characters of *Oroxylum indicum* seed in powdered form

1. Parenchyma in transverse view, 2. Aleurone grains, 3. Parenchyma in longitudinal view, 4. Septate fibre, 5. Stone cells, 6. Fibre

Thin layer chromatographic fingerprint

The ethanolic extract of *O. indicum* was spotted on TLC silica gel 60 GF_{254} plate developed in toluene : chloroform : acetone : formic acid (5 : 4 : 1 : 0.2) and observed under ultraviolet light (254, 365 nm) and dipped with 10% sulfuric acid in ethanol then heated at 100 ºC for 15 minutes (Figure 13).

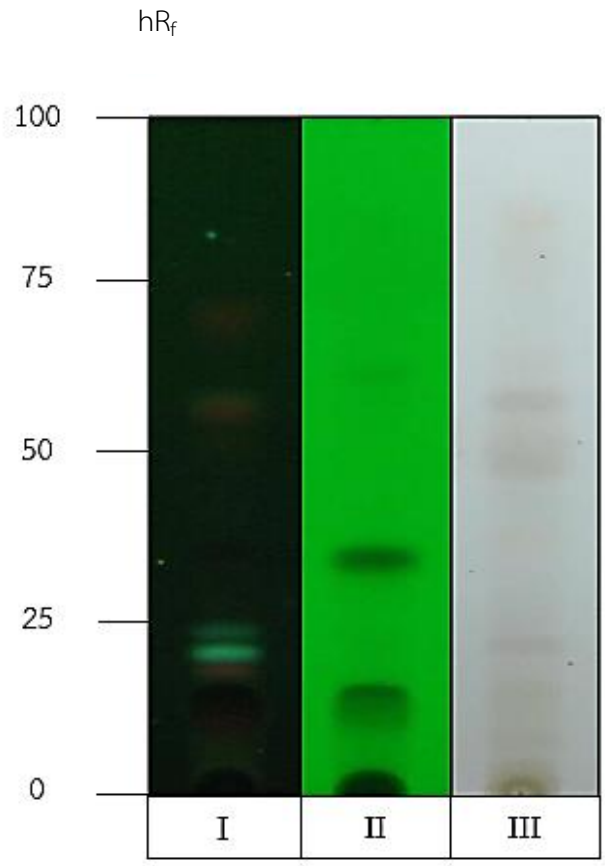

Figure 13 Thin layer chromatographic fingerprint of ethanolic extract of *Oroxylum indicum*

Detection I = detection under UV 365 nm

II = detection under UV 254 nm

III = detection with 10 % sulfuric acid in ethanol and heated

Physico-chemical parameters of dried *Oroxylum indicum* seed

The contents of physico-chemical parameters of *O. indicum* seed were shown in Table 3. The loss on drying, water contents, total ash and acid-insoluble ash should be not more than 3.32, 6.89, 4.40 and 0.47 % of dry weight respectively. The ethanol soluble extractive matter and water soluble extractive matter should be not less than 9.74 and 12.11 % by dry weight respectively.

Table 3 Physico-chemical content of *Oroxylum indicum* seed (% by weight)

Specification	Content (% dry weight) *
Loss on Drying	3.32 ± 0.12
Water Content	6.89 ± 0.80
Total Ash	4.40 ± 0.08
Acid Insoluble Ash	0.47 ± 0.05
Ethanol Soluble Extractive Matter	9.74 ± 0.68
Water Soluble Extractive Matter	12.11 ± 0.80

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*The parameters were shown as grand mean ± pooled SD. Samples were collected from 15 different sources in Thailand. Each sample was tested in triplicate.

Ethanolic extraction of *Oroxylum indicum* seed

The percent yield of 95 % ethanolic extract of *O. indicum* seed by Soxhlet extraction was 29.14 ± 5.43 % by weight in average (Table 4).

Table 4 The ethanolic extract of *Oroxylum indicum* seed from 15 different sources

No.	Source	Weight of sample (g)	Weight of extractive matter (g)	% yield
1	Bangkok	5.01	1.28	25.60
2	Rayong	5.02	1.26	25.09
3	Satun	5.02	1.28	25.42
4	Phuket	5.04	1.38	27.26
5	Khonkaen	5.01	1.64	32.70
6	Lumpang	5.00	1.24	24.75
7	Chiang Mai	5.00	1.19	23.75
8	Songkla	5.00	1.18	23.55
9	Chanthaburi	5.00	1.52	30.36
10	Nakhon Ratchasima	5.00	1.24	24.86
11	Suratthani 1	5.01	2.09	41.70
12	Chaiyaphum	5.01	1.77	35.26
13	Nakhon Pathom	5.00	1.83	36.51
14	Suratthani 2	5.00	1.45	28.93
15	Phetchaboon	5.00	1.57	31.42
			Average	**29.14 ± 5.43**
			Min	**23.55**
			Max	**41.70**

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The content of chrysin in *Oroxylum indicum* seed by TLC densitometry

Standard chrysin and the ethanolic extracts were developed in toluene : chloroform : acetone : formic acid (5 : 4 : 1 : 0.2). TLC plate was scanned by CAMAG TLC scanner under 269 nm. TLC densitograms of chrysin standards and 15 samples of *O. indicum* seed performed under UV wavelength of 269 nm were shown in Figure 14. The peak areas were computed by WinCATS software. The ethanolic extrats of *O. indicum* seed were determined for the chrysin content in triplicate by TLC densitometry and found to be 0.17 ± 0.05 gram per 100 grams of the crude drug (Table 5).

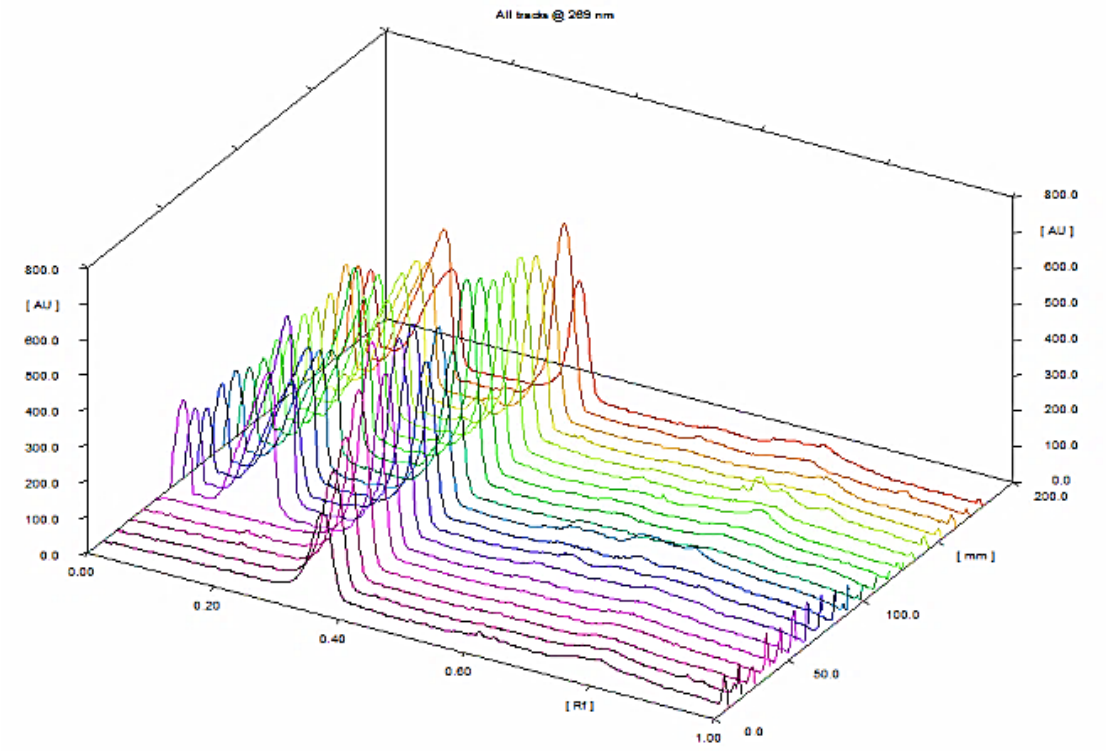

Figure 14 TLC densitograms of chrysin standards and 15 samples of *Oroxylum indicum* seed under UV 269 nm

Table 5 The amount of chrysin in *Oroxylum indicum* seed by TLC densitometry (% by weight)

Source	Chrysin in the ethanolic extract (g/g)	Yield of the ethanolic extract (g/100 g of dried crude drug)	Chrysin in *Oroxylum indicum* seed (g/100 g of dried crude drug)
1	0.005	25.60	0.13
2	0.007	25.09	0.18
3	0.009	25.42	0.22
4	0.004	27.26	0.12
5	0.006	32.70	0.18
6	0.004	24.75	0.09
7	0.008	23.75	0.19
8	0.007	23.55	0.17
9	0.006	30.36	0.17
10	0.006	24.86	0.15
11	0.007	41.70	0.30
12	0.005	35.26	0.19
13	0.004	36.51	0.14
14	0.007	28.93	0.19
15	0.003	31.42	0.09
	Average		**0.17 ± 0.05**

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Method validation (TLC densitometry)

The calibration range, specificity, accuracy, precision, LOD, LOQ and robustness were examined for the validation of an analytical method following ICH guideline.

Calibration range

The calibration curve of chrysin obtained by the peak area of standard (0.3 – 1.2 µg/spot) was polynomial with the regression equation of $y = -4744.6x^2 + 21102x + 3735.3$ (Figure 15). The coefficient of determination (R^2) of chrysin was 0.9999.

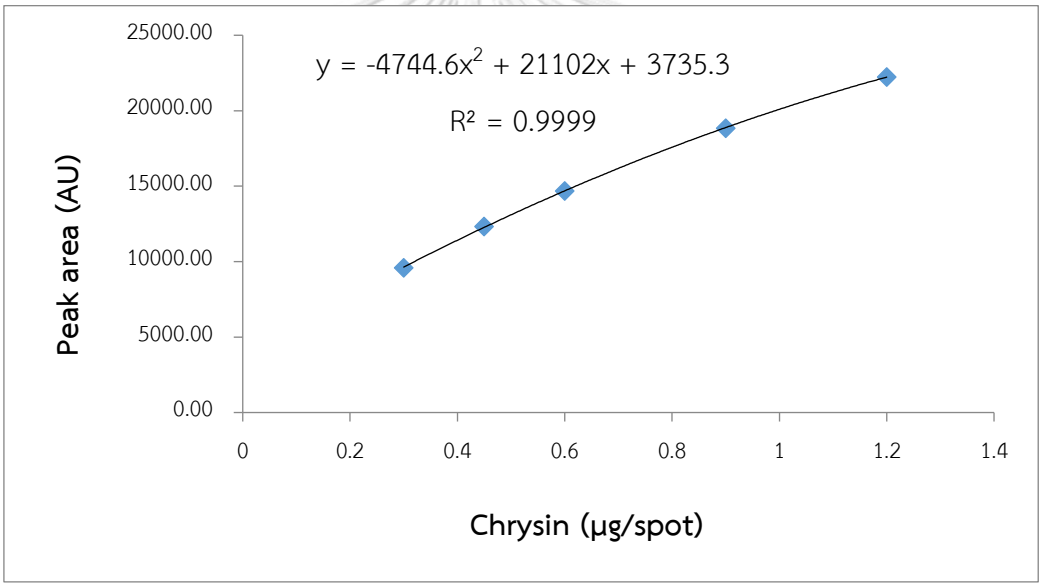

Figure 15 The calibration curve of chrysin in *Oroxylum indicum* seed by TLC densitometry

Detection limit and quantitation limit

The detection limit and quantitation limit determination were based on the standard deviation of regression line and the slope of the calibration curve. The LOD value, considered as the lowest concentration for analyte in a sample that could be detected was found to be 0.015 µg/spot. The LOQ value, the lowest concentration of analyte in a sample that could be quantitatively defined was 0.046 µg/spot.

Accuracy

The accuracy was tested by recovery method. Standard chrysin was spiked into the extract to have three different levels of chrysin (low, medium, high). The recovery values were 109.2 – 109.7 % as demonstrated in Table 6.

Table 6 Accuracy of quantitation of chrysin in *Oroxylum indicum* seed by TLC densitometry (n=3)

Chrysin added (µg/spot)	Chrysin found (µg/spot)	% Recovery
0.00	0.42 ± 0.01	-
0.15	0.62 ± 0.01	109.7 ± 3.6
0.30	0.79 ± 0.01	109.7 ± 2.2
0.60	1.11 ± 0.03	109.2 ± 2.4
	Average	**109.6 ± 0.3**

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Precision

The precision was examined by repeatability (intra-day) and intermediate precision (inter-day). The method was performed by analyzing sample solution of three concentrations in three replicates on the same day and three different days respectively. The repeatability and intermediate precision were showed in Table 7.

Table 7 Repeatability and intermediate precision of quantitation of chrysin in *Oroxylum indicum* seed by TLC densitometry (n=3)

Repeatability		**Intermediate precision**	
Amount (µg/spot)	**%RSD**	**Amount (µg/spot)**	**%RSD**
0.62 ± 0.01	1.28	0.60 ± 0.02	3.79
0.79 ± 0.01	1.35	0.76 ± 0.02	2.93
1.11 ± 0.03	2.50	1.09 ± 0.08	7.60
Average	**1.98 ± 0.78**		**4.30 ± 2.24**

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Specificity

Peak identity

The specificity was executed by peak identity and peak purity checking. The identity in absorbance spectra determined at the peak apex among chrysin standards and samples was illustrated in Figure 16. The maximum absorbance of chrysin was at the wavelength 269 nm.

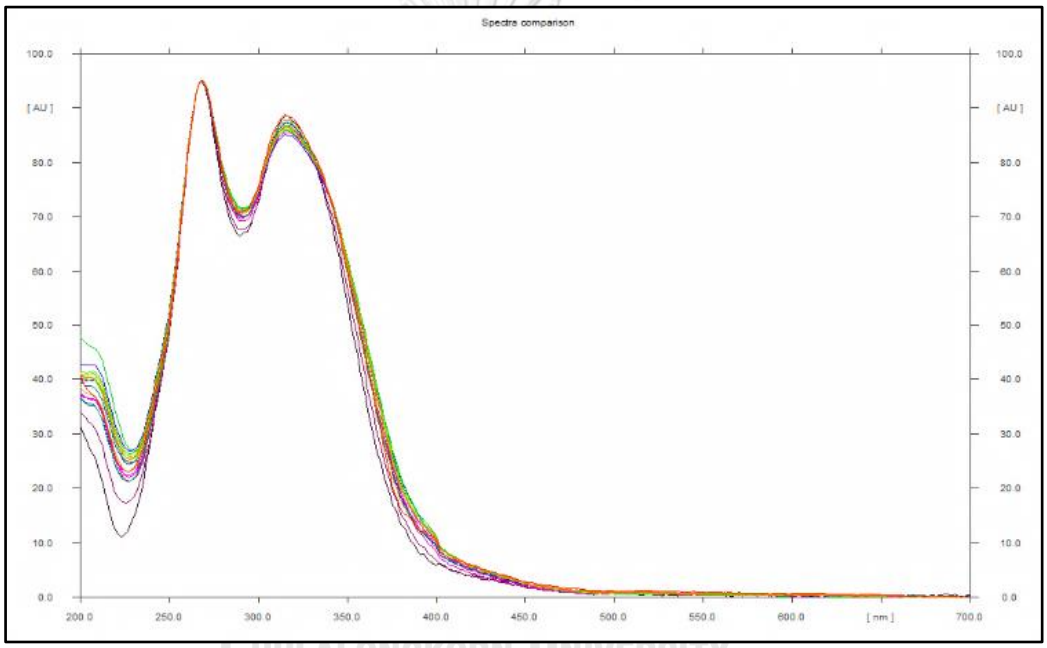

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Figure 16 The absorbance spectra of chrysin in *Oroxylum indicum* seed extracts from 15 different sources and standard chrysin representing peak identity

Peak purity

Peak purity of chrysin was represented in Figure 17. The absorbance spectra from up-slope, apex and down-slope of the peak were identical. The percent peak purity of chrysin was 0.9990.

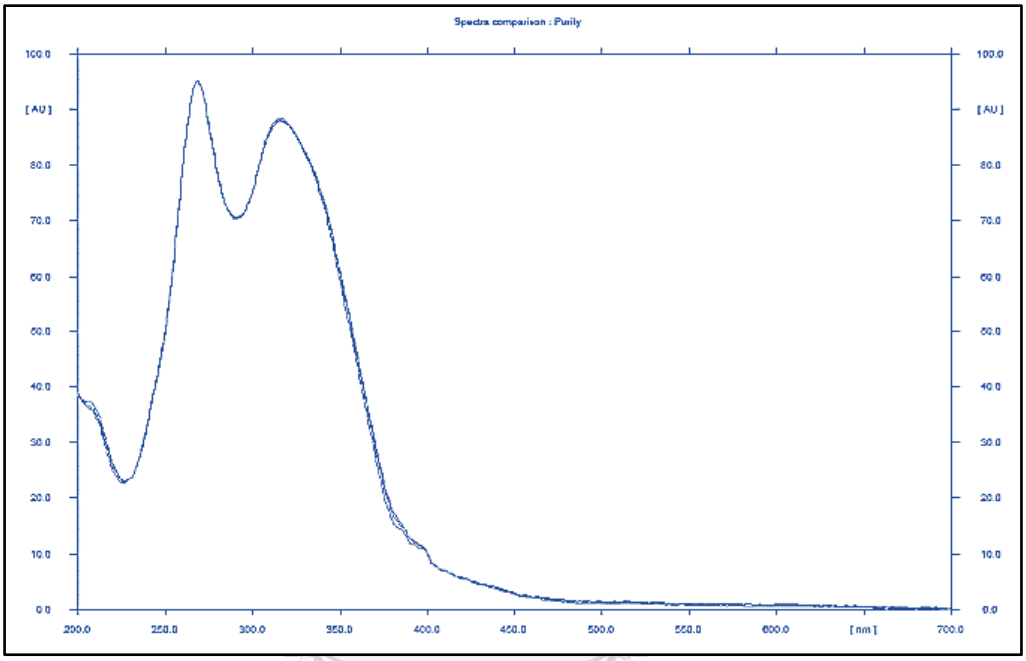

Figure 17 Peak purity measurement using up-slope, apex and down-slope of the peak.

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Robustness

The robustness of chrysin quantitation in *O. indicum* seed by TLC densitometric analysis was examined by changing the mobile phase ratio. The selected mobile phase ratio was shown in Table 8. The result of robustness was 2.05 % RSD of peak area.

Table 8 Robustness of chrysin in *Oroxylum indicum* seed by TLC densitometry

Mobile phase composition **Toluene : Chloroform : Acetone : Formic acid**	**Peak area**
5 : 4 : 1 : 0.2	16812.6
4.5 : 3.5 : 0.5 : 0.1	16655.0
5.5 : 4.5 : 1.5 : 0.3	16163.9
Mean ± SD	**16543.8 ± 338.3**
% RSD	**2.05**

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The content of chrysin in *Oroxylum indicum* seed by TLC image analysis

TLC plate containing standard chrysin and the ethanolic extracts which were developed in toluene : chloroform : acetone : formic acid (5: 4 : 1 : 0.2) were photographed under UV 254 nm in UV viewing cabinet. The image of TLC plate was analyzed for chrysin peak areas by Image J software. The amounts of chrysin were found to be 0.20 ± 0.07 grams in 100 grams of *O. indicum* seed crude drug (Table 9).

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Table 9 The amount of chrysin in *Oroxylum indicum* seed by TLC image analysis (% by weight)

Source	Chrysin in the ethanolic extract (g/g)	Yield of the ethanolic extract (g/100 g of dried crude drug)	Chrysin in *Oroxylum indicum* seed (g/100 g of dried crude drug)
1	0.006	25.60	0.16
2	0.009	25.09	0.22
3	0.009	25.42	0.24
4	0.005	27.26	0.15
5	0.007	32.70	0.21
6	0.004	24.75	0.11
7	0.009	23.75	0.22
8	0.008	23.55	0.19
9	0.007	30.36	0.22
10	0.007	24.86	0.18
11	0.009	41.70	0.39
12	0.007	35.26	0.24
13	0.005	36.51	0.18
14	0.008	28.93	0.23
15	0.004	31.42	0.12
	Average		**0.20 ± 0.07**

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Method validation (TLC image analysis)

Calibration range

The calibration curve of chrysin obtained by the peak area of standard (0.3 – 1.2 µg/spot) was polynomial with the regression equation of $y = -4000x^2 + 27499x - 1541.6$ (Figure 18). The coefficient of determination (R^2) of chrysin was 0.9999.

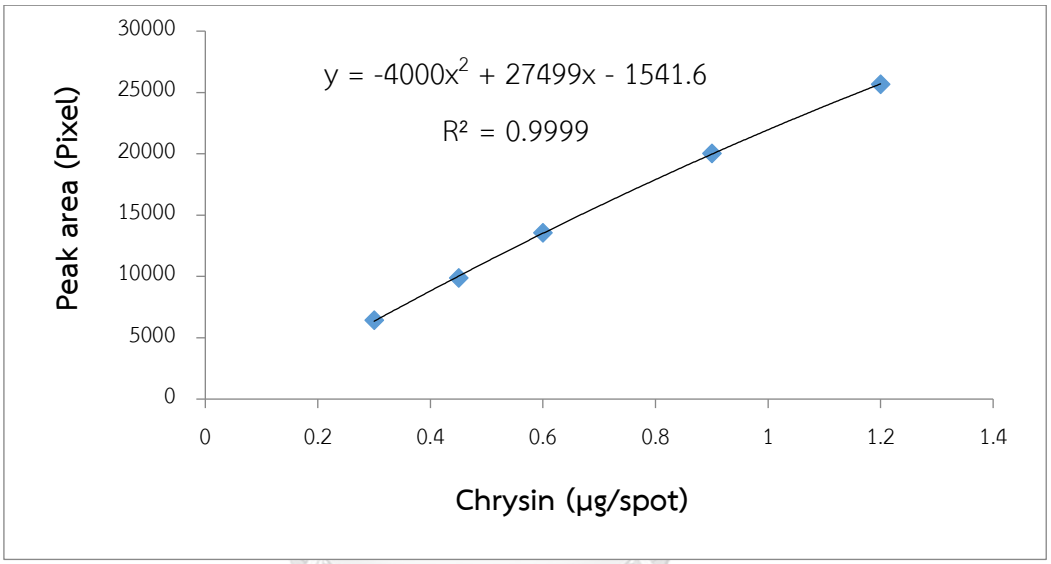

Figure 18 The calibration curve of chrysin in *Oroxylum indicum* seed by TLC image analysis

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Detection limit and quantitation limit

The detection limit and quantitation limit determination were based on the standard deviation of regression line and the slope of the calibration curve. The LOD value, considered as the lowest concentration for analyte in a sample that could be detected was found to be 0.016 µg/spot. The LOQ value, the lowest concentration of analyte in a sample that could be quantitatively defined was 0.048 µg/spot.

Accuracy

The accuracy was tested by recovery method. Standard chrysin was spiked into the extract to have three different levels of chrysin (low, medium, high). The recovery values were 96.7 – 116.0 % as demonstrated in Table 10.

Table 10 Accuracy of quantitation of chrysin in *Oroxylum indicum* seed by TLC image analysis (n=3)

Chrysin added (µg/spot)	Chrysin found (µg/spot)	% Recovery
0.00	0.47 ± 0.03	-
0.15	0.60 ± 0.02	96.7 ± 2.6
0.30	0.89 ± 0.01	116.0 ± 2.7
0.60	1.18 ± 0.01	110.4 ± 4.0
Average		**107.7 ± 9.9**

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Precision

The precision was examined by repeatability (intra-day) and intermediate precision (inter-day). The method was performed by analyzing sample solution of three concentrations in three replicates on the same day and three different days respectively. The repeatability and intermediate precision were showed in Table 11.

Table 11 Repeatability and intermediate precision of quantitation of chrysin in *Oroxylum indicum* seed by TLC image analysis (n=3)

Repeatability		**Intermediate precision**	
Amount (µg/spot)	**%RSD**	**Amount (µg/spot)**	**%RSD**
0.60 ± 0.02	3.51	0.67 ± 0.06	8.30
0.89 ± 0.01	1.37	0.89 ± 0.02	2.66
1.18 ± 0.01	1.17	1.15 ± 0.01	1.24
Average	**3.00 ± 2.23**		**5.12 ± 3.71**

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Robustness

The robustness of chrysin quantitation in *O. indicum* seed by TLC image analysis was examined by changing the mobile phase ratio. The selected mobile phase ratio was shown in Table 12. The result of robustness was 4.07 % RSD of peak area.

Table 12 Robustness of chrysin in *Oroxylum indicum* seed by TLC image analysis

Mobile phase composition **Toluene : Chloroform : Acetone : Formic acid**	**Peak area**
5 : 4 : 1 : 0.2	10412.5
4.5 : 3.5 : 0.5 : 0.1	10974.5
5.5 : 4.5 : 1.5 : 0.3	11287.9
Mean ± SD	**10891.6 ± 443.6**
% RSD	**4.07**

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Chrysin contents between TLC densitometry and TLC image analysis

The contents of chrysin determined by TLC densitometry and TLC image analysis were in agreement (Table 13). However, the results from TLC image analysis tended to be a few higher than TLC densitometry ($P < 0.01$ by paired *t*-test).

Table 13 The comparison of chrysin contents in *Oroxylum indicum* seed between TLC densitometry and TLC image analysis

Source	Chrysin in the ethanolic extract (g/g)	
	TLC densitometry	TLC image analysis
1	0.13	0.16
2	0.18	0.22
3	0.22	0.24
4	0.12	0.15
5	0.18	0.21
6	0.09	0.11
7	0.19	0.22
8	0.17	0.19
9	0.17	0.22
10	0.15	0.18
11	0.30	0.39
12	0.19	0.24
13	0.14	0.18
14	0.19	0.23
15	0.09	0.12
Average	**0.17 ± 0.05**	**0.20 ± 0.07**

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Fatty acid analysis of *Oroxylum indicum* seed oil by GC/MS

The percent yield of petroleum ether extract of *O. indicum* seed oil by Soxhlet extraction was found to be 11.74 ± 2.66 % by weight (Table 14). The methyl ester of fatty acid in *Oroxylum indicum* seed oil analyzed by GC/MS revealed that the seed oil consist of palmitic acid, linoleic acid, oleic acid, stearic acid, behenic acid, gondoic acid, arachidic acid, lignoceric acid and 9, 10-dihydroxystearic acid as shown in Table 15. The main component was oleic acid 67.99 ± 5.98. GC/MS chromatogram of *Oroxylum indicum* seed oil was shown in Figure 19. The mass spectrum of retention time of palmitic acid, linoleic acid, oleic acid, stearic acid, behenic acid, gondoic acid, arachidic acid, lignoceric acid and 9, 10-dihydroxystearic acid were shown in Figure 20-28.

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Table 14 The percent yield of *Oroxylum indicum* seed oil from 15 different sources throughout Thailand

No.	Source	Weight of sample (g)	Amount of extractive value (ml)	% yield
1	Bangkok	5.02	0.70	13.94
2	Rayong	5.03	0.70	13.93
3	Satun	5.03	0.75	14.93
4	Phuket	5.02	0.65	12.94
5	Khonkaen	5.03	0.80	15.90
6	Lumpang	5.03	0.60	11.93
7	Chiang Mai	5.02	0.40	7.96
8	Songkla	5.03	0.40	7.95
9	Chanthaburi	5.03	0.70	13.91
10	Nakhon Ratchasima	5.02	0.60	11.96
11	Suratthani 1	5.02	0.55	10.96
12	Chaiyaphum	5.03	0.65	12.93
13	Nakhon Pathom	5.02	0.40	7.96
14	Suratthani 2	5.03	0.45	8.96
15	Phetchaboon	5.02	0.50	9.95
			Average	**11.74 ±**
		2.66		
			Min	**7.95**
			Max	**15.90**

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Table 15 The fatty acid constituents of *Oroxylum indicum* seed oil

No.	Fatty acid	IUPAC Name	Delta notation	RT[a] (min)	KI[b]	Peak area[c] (%)
1	Palmitic acid	Hexadecanoic acid	16:0	20.83 ± 0.04	1938	10.22 ± 0.88
2	Linoleic acid	9, 12-Octadecadienoic acid	18:2-delta-9c, 12c	37.14 ± 0.05	2092	2.69 ± 0.77
3	Oleic acid	9-Octadecenoic acid	18:1-delta-9c	38.50 ± 0.14	2097	67.99 ± 5.98
4	Stearic acid	Octadecanoic acid	18:0	41.77 ± 0.03	2134	1.86 ± 0.42
5	Behenic acid	Docosanoic acid	22:0	43.11 ± 0.29	2513	7.28 ± 5.60
6	Gondoic acid	11-Eicosenoic acid	20:1- delta-11c	59.83 ± 0.03	2279	5.60 ± 1.28
7	Arachidic acid	Eicosanoic acid	20:0	62.79 ± 0.01	2332	1.02 ± 0.31
8	Lignoceric acid	Tetracosanoic acid	24:0	64.74 ± 0.43	2692	3.11 ± 2.83
9	9, 10-dihydroxystearic acid	9,10-dihydroxy, octadecanoic acid	9, 10-di-OH-18:0	69.77 ± 0.02	2612	0.41 ± 0.20

[a]The retention time of fatty acid methyl ester were shown as mean ± SD. Samples were from 15 different sources.

[b]Kovat's index: Retention indices determined relative to n-alkane (C_6-C_{24}) on Zebron ZB-5 MS fuse silica column [64]

[c]The peak area were shown as mean ± SD in percentage sample were from 15 different sources.

Fatty acid methyl ester fingerprint of *Oroxylum indicum* seed oil

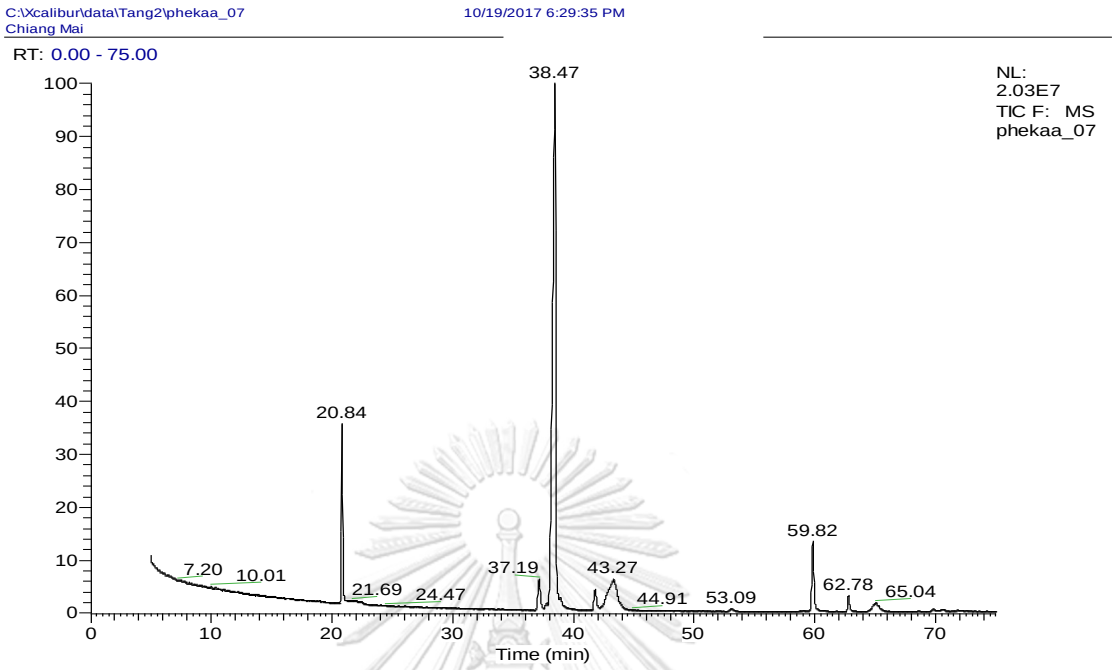

Figure 19 GC/MS chromatogram of *Oroxylum indicum* seed oil

Mass spectrum of retention time 20.84 was palmitic acid methyl ester

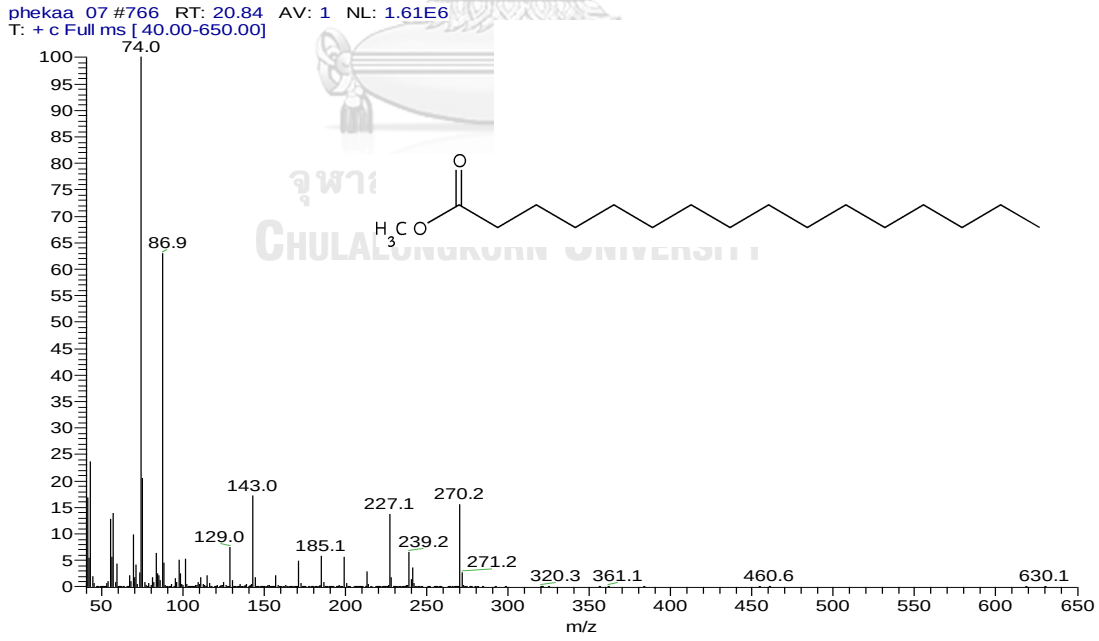

Figure 20 Mass spectrum of palmitic acid methyl ester

Mass spectrum of retention time 37.19 was linoleic acid methyl ester

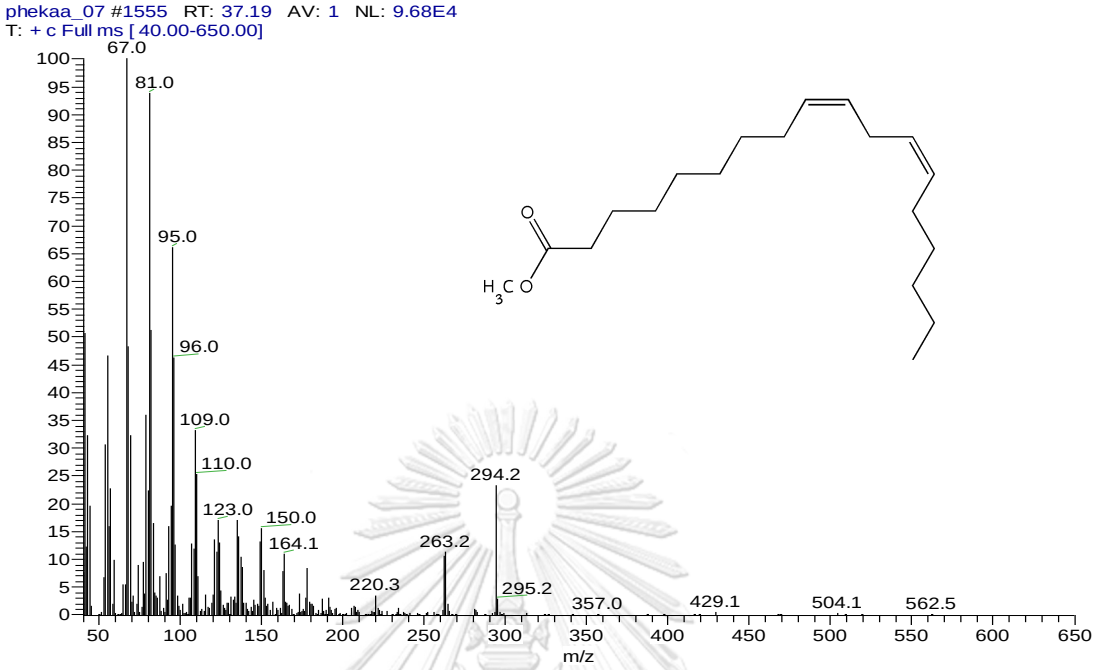

Figure 21 Mass spectrum of linoleic acid methyl ester

Mass spectrum of retention time 38.47 was oleic acid methyl ester

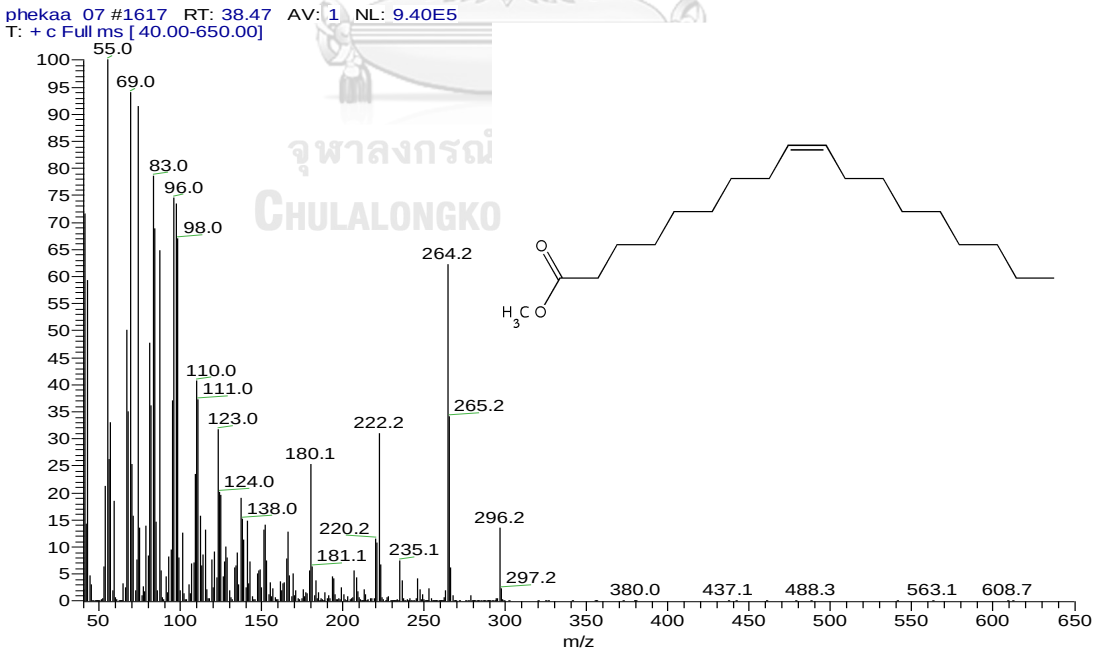

Figure 22 Mass spectrum of oleic acid methyl ester

Mass spectrum of retention time 41.80 was stearic acid methyl ester

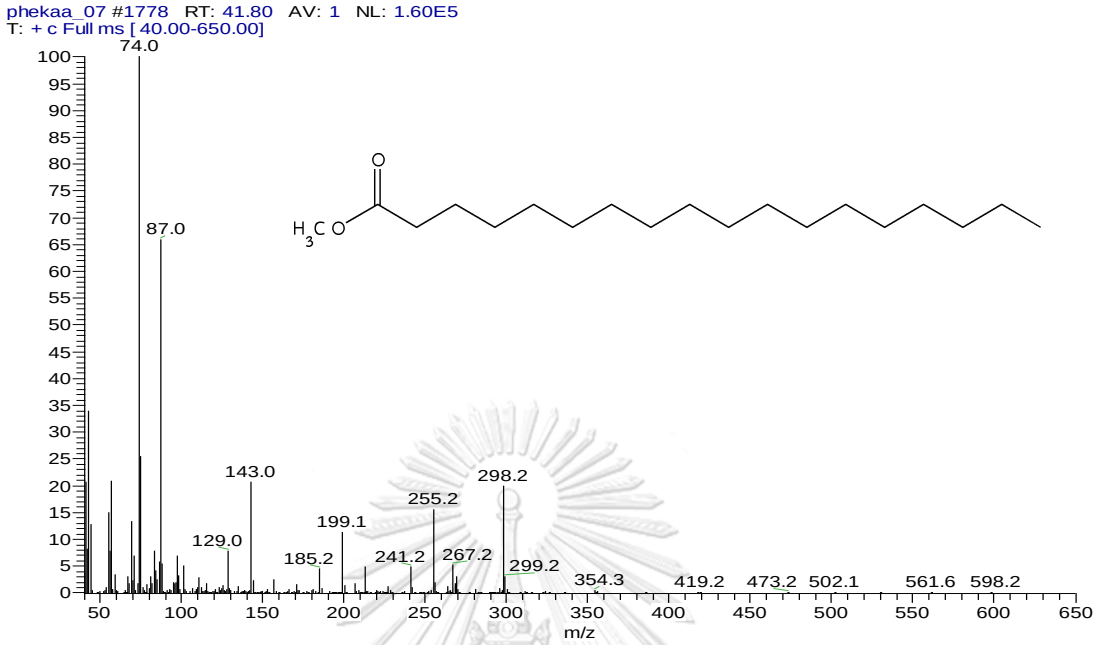

Figure 23 Mass spectrum of stearic acid methyl ester

Mass spectrum of retention time 43.27 was behenic acid methyl ester

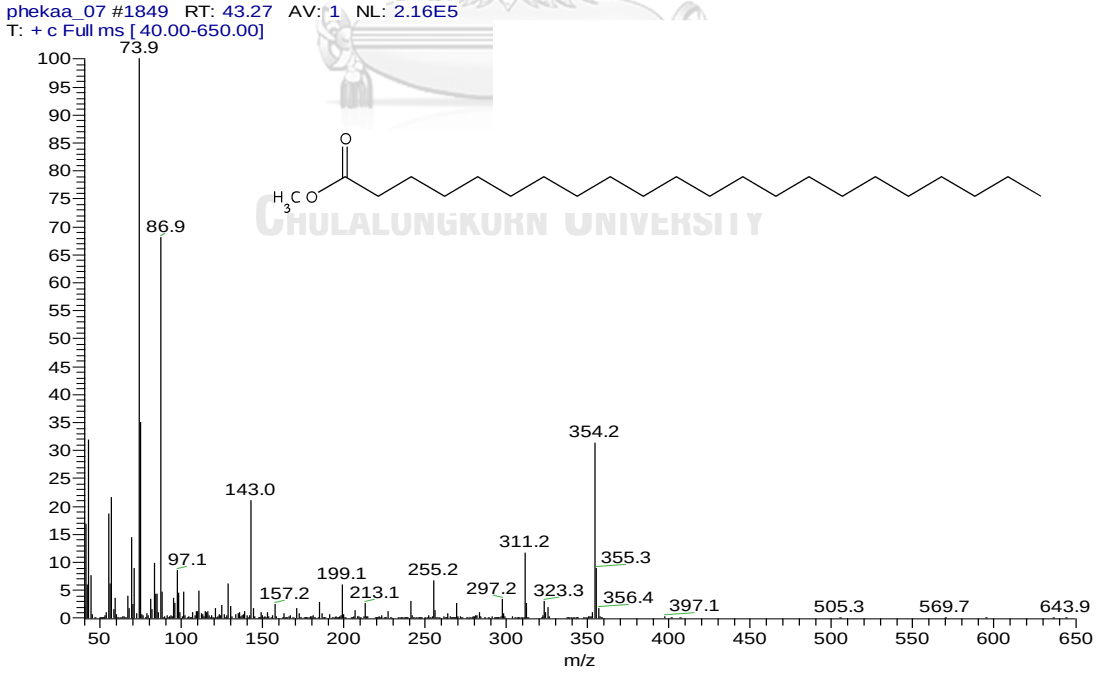

Figure 24 Mass spectrum of behenic acid methyl ester

Mass spectrum of retention time 59.82 was gondoic acid methyl ester

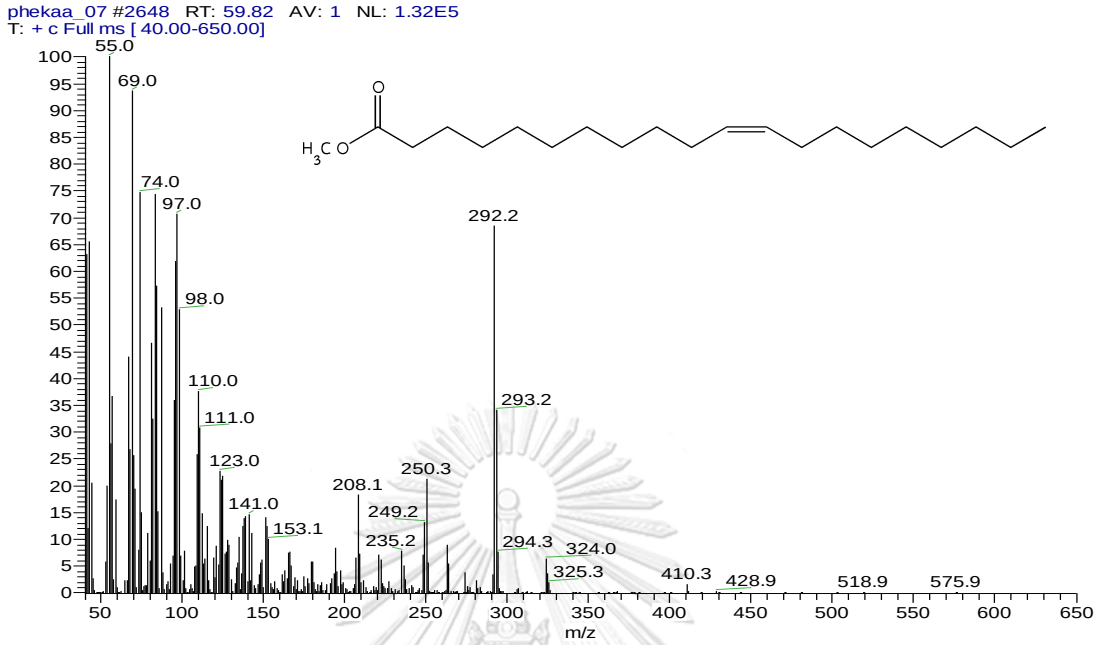

Figure 25 Mass spectrum of gondoic acid methyl ester

Mass spectrum of retention time 62.78 was arachidic acid methyl ester

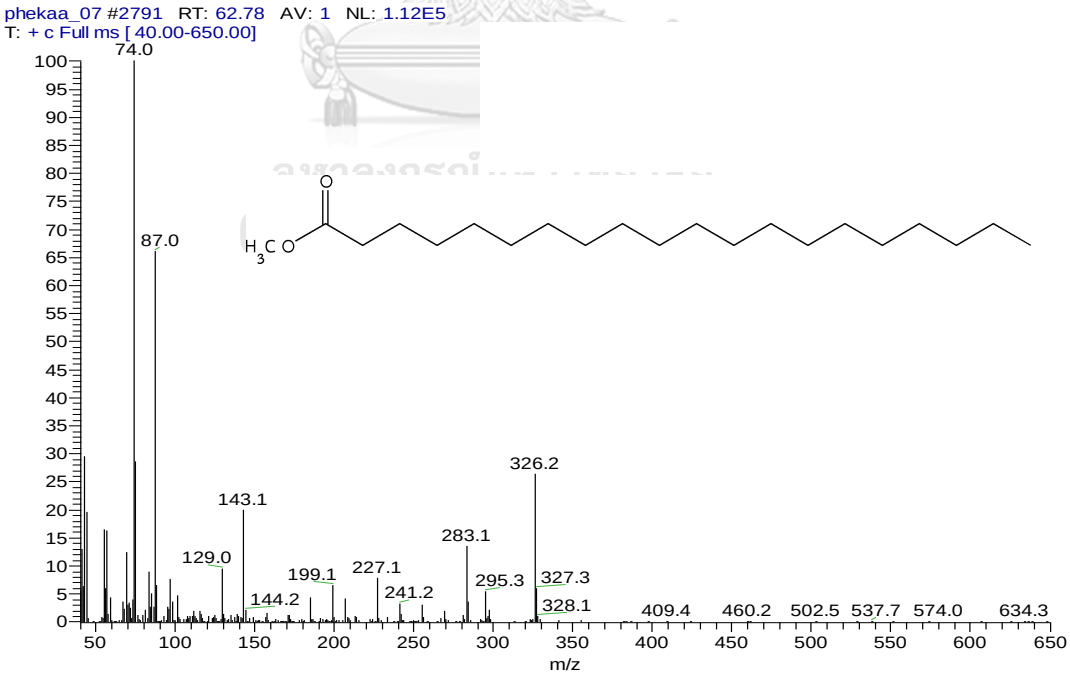

Figure 26 Mass spectrum of arachidic acid methyl ester

Mass spectrum of retention time 65.04 was lignoceric acid methyl ester

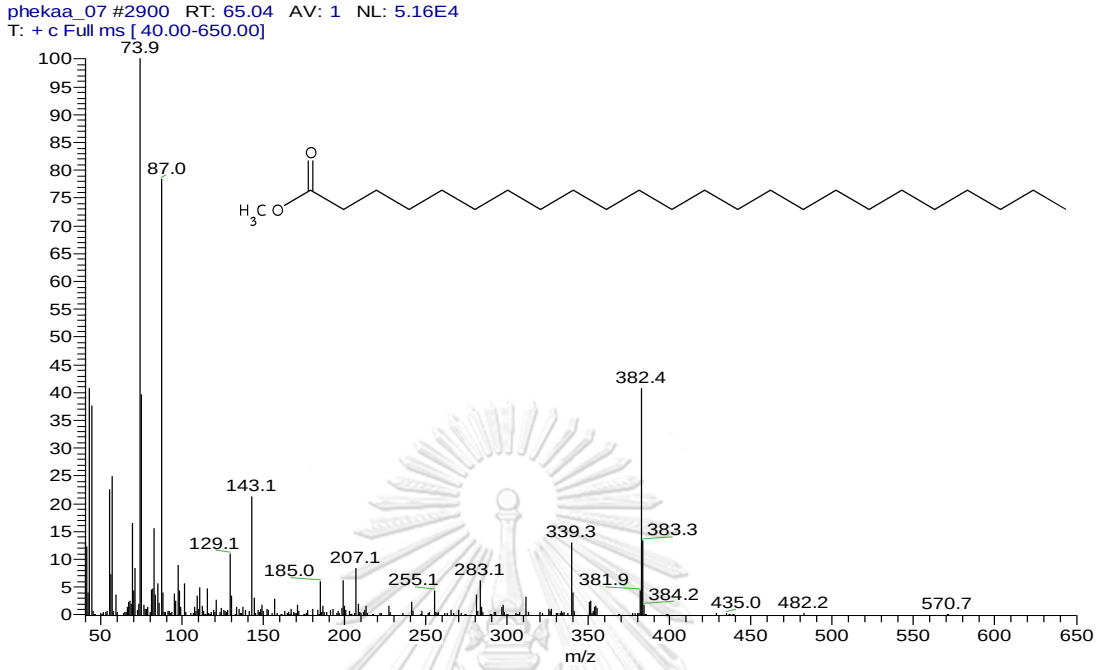

Figure 27 Mass spectrum of lignoceric acid methyl ester

Mass spectrum of retention time 69.76 was 9, 10 dihydroxystearic acid methyl ester

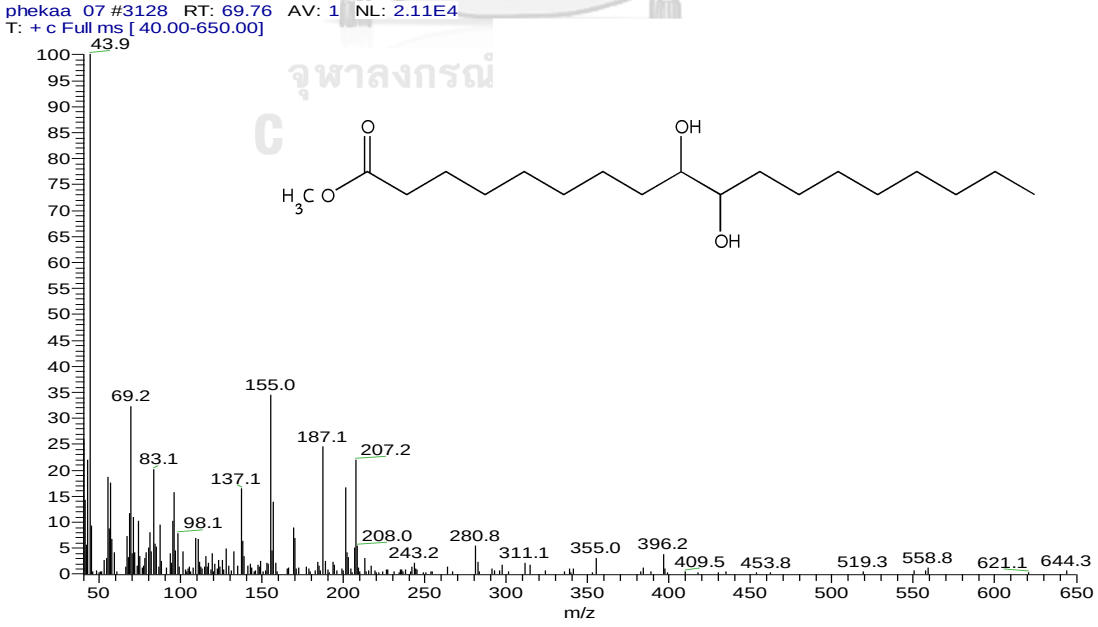

Figure 28 Mass spectrum of 9, 10-dihydroxystearic acid methyl ester

CHAPTER V
DISCUSSION AND CONCLUSION

The quality control and standardization of herbal crude drug are important for quality of herbal medicine. The pharmacognostic specification is set by macroscopic, microscopic characters, physico-chemical parameters, chemical fingerprint profile and eactive chemical constituent. These characteristics are criteria for authentication of crude drug as well as assurance of the purity and quality of crude drug.

Oroxylum indicum (L.) Kurz is one of medicinal plants used in many countries. In Thailand, it is a medicinal plant locally known in Thai name as Phe-kaa. Seed of *O. indicum* has been used as cough, throat infections, hypertension and purgative. The present study provided the information of pharmacognostic specifications, chrysin content of *O. indicum* seed crude drug and fatty acid constituents of fixed oil in *O. indicum* seed.

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The structural specifications are the first step that can help to identify and authenticate plant materials. *O. indicum* seed crude drug was illustrated in Figure 9 for its macroscopic structures as its shape, size, color, margin, length and wide of seed. Microscopic characteristics of the seed crude drug were also demonstrated. The sum part anatomical structure of endodermis, endosperm, cortical parenchyma, cortical fiber and epidermis was shown in Figure 11. The histological characteristics of *O. indicum* seed powder presenting parenchyma in transverse view, aleurone grains,

parenchyma in longitudinal view, septate fibre, stone cells and fibre was shown in Figure 12.

Physico-chemical parameters of *O. indicum* seed crude drug including loss on drying, water, total ash, acid-insoluble ash, ethanol soluble extractive matter, water soluble extractive matter were found to be 3.32 ± 0.12, 6.89 ± 0.80, 4.40 ± 0.08, 0.47 ± 0.05, 9.74 ± 0.68 and 12.11 ± 0.80 % by dry weight, respectively (Table 3). The physico-chemical parameters are a part of quality control for herbal drug. The contents of loss on drying, water, total ash and acid-insoluble ash should be not more than 3.3, 6.9, 4.4 and 0.5 % by dry weight, respectively. The ethanol soluble extractive matter and water soluble extractive matter should be not less than 9.7 and 12.1 % by dry weight, respectively. The results showed water soluble extractive yield higher than ethanol soluble extractive yield, it indicated more polar components in this seed. The data from this study could be used as quality criteria of this crude drug.

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Thin layer chromatography is a simple technique for separation, illustrated pattern of many components and identification of crude drug. Each compound can be separated and presented at different R_f value which can be used as marker for quality control. This study established TLC fingerprint of ethanolic extracts of *O. indicum* seed for crude drug identification according to qualitative evaluated components. The advantages of TLC are its simplicity, versatility, high velocity, specific sensitivity and simple sample preparation [65]. Thus, TLC is a convenient method for

determination of the quality and possible adulteration of plant materials [66]. In this study, TLC fingerprint of ethanolic extracts of *O. indicum* seed was performed using toluene : chloroform : acetone : formic acid (5:4:1:0.2) as mobile phase. The bands were clearly detected under ultraviolet lights (254 nm and 365 nm) and 10 % sulfuric acid in ethanol.

In this study, Soxhlet extraction of *O. indicum* seed with 95% ethanol was performed for the determination of chrysin content. The percentage of the ethanolic extract yields was 29.1 ± 5.4 % by weight. The advantages of this extraction include continuous process, basic, simple and cheap [67]. The disadvantages include time consuming, uses large amount of solvent, the Soxhlet apparatus cannot provide agitation to accelerate the process, not suitable for thermolabile compounds as long time heating may lead to degradation of compounds and exposure to hazardous and flammable liquid organic solvents with potential toxic emissions during extraction [68].

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For quantitative analysis of chrysin content in *O. indicum* seed crude drug, TLC-densitometry and TLC image analysis by Image J software were used as analytical techniques. 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 chrysin content in *O. indicum* seed owing to its convenient and cost-effective. Although TLC image analysis are not as sensitive as TLC-densitometry, it can be used to quantify only in

the visible range and specified UV wavelengths at 254 and 365 nm. In this study, TLC-densitometry and TLC image analysis using image J software were performed and validated to confirm that these analytical techniques provided reliable and accurate results. By TLC-densitometry and TLC image analysis in this study, chrysin content in *O. indicum* seed were found to be 0.17 ± 0.05 and 0.20 ± 0.07 % by dry weight, respectively (Table 13). TLC image analysis by image J software can be used as an alternative method to TLC densitometry. However, it was found that the results from TLC image analysis tended to be higher than the results from TLC densitometry ($P < 0.01$ by paired *t*-test). The previous study, Kruger and Ganzera analyzed chrysin in the methanolic extracts of *O. indicum* seeds from four different areas in India by HPLC, and indicated the content of 0.03, 0.10, 0.14 and 0.14 % dry weight [69]. Srinivas and Aparna analyzed chrysin in other plant parts by HPTLC and found that the amount of chrysin in the ethanolic extracts of *O. indicum* root, stem and leaf were 0.011-0.014, 0.002-0.004 and 0.006-0.007 % by dry weight respectively [70]. *O. indicum* seed obviously contained higher amount of chrysin compared to that of the root, stem and leaf. Zaveri *et al*. previously reported that baicalein, chrysin, scutellarine and oroxylin-A could be present in stem bark and leaves of *O. indicum* and validated HPLC method for quantification of their compounds in *O. indicum* stem bark and leaves [71].

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TLC-densitometry and TLC image analysis in this study were validated according to ICH guideline including accuracy, precision, limit of detection (LOD), limit of

quantitation (LOQ), specificity and robustness. The accuracy was evaluated by recovery of spiking known three concentrations of standard chrysin in sample matrix. The percent recovery values by two methods were 109.6 and 107.7 % respectively. The results were accepted in range of 80-120% [62], thus these methods were accurate. The precision of chrysin quantitative analysis was conducted by determination of 3 concentrations × 3 replicates at the same and different days of tests. The repeatability or intra-day precision and the intermediate precision or inter-day precision of two methods were 1.98 ± 0.78, 3.00 ± 2.23 and 4.30 ± 2.24, 5.12 ± 3.71 %RSD respectively, that were not more than 15 %RSD of standard criteria [17]. LOD and LOQ were calculated based on the residual standard deviation of a regression line. LOD value of TLC-densitometry and TLC image analysis, regard as the lowest concentration of analyte in a sample which could be detected were found to be 0.015 and 0.016 µg/spot, respectively. LOQ value of TLC-densitometry and TLC image analysis, regard as the lowest concentration of analyte in a sample which could be quantitatively determined were 0.046 and 0.048 µg/spot, respectively. The robustness estimated by analysis of the peak area after deliberate variation of mobile phase ratio (9:7:1:0.2, 10:8:2:0.4, 11:9:3:0.6) showed the values of 2.05 and 4.07 %RSD of sample peak area. The calibration curves were polynomial with the range of 0.3 - 1.2 µg/spot. Specificity of the method was validated through UV absorbance spectra under the range of 200-700 nm. The results revealed that the identical spectra among standard chrysin and chrysin in the ethanolic extract. The identity in absorbance spectra which

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determined at up-slope, apex and down-slope of the peak represented chromatographic peak purity of chrysin. Maximum absorption wavelength of chrysin was 269 nm in agreement with the previous study of 262 nm [72]. However, the results from method validation indicated that TLC-densitometry and TLC image analysis could be efficient, reliable and suitable technique for quantitative analysis of chrysin in *O. indicum* seed.

Gas chromatography-mass spectrometry is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of components can be separated, identified and quantified. This technique revolutionized the study of lipids by making it possible to determine the complete fatty acid composition of a lipid in a very short time [73]. For this purpose, the fatty acid components of lipids are converted to the simplest convenient volatile derivative, usually methyl esters, although other esters may be preferred for specific purposes. The preparation of such esters has, therefore, become by far the most common type of chemical reaction for lipid analysts. *O. indicum* seed oil was exhaustively extracted with petroleum ether using Soxhlet apparatus. The percentage yield of *O. indicum* seed oil was found to be 11.74 ± 2.66 % by weight. The seed oil was analyzed for fatty acid composition by GC/MS in the form of methyl ester derivative and 9 components were identified. It was indicated that the main compositions of the fatty acids in *O. indicum* seed oil were oleic acid (67.99 ± 5.98%),

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palmitic acid (10.22 ± 0.88%), behenic acid (7.28 ± 5.60%), gondoic acid (5.60 ± 1.28%), lignoceric acid (3.11 ± 2.83%), linoleic acid (2,69 ± 0.77%), stearic acid (1.86 ± 0.42%), arachidic acid (1.02 ± 0.31%) and 9, 10-dihydroxystearic acid (0.41 ± 0.20%) respectively. The result was related to the previous study of fatty acid of *O. indicum* seed oil in China which reported oleic acid (57.480%), palmitic acid (9.418%), behenic acid (6.882%), gondoic acid (8.531%), linoleic acid (4.053%), lignoceric acid (3.945%), erucic acid (2.934%), stearic acid (1.741%), α-linolenic acid (1.459%) and arachidic acid (1.361%) respectively [74].

In conclusion, the pharmacognostic specification of *O. indicum* seed in Thailand is established. This standard can further be used for quality control of this crude drug. Quantitative TLC can be used as a precise specific and reliable technique for analysis of chrysin content in this crude drug. The fatty acid composition of *O. indicum* seed oil was demonstrated.

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APPENDICES

จุฬาลงกรณ์มหาวิทยาลัย
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APPENDIX A

Data of pharmacognostic specification of *Oroxylum indicum* seed

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Table 16 Physico-chemical parameters of *Oroxylum indicum* dried seed

Source		No.	% by weight					
			Water content	Loss on drying	Total ash	Acid insoluble ash	ethanol soluble extractive matter	Water soluble extractive matter
1	Bangkok	1	7.33	3.48	4.53	0.39	11.31	10.51
		2	8.66	3.11	4.26	0.48	12.33	8.47
		3	7.67	2.95	4.15	0.44	12.12	8.29
2	Rayong	1	6.00	4.87	4.58	0.50	10.73	7.34
		2	5.33	5.01	4.54	0.41	10.19	7.79
		3	6.00	5.04	4.59	0.44	10.27	6.62
3	Satun	1	6.00	4.61	4.31	0.35	10.85	5.95
		2	6.67	4.58	4.31	0.36	10.09	6.90
		3	7.33	4.62	4.20	0.23	10.25	6.83
4	Phuket	1	6.67	4.65	4.12	0.37	10.91	9.66
		2	5.33	4.70	4.09	0.43	11.08	8.50
		3	6.00	4.84	4.08	0.38	9.27	9.00
5	Khonkaen	1	7.00	4.56	4.63	0.60	12.64	10.19
		2	6.33	4.64	4.66	0.50	12.28	11.10
		3	8.00	4.75	4.72	0.50	11.53	9.34
6	Lumpang	1	7.31	2.97	4.72	0.61	8.60	17.67
		2	6.00	3.13	4.55	0.49	7.39	16.86
		3	7.32	2.97	4.64	0.47	7.62	16.38
7	Chiang Mai	1	6.67	2.25	4.35	0.58	11.11	13.03
		2	6.00	2.37	4.56	0.63	11.27	12.52
		3	7.31	2.21	4.45	0.60	11.77	14.24
8	Songkla	1	6.33	3.30	4.51	0.48	8.68	14.87
		2	5.66	3.26	4.55	0.51	8.00	15.57
		3	6.65	3.09	4.49	0.54	8.29	14.98

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	Source	No.	% by weight					
			Water content	Loss on drying	Total ash	Acid insoluble ash	Ethanol soluble extractive matter	Water soluble extractive matter
9	Chanthaburi	1	4.99	3.11	4.05	0.52	10.36	13.91
		2	5.99	2.92	4.14	0.53	12.19	14.99
		3	5.67	2.94	4.20	0.52	12.19	15.65
10	Nakhon Ratchasima	1	7.32	2.58	4.44	0.49	7.94	14.16
		2	5.98	2.91	4.30	0.50	8.11	16.11
		3	8.66	2.94	4.35	0.50	8.75	15.68
11	Suratthani 1	1	6.66	2.86	4.45	0.43	10.30	13.16
		2	6.66	2.69	4.45	0.42	9.01	10.94
		3	7.98	2.84	4.49	0.38	10.33	11.68
12	Chaiyaphum	1	6.66	2.64	3.93	0.29	9.33	11.47
		2	5.98	2.83	3.90	0.36	8.10	11.39
		3	7.97	2.75	3.87	0.33	7.46	11.43
13	Nakhon Pathom	1	8.64	3.05	4.42	0.53	8.06	14.04
		2	8.00	2.88	4.53	0.56	7.39	12.47
		3	9.99	3.03	4.68	0.62	7.57	12.92
14	Suratthani 2	1	6.65	2.59	4.67	0.60	7.87	13.61
		2	7.32	2.55	4.52	0.49	9.87	13.79
		3	7.98	2.41	4.56	0.46	8.31	14.44
15	Phetchaboon	1	7.32	2.38	4.40	0.45	8.19	12.44
		2	6.00	2.40	4.45	0.44	9.48	14.53
		3	7.99	2.20	4.42	0.45	8.94	13.74
	Grand mean		**6.89**	**3.32**	**4.40**	**0.47**	**9.74**	**12.11**
	Pooled SD		**0.80**	**0.12**	**0.08**	**0.05**	**0.68**	**0.80**

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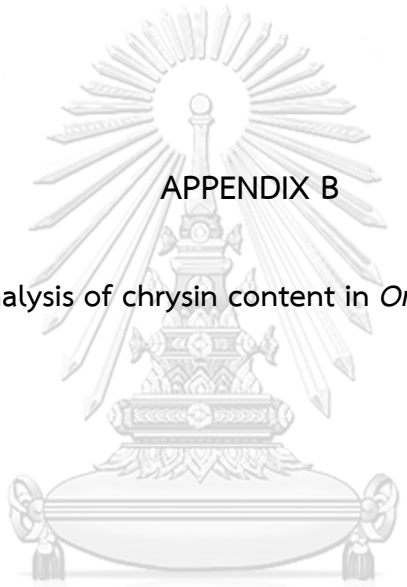
APPENDIX B

Quantitative analysis of chrysin content in *Oroxylum indicum* seed



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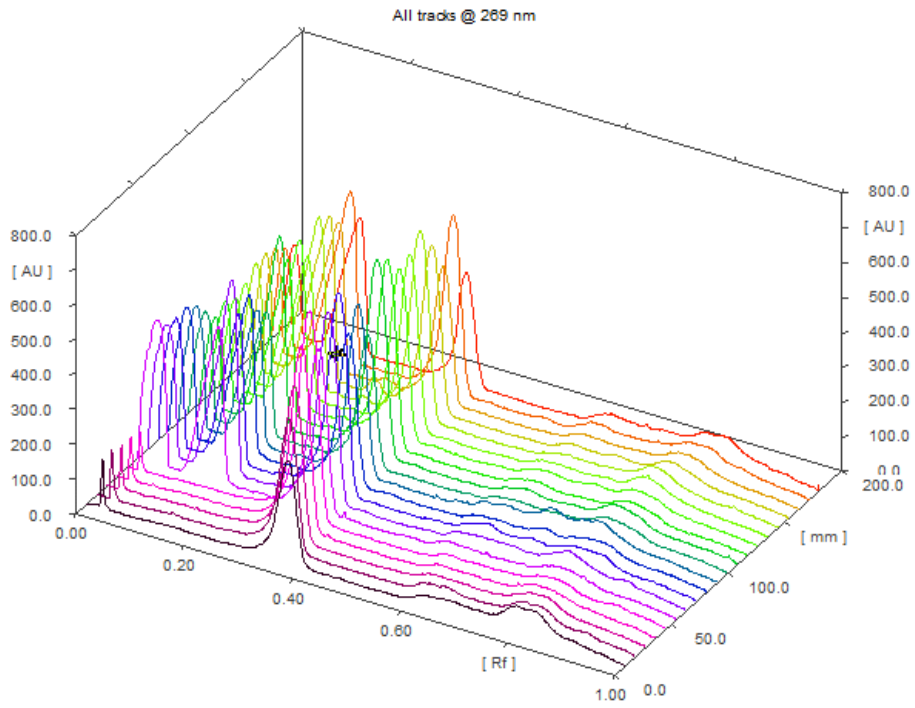

Figure 29 3D TLC-densitometry chromatogram of chrysin in *Oroxylum indicum* seed extracts from 15 different sources and standard chrysin for calibration range (Plate A – standard chrysin NO.1-5, sample NO.6-20)

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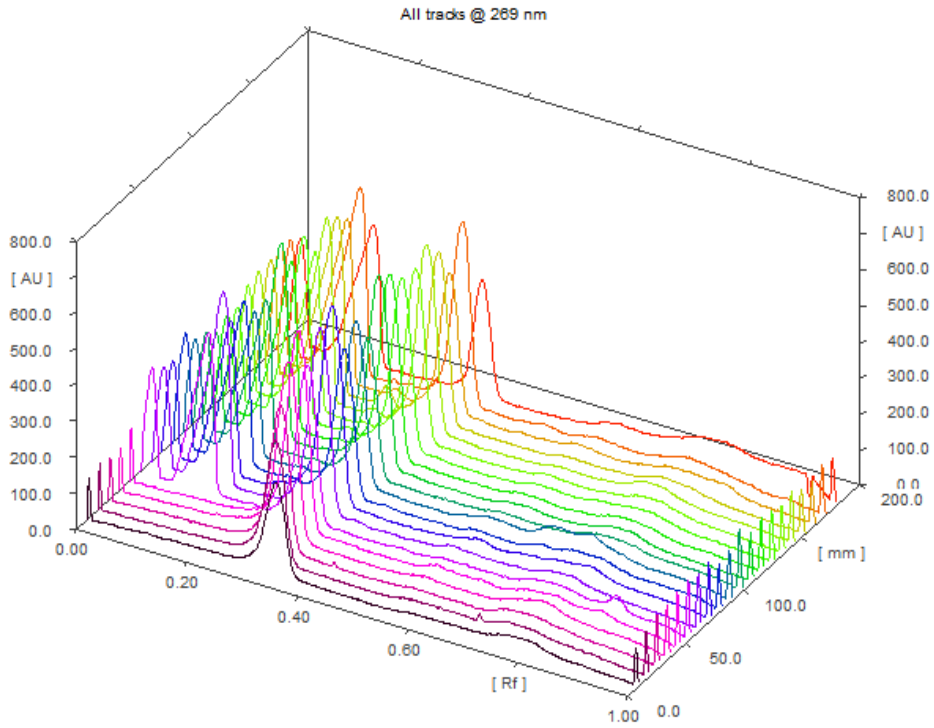

Figure 30 3D TLC-densitometry chromatogram of chrysin in *Oroxylum indicum* seed extracts from 15 different sources and standard chrysin for calibration range (Plate B – standard chrysin NO.1-5, sample NO.6-20)

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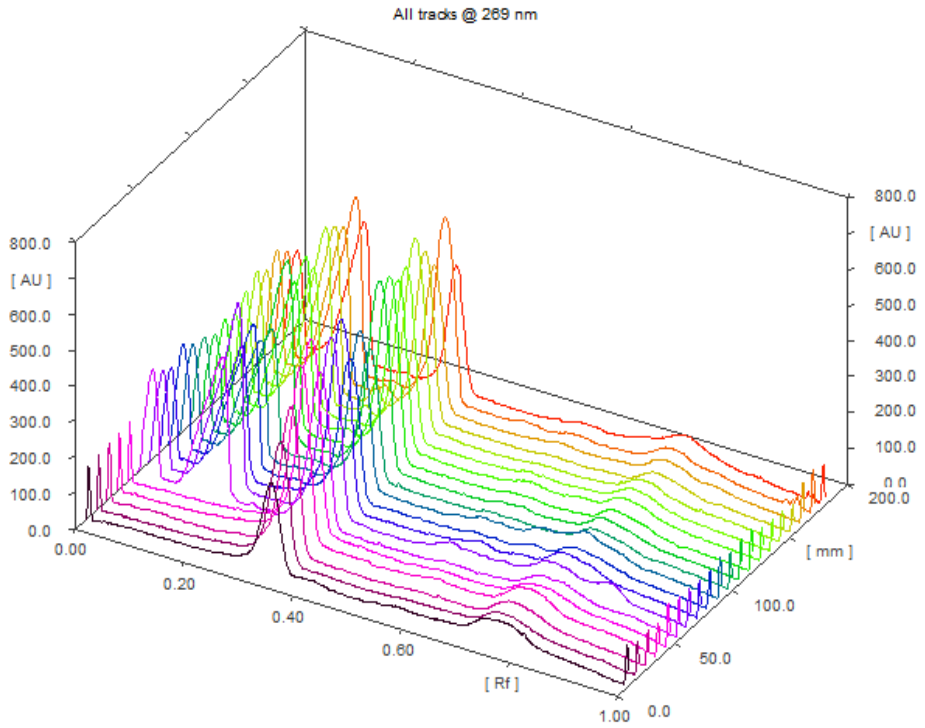

Figure 31 3D TLC-densitometry chromatogram of chrysin in *Oroxylum indicum* seed extracts from 15 different sources and standard chrysin for calibration range (Plate C – standard chrysin NO.1-5, sample NO.6-20)

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Table 17 Accuracy by TLC densitometry

Chrysin added (µg/spot)	1	2	3	Average	%Recovery			
sample	0.42	0.41	0.43	0.42	**1**	**2**	**3**	**Average**
0.15	0.63	0.63	0.62	0.62	110.49	112.93	105.79	109.73
0.30	0.78	0.80	0.80	0.79	108.21	112.20	108.76	109.73
0.60	1.08	1.12	1.14	1.14	106.41	110.88	110.30	109.20
							Average	109.55
							SD	0.31

Table 18 Repeatability by TLC densitometry

Chrysin added (µg/spot)	Repeatability					
	1	**2**	**3**	**Mean**	**SD**	**%RSD**
0.000	0.418	0.408	0.432	0.419	0.012	2.81
0.150	0.628	0.630	0.615	0.624	0.008	1.28
0.300	0.777	0.795	0.796	0.789	0.011	1.35
0.600	1.083	1.118	1.138	1.113	0.028	2.50
					Average	**1.98**
					SD	**0.78**

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Table 19 Intermediate precision by TLC densitometry

Chrysin added (µg/spot)	Intermediate precision					
	1	2	3	Mean	SD	%RSD
0.000	0.380	0.384	0.401	0.388	0.011	2.87
0.150	0.616	0.576	0.615	0.602	0.023	3.79
0.300	0.787	0.753	0.745	0.762	0.022	2.93
0.600	1.088	1.167	1.002	1.086	0.083	7.60
					Average	4.30
					SD	2.24

Table 20 Robustness by TLC densitometry

Mobile phase (v/v)	Robustness					Peak area of chrysin (AU)
	0.1	0.2	0.3	Sample14	Sample15	
M1	8548.88	13172.20	16812.55	16869.79	10429.06	16812.55
M2	8701.20	12960.47	16654.96	15484.77	9817.29	16654.96
M3	7715.65	12298.82	16163.86	15416.46	9406.58	16163.86
Mean	8321.91	12810.50	16543.79	15923.67	9884.31	16543.79
SD	530.53	455.60	338.33	820.07	514.52	338.33
%RSD	6.38	3.56	2.05	5.15	5.21	2.05

* M1 is a mobile phase ratio of toluene : chroloform : acetone : formic acid (10:8:2:0.4)

M2 is a mobile phase ratio of toluene : chroloform : acetone : formic acid (9:7:1:0.2)

M3 is a mobile phase ratio of toluene : chroloform : acetone : formic acid (11:9:3:0.6)

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Table 21 Accuracy by TLC image analysis

Chrysin added (µg/spot)	1	2	3	Average	%Recovery			
Sample	0.49	0.48	0.44	0.47	1	2	3	Average
0.15	0.62	0.59	0.58	0.60	97.49	93.82	98.80	96.70
0.30	0.90	0.90	0.88	0.89	114.02	114.96	119.09	116.02
0.60	1.16	1.18	1.19	1.18	107.10	109.31	114.83	110.41
							Average	107.71
							SD	9.94

Table 22 Repeatability by TLC image analysis

Chrysin added (µg/spot)	Repeatability					
	1	2	3	Mean	SD	%RSD
0.000	0.485	0.480	0.435	0.467	0.028	5.94
0.150	0.619	0.591	0.578	0.596	0.021	3.51
0.300	0.895	0.897	0.875	0.889	0.012	1.37
0.600	1.162	1.181	1.188	1.177	0.014	1.17
					Average	3.00
					SD	2.23

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Table 23 Intermediate precision by TLC image analysis

Chrysin added (µg/spot)	Intermediate precision					
	1	2	3	Mean	SD	%RSD
0.000	0.416	0.489	0.471	0.459	0.038	8.29
0.150	0.629	0.652	0.735	0.672	0.056	8.30
0.300	0.878	0.876	0.918	0.891	0.024	2.66
0.600	1.131	1.159	1.149	1.146	0.014	1.24
					Average	5.12
					SD	3.71

Table 24 Robustness by TLC image analysis

Mobile phase (v/v)	Robustness					Peak area of chrysin (Pixel)
	0.1	0.2	0.3	Sample14	Sample15	
M1	5350.60	10412.50	14960.91	15588.53	7374.19	10412.50
M2	5801.24	10974.51	13046.58	13301.04	7298.28	10974.51
M3	6312.75	11287.90	14507.69	13498.53	8655.17	11287.90
Mean	5821.54	10891.64	14171.73	14129.37	7775.88	10891.64
SD	481.38	443.55	1000.41	1267.52	762.43	443.55
%RSD	8.27	4.07	7.06	8.97	9.81	4.07

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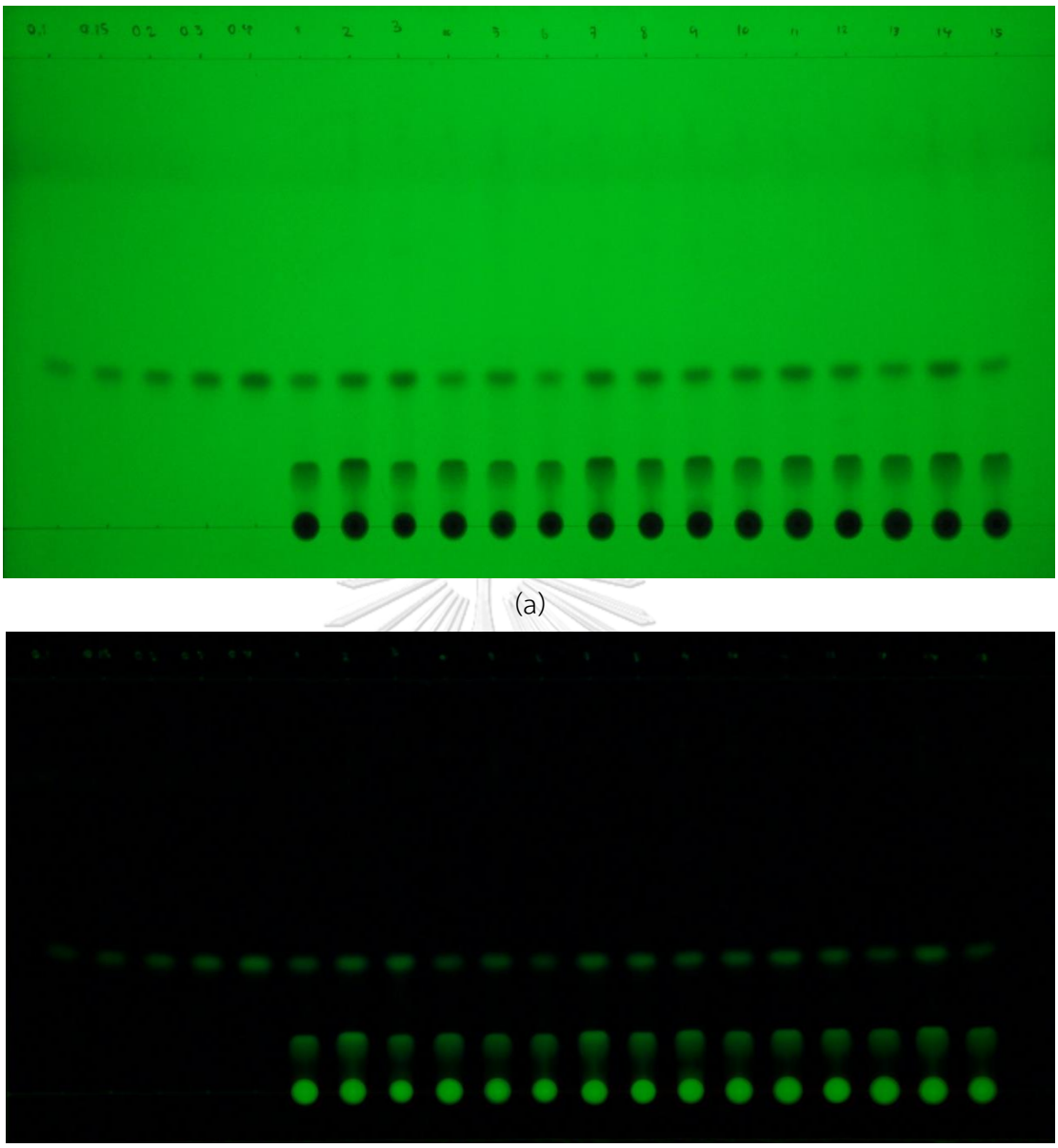
* M1 is a mobile phase ratio of toluene : chroloform : acetone : formic acid (10:8:2:0.4)

M2 is a mobile phase ratio of toluene : chroloform : acetone : formic acid (9:7:1:0.2)

M3 is a mobile phase ratio of toluene : chroloform : acetone : formic acid (11:9:3:0.6)



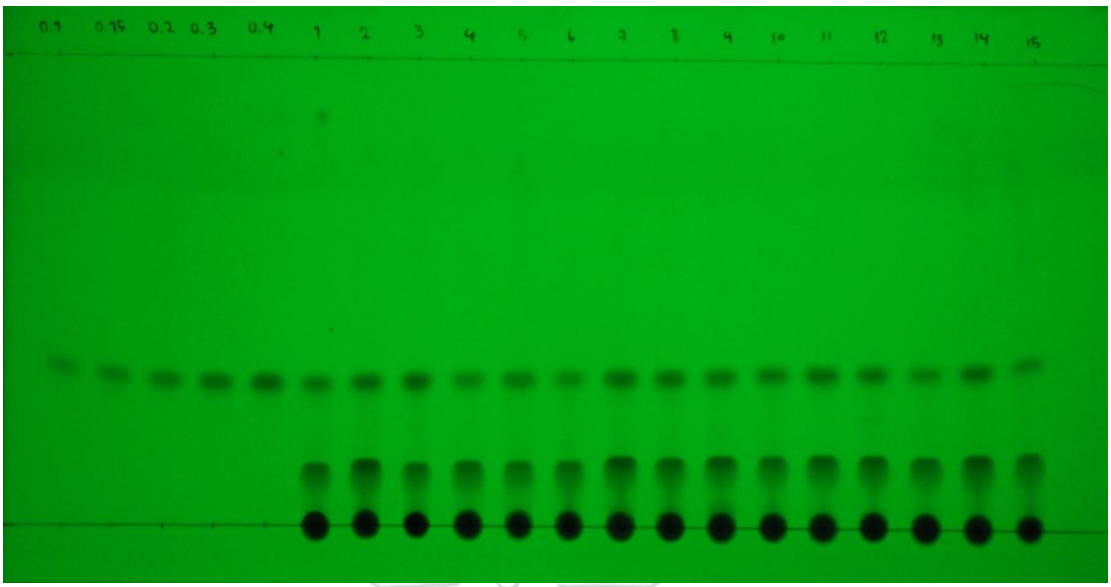
(a)

(b)

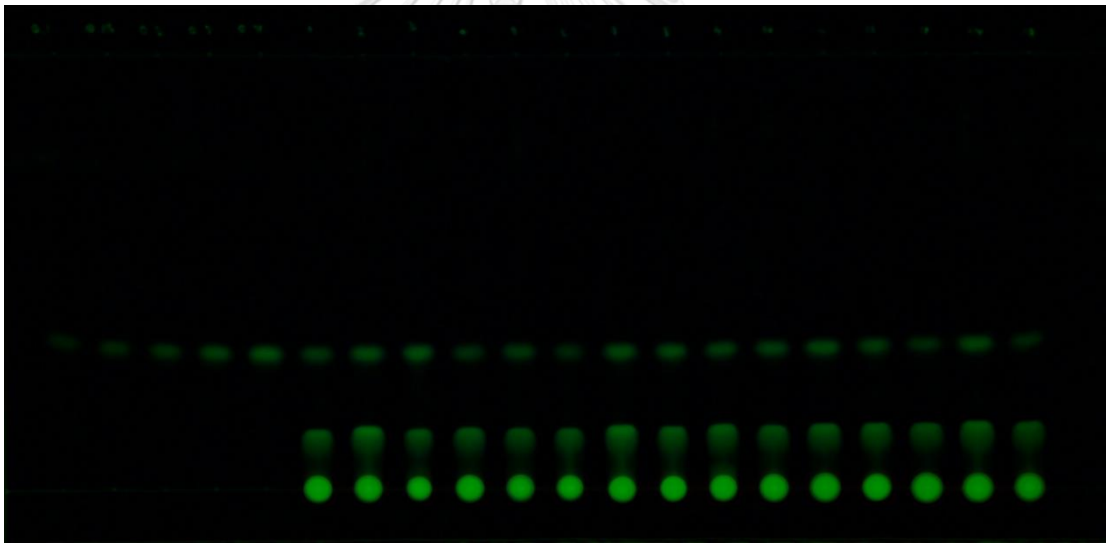
Figure 32 The TLC Plate A

(standard chrysin NO.1-5, sample NO.6-20) visual under UV 254 nm (a),

with subtract background by Image J software (b)



(a)

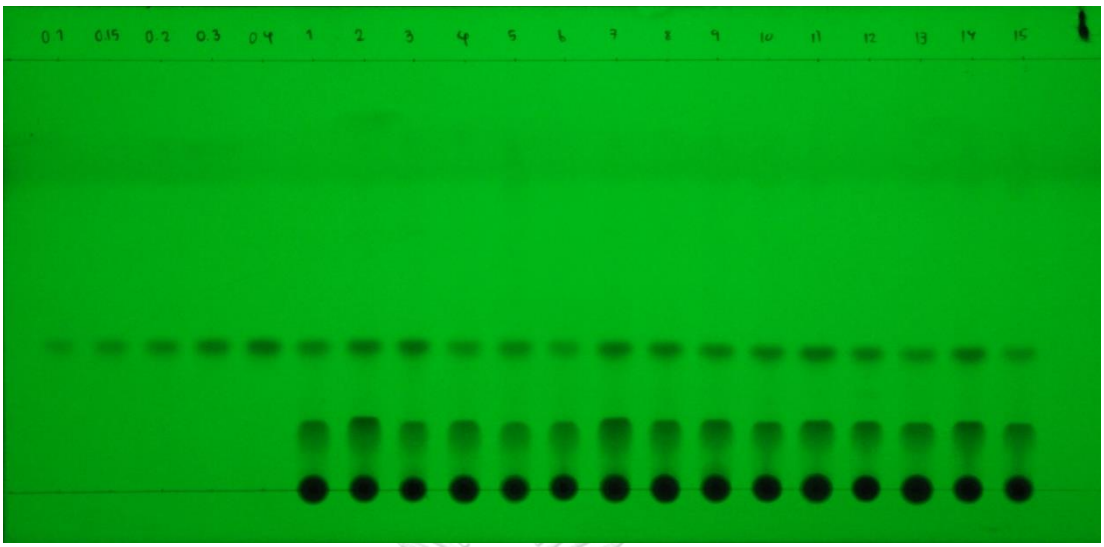


(b)

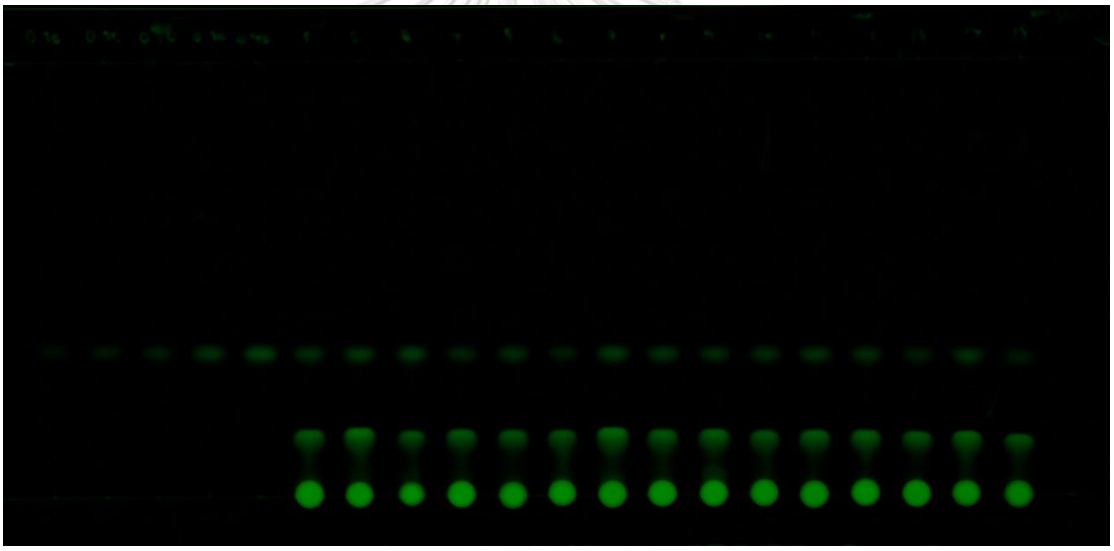
Figure 33 The TLC Plate B

(standard chrysin NO.1-5, sample NO.6-20) visual under UV 254 nm (a),

with subtract background by Image J software (b)

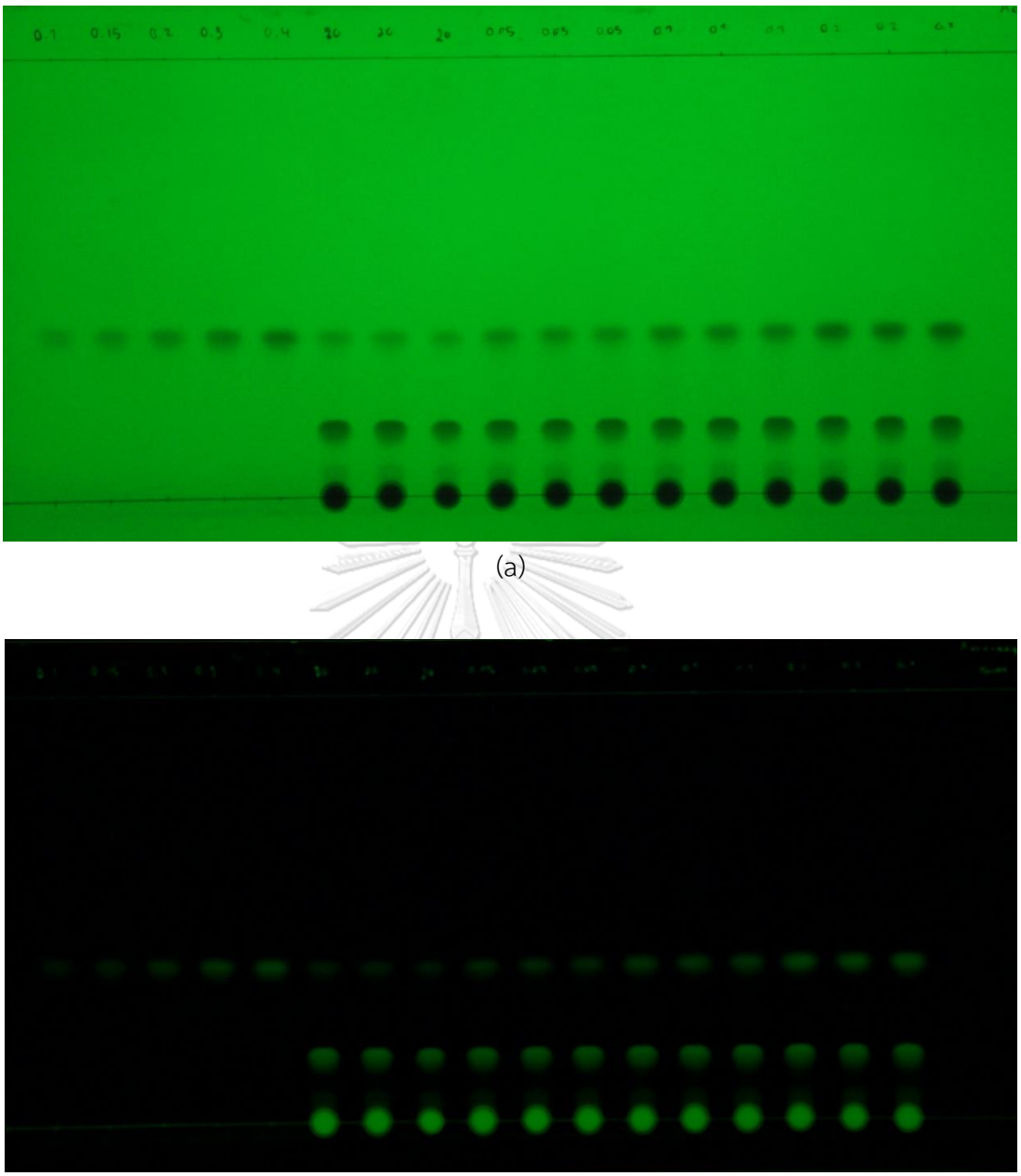


(a)



(b)

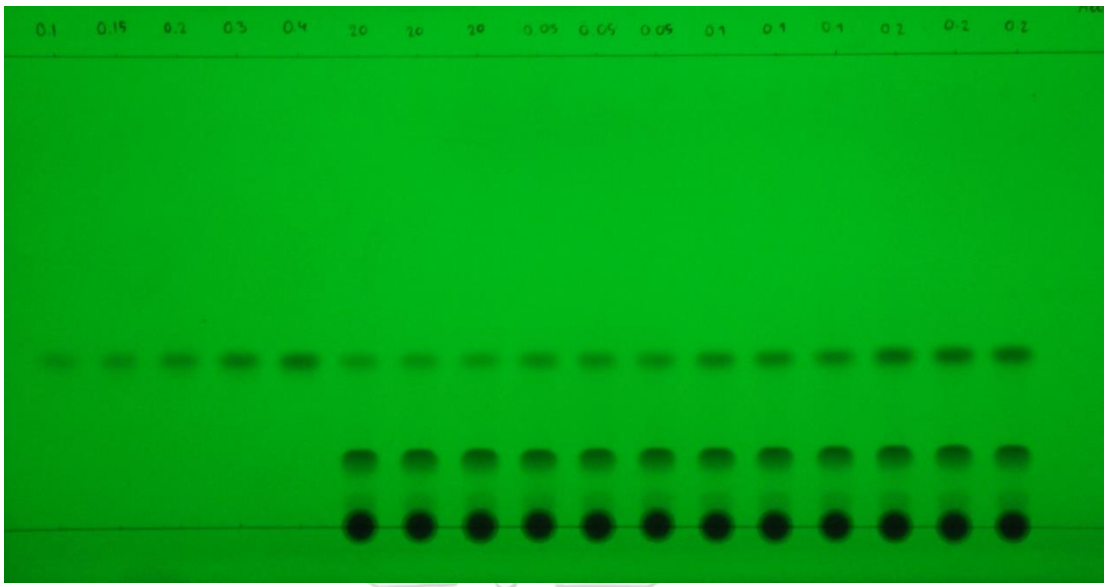
Figure 34 The TLC Plate C
(standard chrysin NO.1-5, sample NO.6-20) visual under UV 254 nm (a),
with subtract background by Image J software (b)



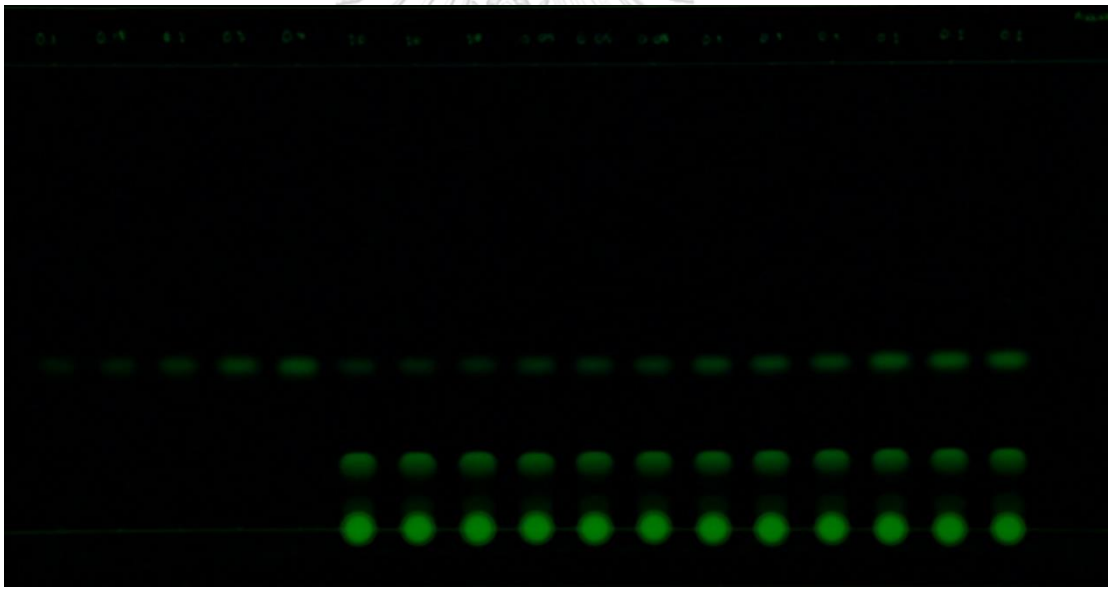
(a)

(b)

Figure 35 The TLC Plate D Accuracy
(standard chrysin NO.1-5, sample NO.6-8, spiked standard 0.15 µg/spot NO. 9-11, spiked standard 0.30 µg/spot NO. 12-14, spiked standard 0.60 µg/spot NO. 15-17) visual under UV 254 nm (a), with subtract background by Image J software (b)

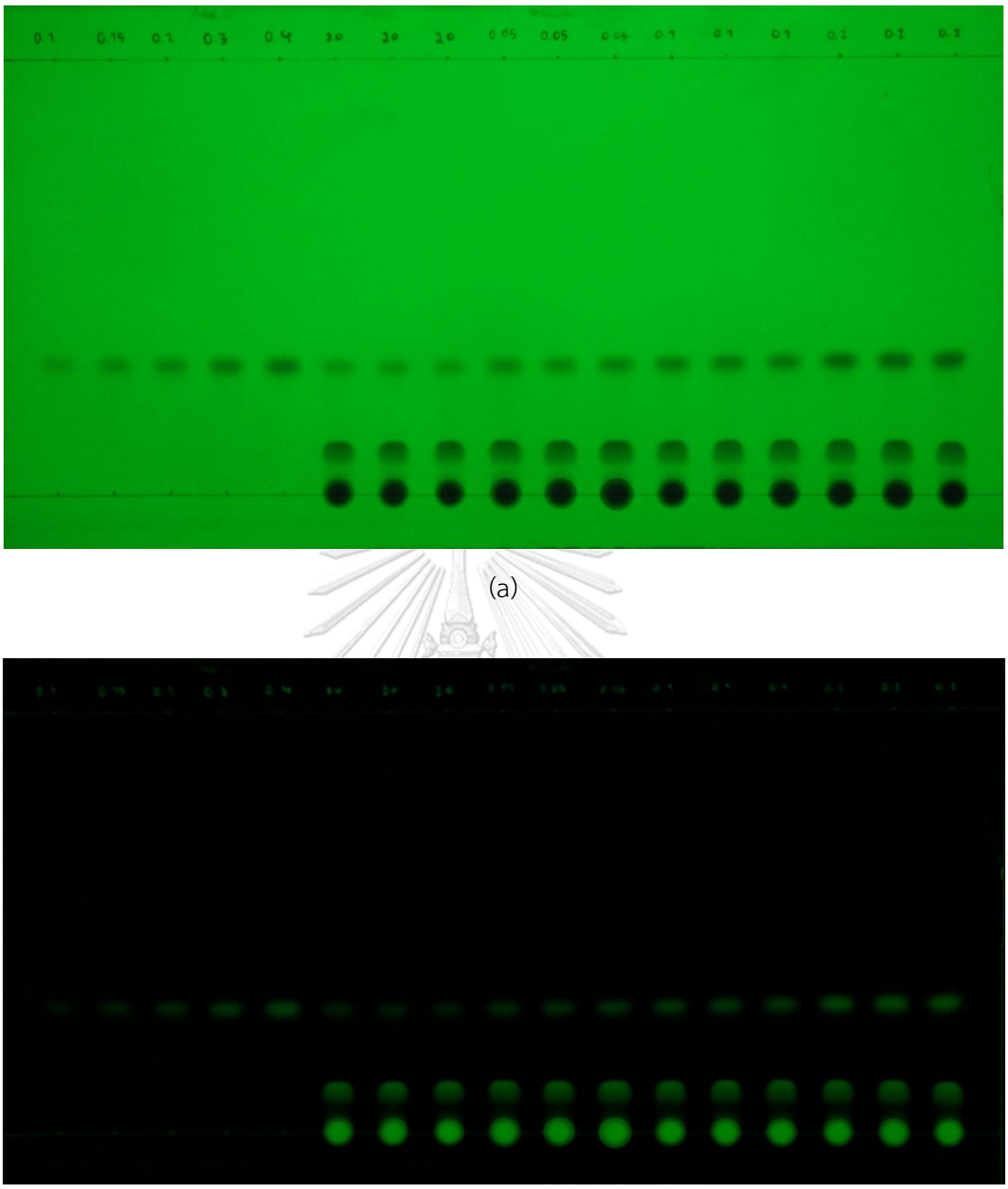


(a)



(b)

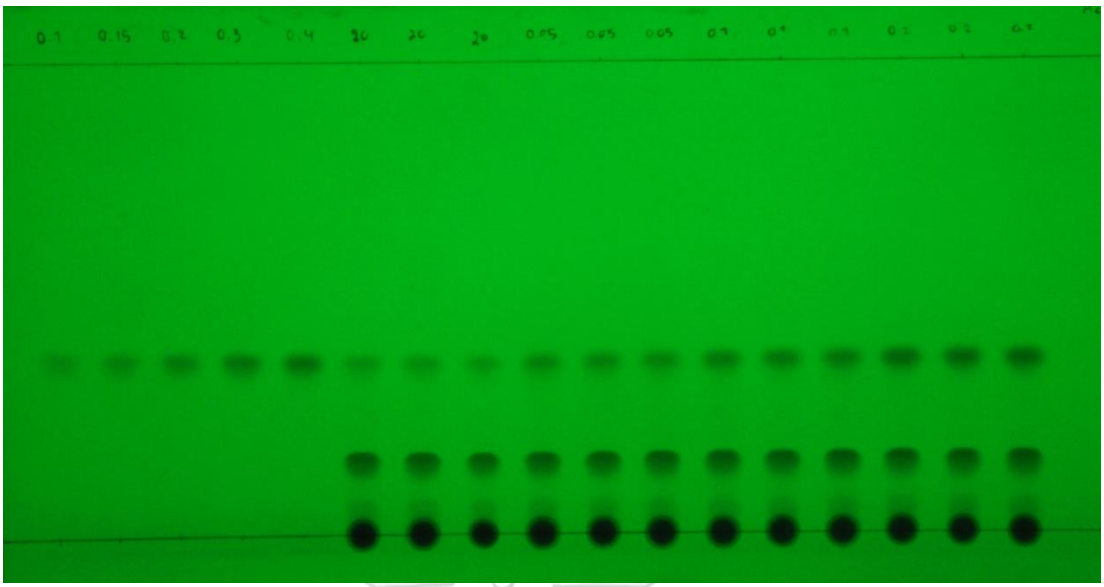
Figure 36 The TLC Plate E Precision 1
(standard chrysin NO.1-5, sample NO.6-8, spiked standard 0.15 µg/spot NO. 9-11, spiked standard 0.30 µg/spot NO. 12-14, spiked standard 0.60 µg/spot NO. 15-17) visual under UV 254 nm (a), with subtract background by Image J software (b)



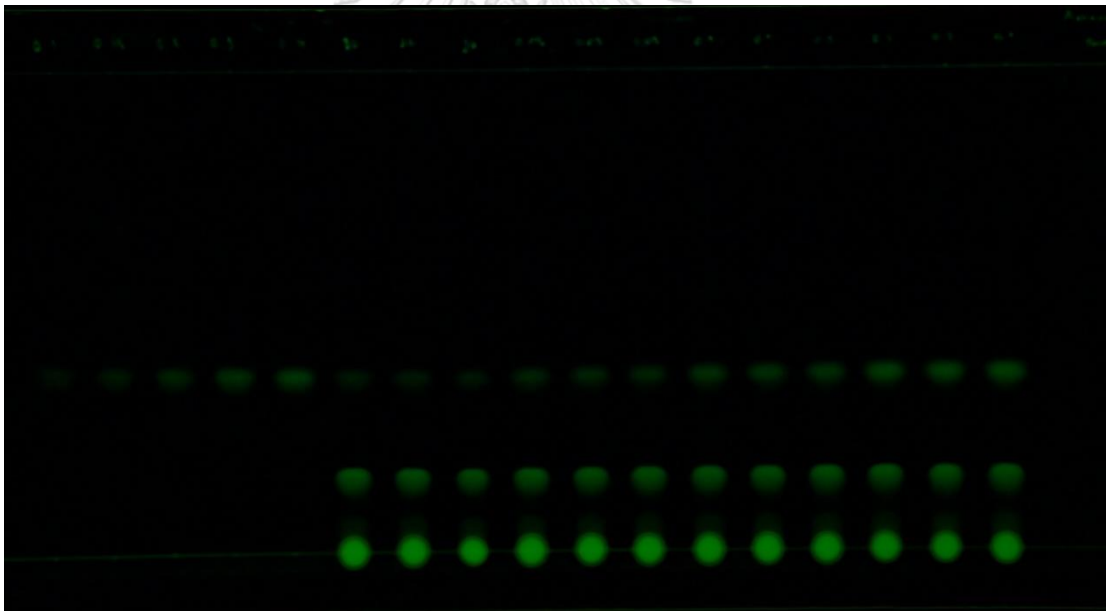
(a)

(b)

Figure 37 The TLC Plate F Precision 2
(standard chrysin NO.1-5, sample NO.6-8, spiked standard 0.15 µg/spot NO. 9-11, spiked standard 0.30 µg/spot NO. 12-14, spiked standard 0.60 µg/spot NO. 15-17) visual under UV 254 nm (a), with subtract background by Image J software (b)



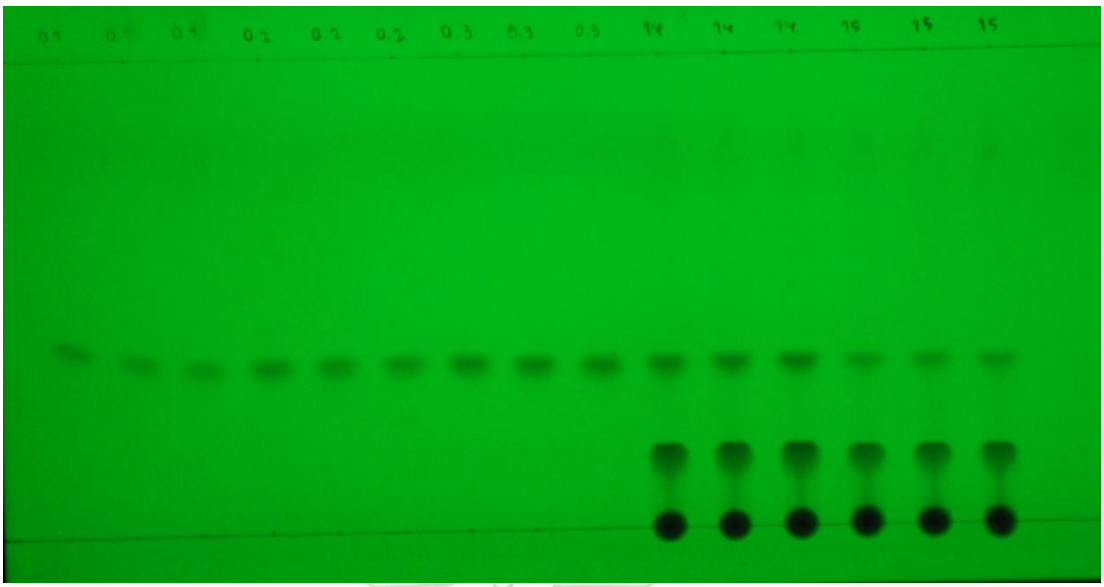
(a)



(b)

Figure 38 The TLC Plate G Precision 3

(standard chrysin NO.1-5, sample NO.6-8, spiked standard 0.15 µg/spot NO. 9-11, spiked standard 0.30 µg/spot NO. 12-14, spiked standard 0.60 µg/spot NO. 15-17) visual under UV 254 nm (a), with subtract background by Image J software (b)



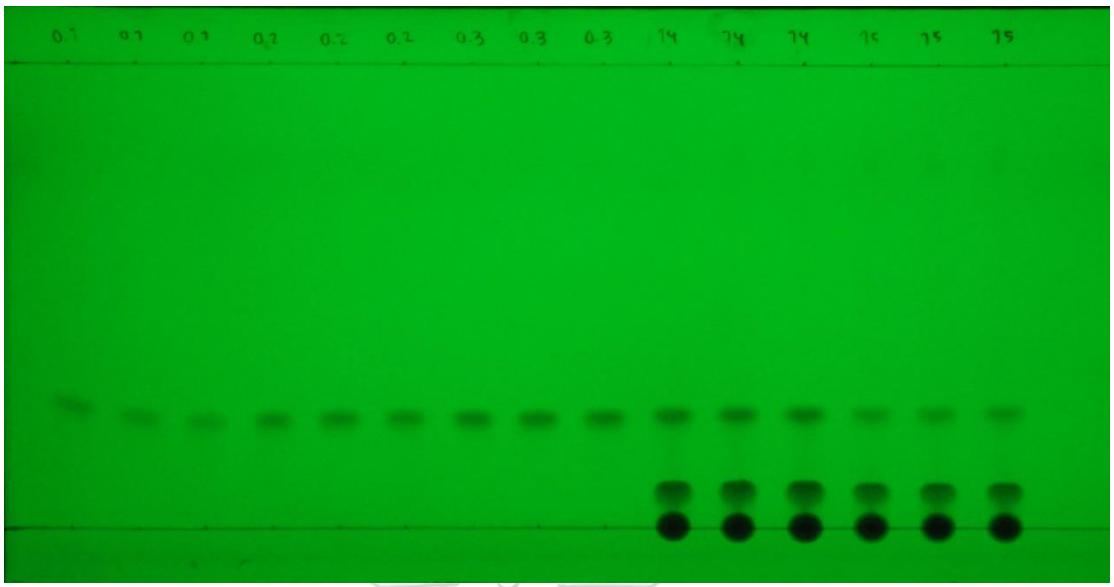
(a)

(b)

Figure 39 The TLC Plate H Robustness 1

(standard chrysin NO.1-9, sample NO.10-15, Mobile phase ratio toluene : chroloform : acetone : formic acid (10:8:2:0.4) visual under UV 254 nm (a),

with subtract background by Image J software (b)



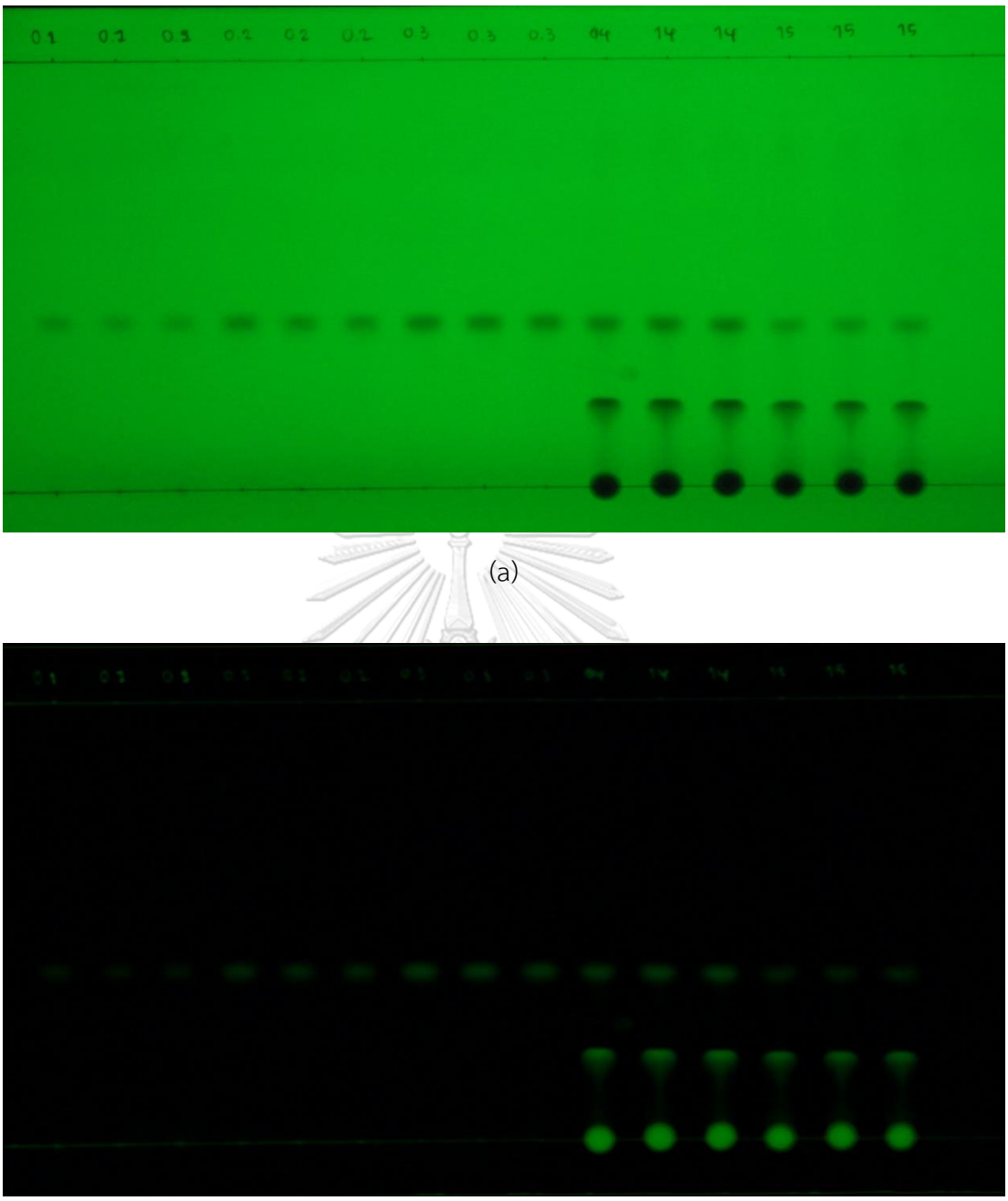
(a)

(b)

Figure 40 The TLC Plate I Robustness 2

(standard chrysin NO.1-9, sample NO.10-15, Mobile phase ratio toluene : chroloform : acetone : formic acid (9:7:1:0.2) visual under UV 254 nm (a),

with subtract background by Image J software (b)



(a)

(b)

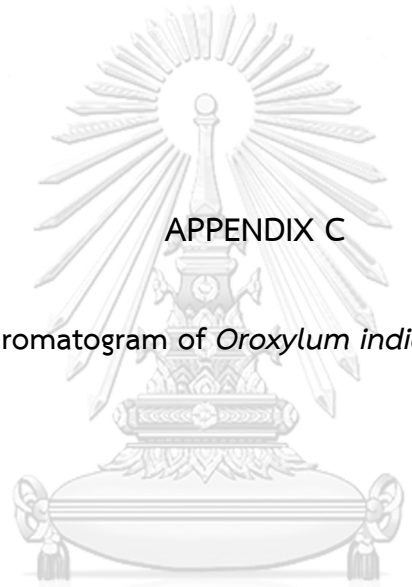
Figure 41 The TLC Plate J Robustness 3

(standard chrysin NO.1-9, sample NO.10-15, Mobile phase ratio toluene : chroloform : acetone : formic acid (11:9:3:0.6) visual under UV 254 nm (a),

with subtract background by Image J software (b)

APPENDIX C

GC chromatogram of *Oroxylum indicum* seed oil



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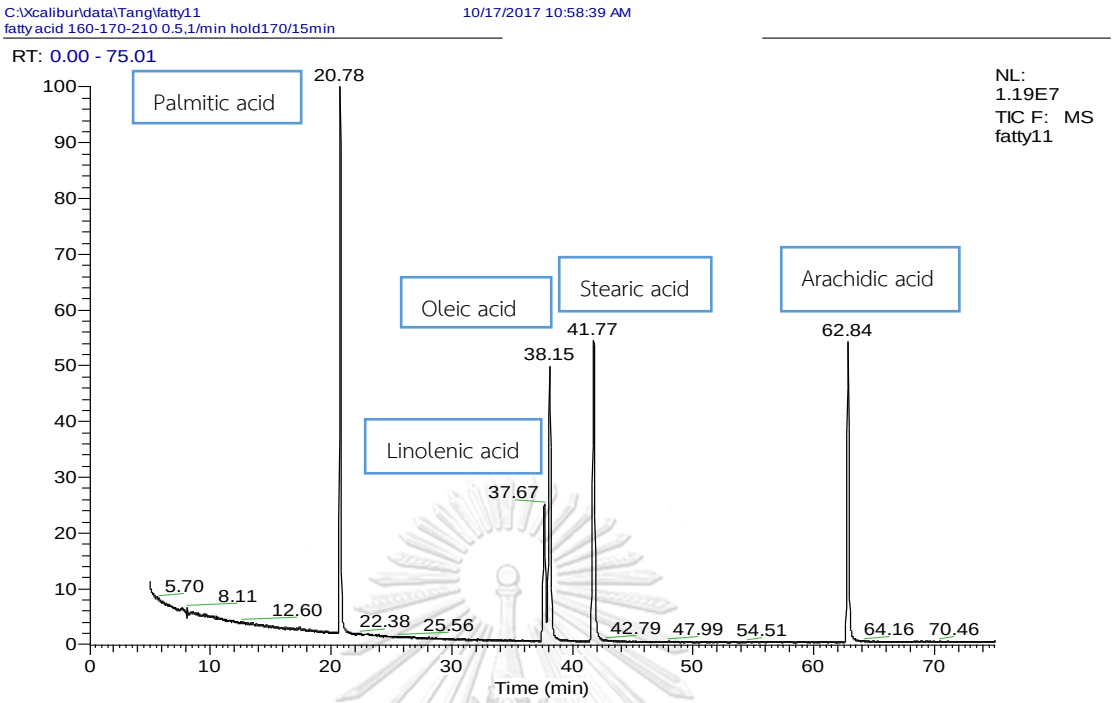

Figure 42 GC chromatogram of fatty acid methyl ester Mix GLC-20

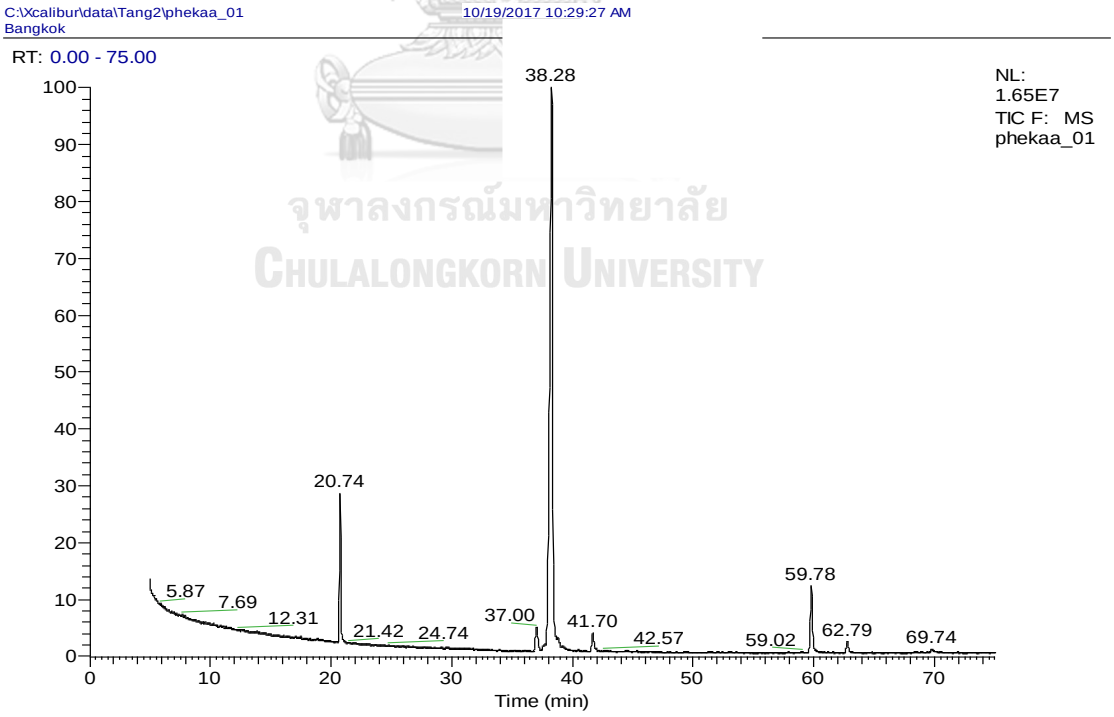

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Figure 43 GC chromatogram of *Oroxylum indicum* seed oil from Bangkok (Source 01)

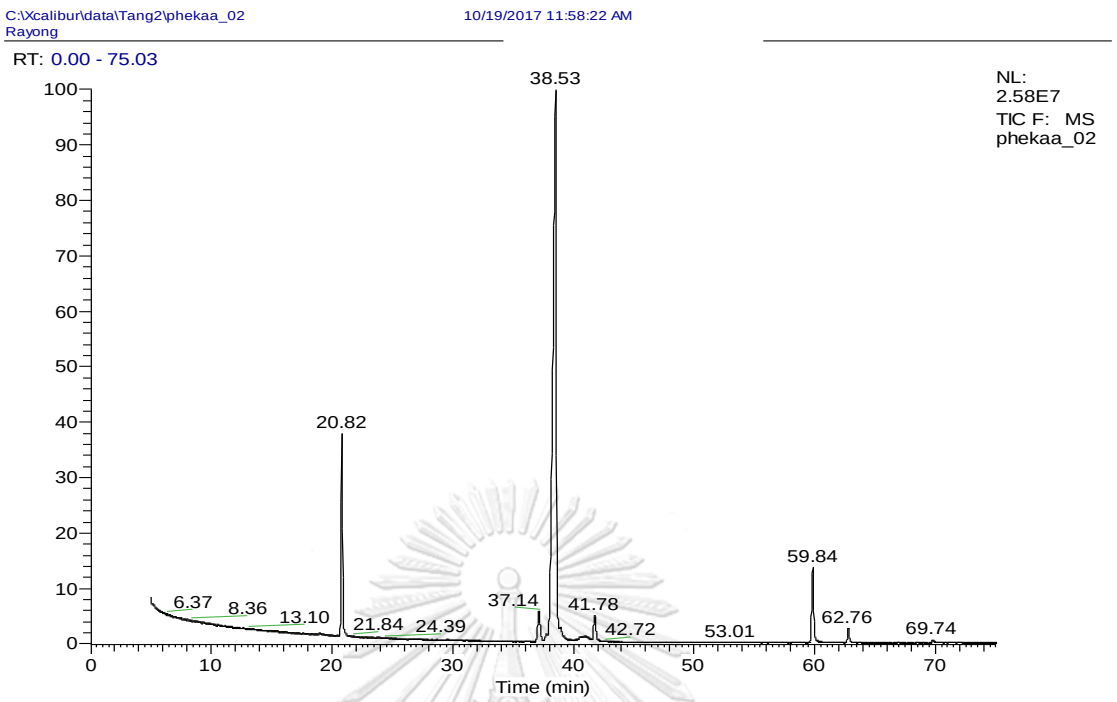

Figure 44 GC chromatogram of *Oroxylum indicum* seed oil from Rayong (Source 02)

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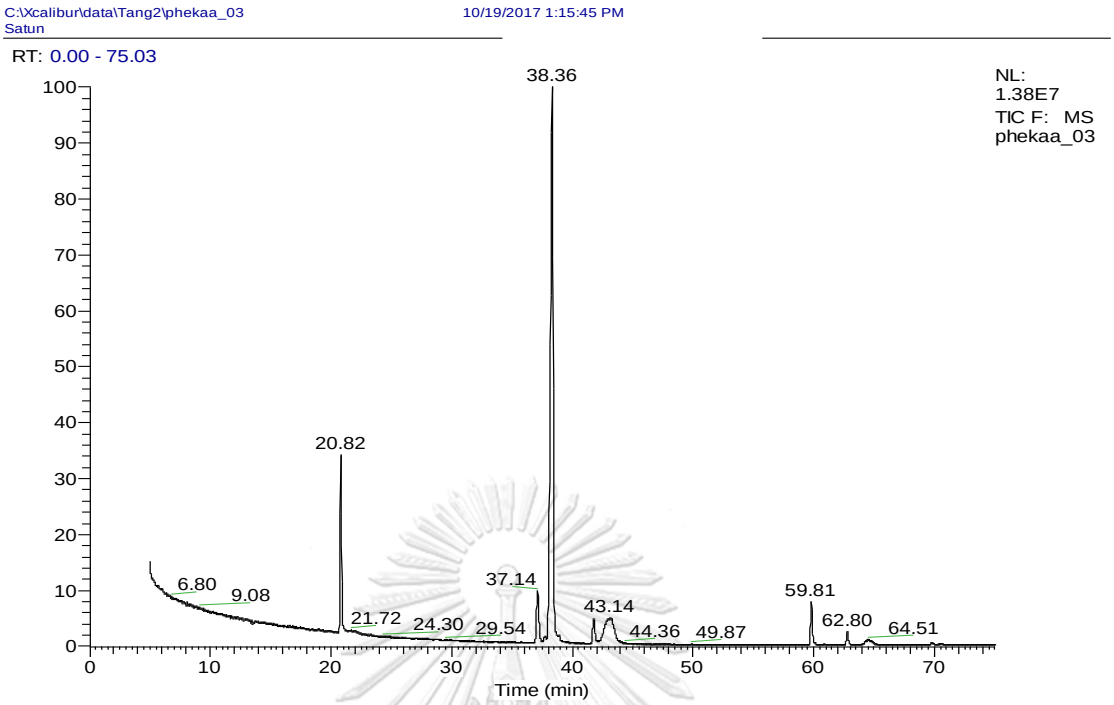

Figure 45 GC chromatogram of *Oroxylum indicum* seed oil from Satun (Source 03)

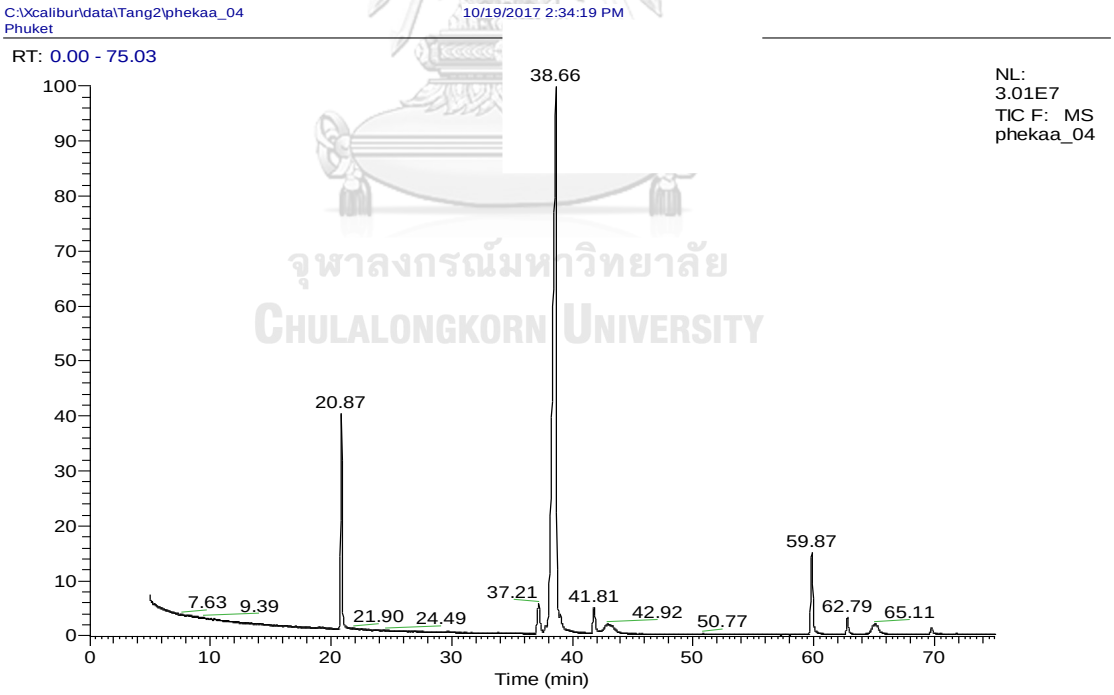

Figure 46 GC chromatogram of *Oroxylum indicum* seed oil from Phuket (Source 04)

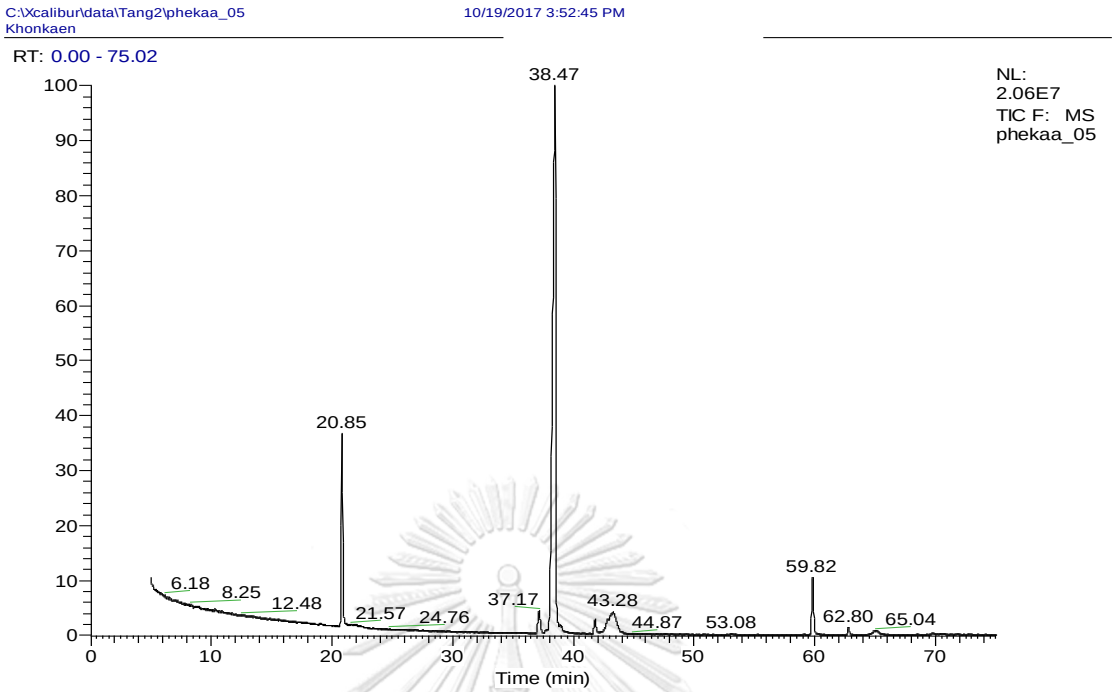

Figure 47 GC chromatogram of *Oroxylum indicum* seed oil from Khonkaen (Source 05)

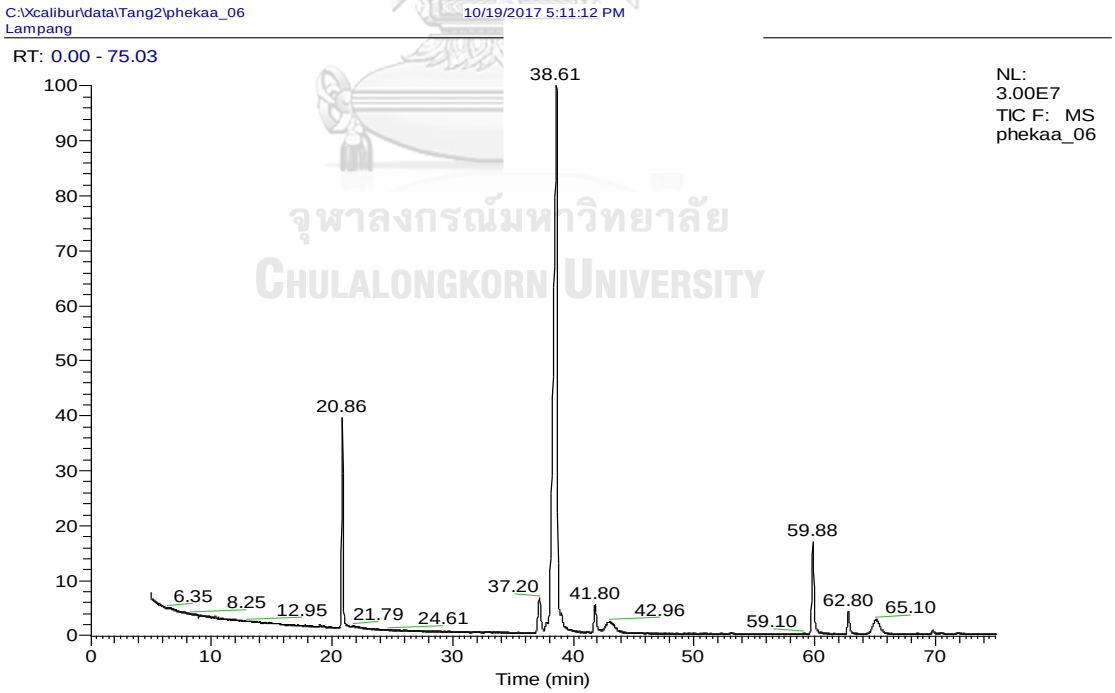

Figure 48 GC chromatogram of *Oroxylum indicum* seed oil from Lampang (Source 06)

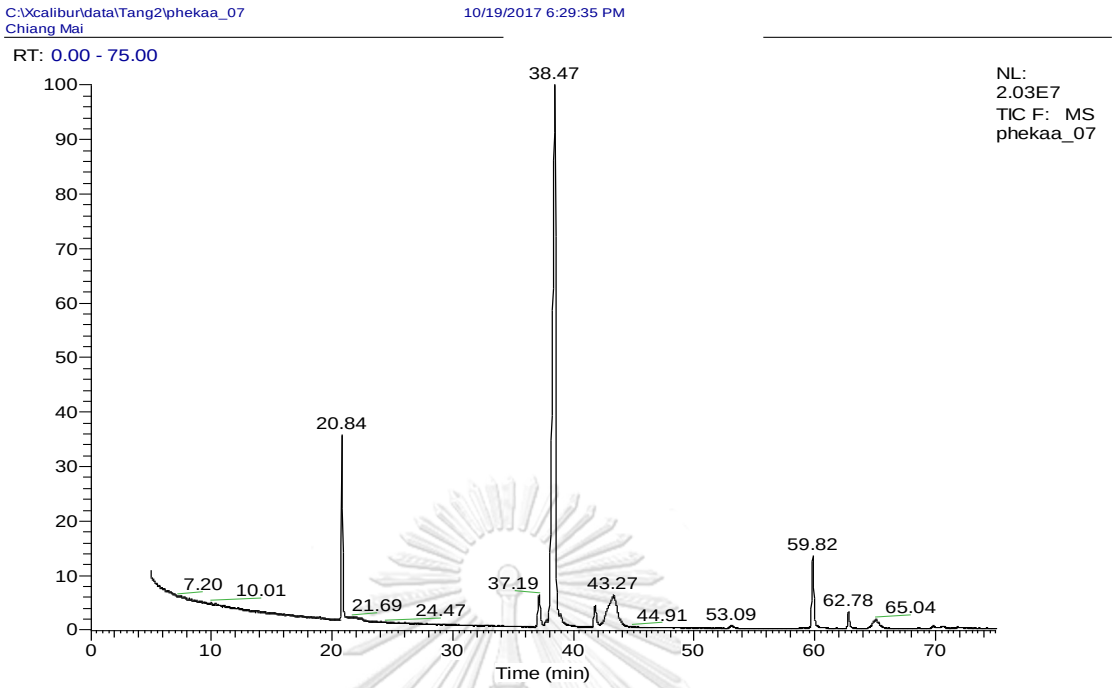

Figure 49 GC chromatogram of *Oroxylum indicum* seed oil from Chiang Mai (Source 07)

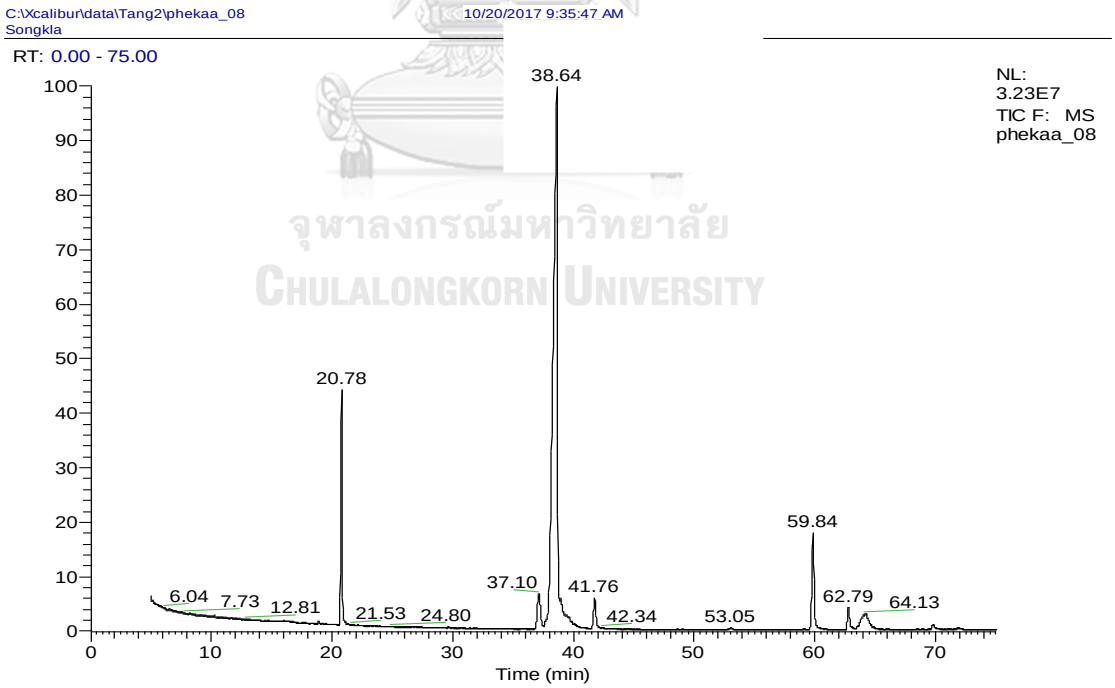

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Figure 50 GC chromatogram of *Oroxylum indicum* seed oil from Songkla (Source 08)

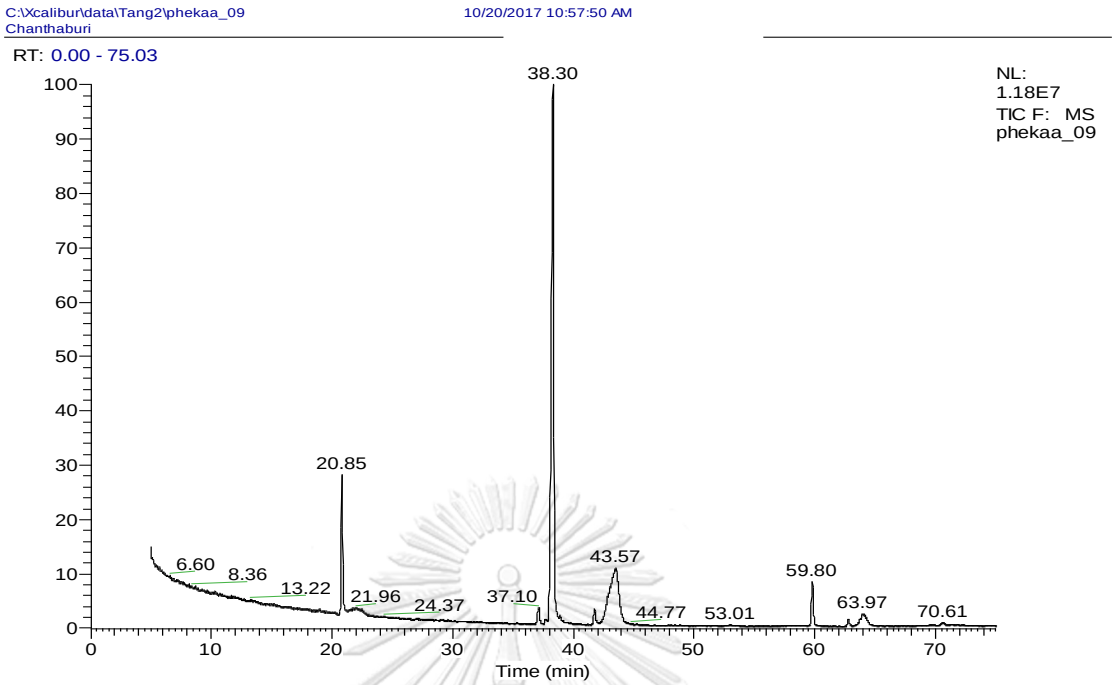

Figure 51 GC chromatogram of *Oroxylum indicum* seed oil from Chanthaburi (Source 09)

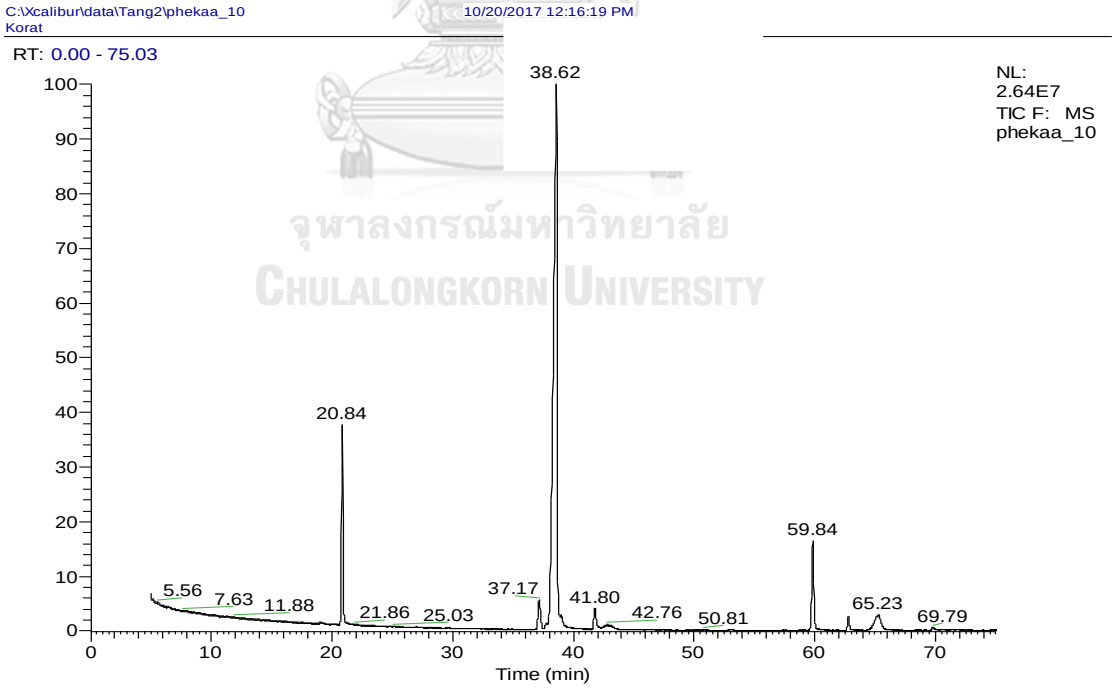

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Figure 52 GC chromatogram of *Oroxylum indicum* seed oil from Nakhon Ratchasima (Source 10)

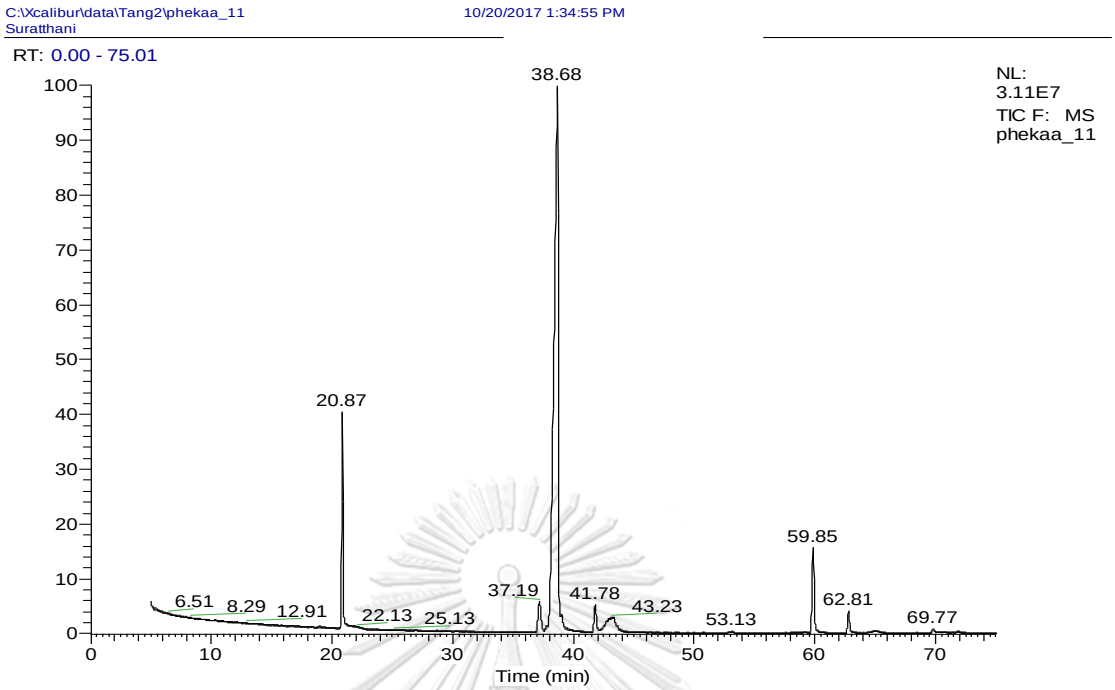

Figure 53 GC chromatogram of *Oroxylum indicum* seed oil from Suratthani 1 (Source 11)

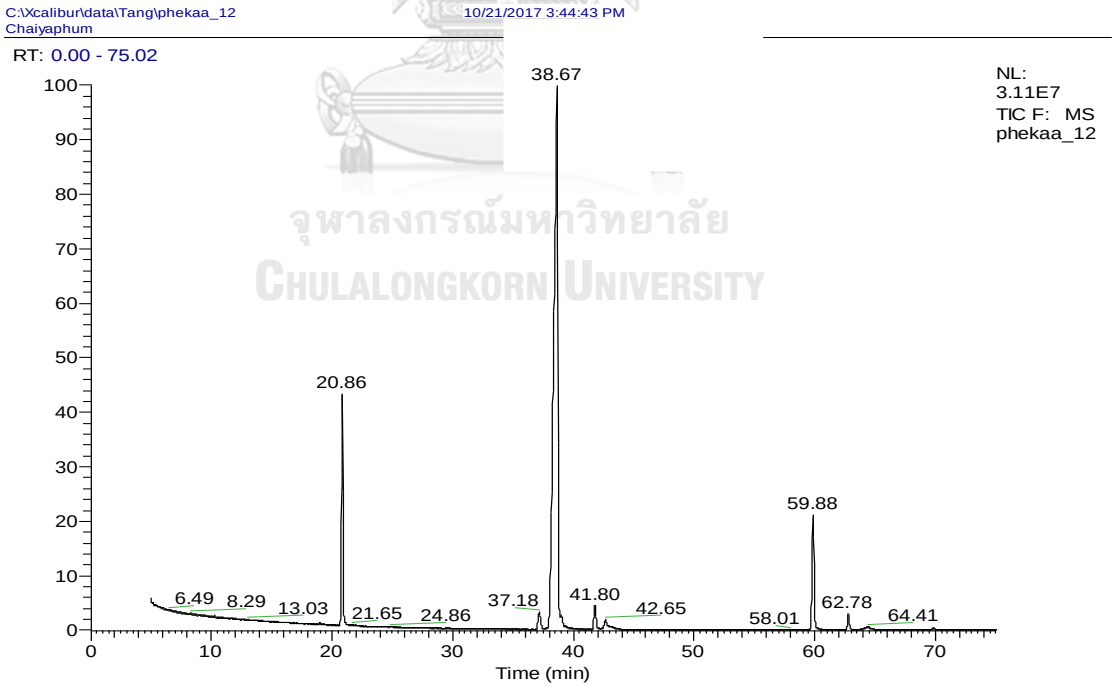

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Figure 54 GC chromatogram of *Oroxylum indicum* seed oil from Chaiyaphum (Source 12)

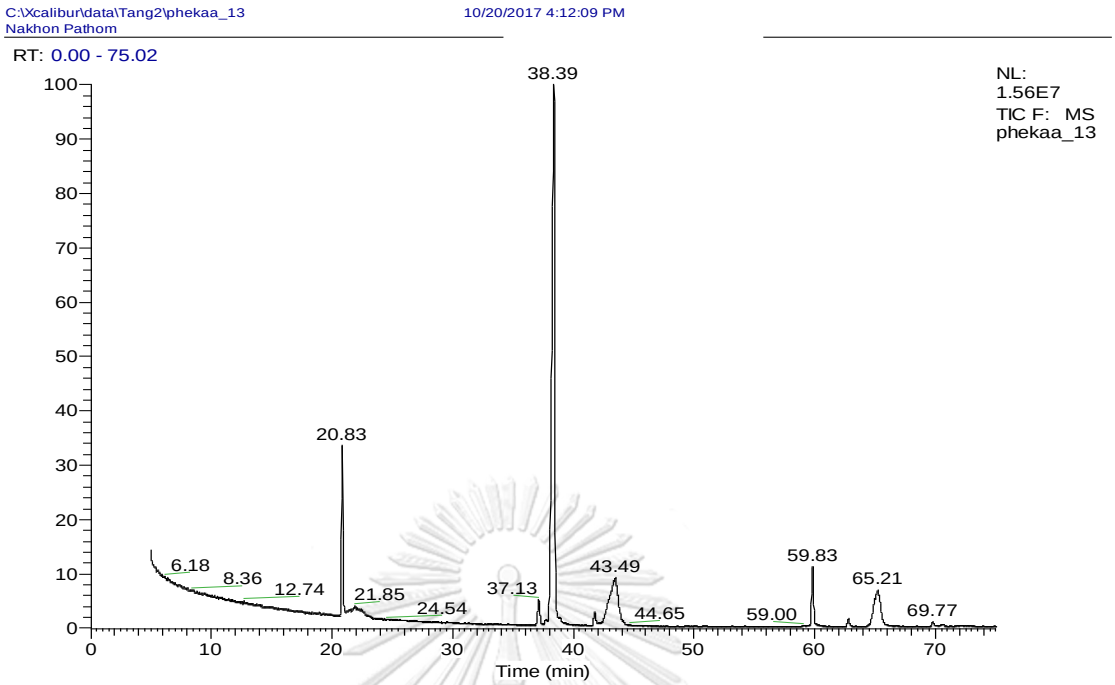

Figure 55 GC chromatogram of *Oroxylum indicum* seed oil from Nakhon Pathom (Source 13)

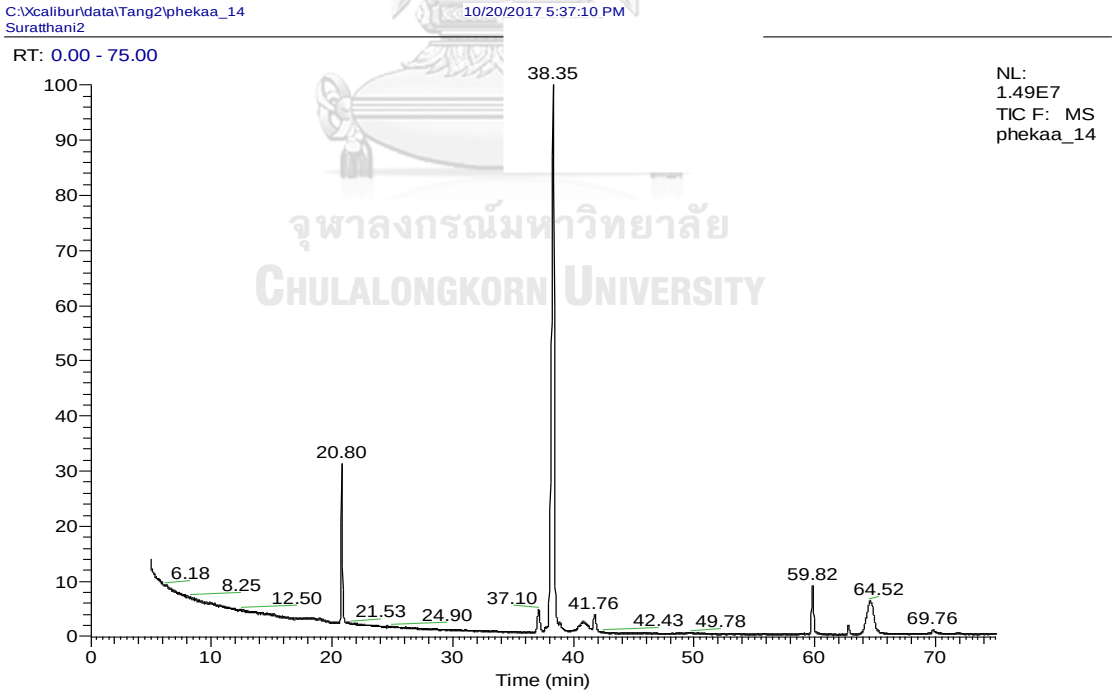

Figure 56 GC chromatogram of *Oroxylum indicum* seed oil from Suratthani 2 (Source 14)

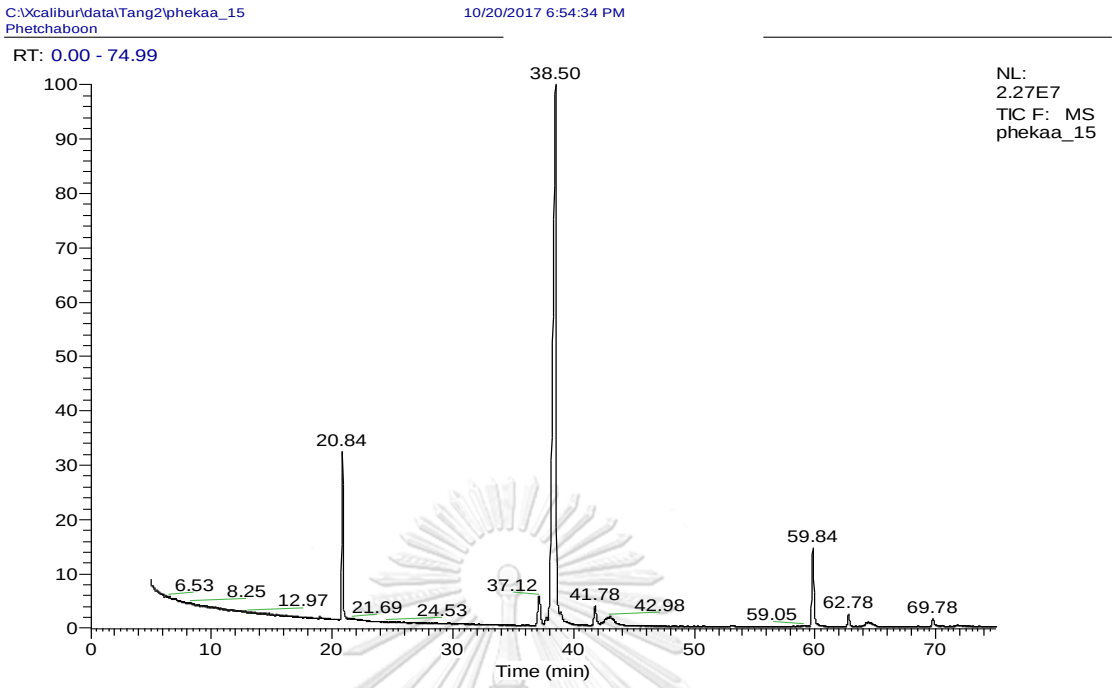

Figure 57 GC chromatogram of *Oroxylum indicum* seed oil from Phetchaboon (Source 15)

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Table 25 The retention time of *Oroxylum indicum* seed oil from 15 sources

Source	Retention time (min)								
	Palmitic acid	Linoleic acid	Oleic acid	Stearic acid	Beheric acid	Gondoic acid	Arachidic acid	Lignoceric acid	9, 10-dihydroxystearic acid
1	20.74	37.00	38.28	41.70	-	59.78	62.79	-	69.74
2	20.82	37.14	38.53	41.78	-	59.84	62.76	-	69.74
3	20.82	37.14	38.36	41.75	43.14	59.81	62.80	64.51	-
4	20.87	37.21	38.66	41.81	42.92	59.87	62.79	65.11	69.79
5	20.85	37.17	38.47	41.78	43.28	59.82	62.80	65.04	-
6	20.86	37.20	38.61	41.80	42.96	59.88	62.80	65.10	69.79
7	20.84	37.14	38.47	41.80	43.27	59.82	62.78	65.04	69.76
8	20.78	37.10	38.64	41.76	-	59.84	62.79	64.13	69.77
9	20.85	37.10	38.30	41.76	43.57	59.80	62.79	63.97	-
10	20.84	37.17	38.62	41.80	-	59.84	62.79	65.23	69.79
11	20.87	37.19	38.68	41.78	43.23	59.85	62.81	64.96	69.77
12	20.86	37.18	38.67	41.80	42.65	59.88	62.78	64.41	69.78
13	20.83	37.13	38.39	41.75	43.49	59.81	62.79	65.21	69.77
14	20.80	37.10	38.35	41.76	-	59.80	62.78	64.52	69.76
15	20.84	37.12	38.50	41.78	42.98	59.84	62.78	64.37	69.78
Mean	**20.83**	**37.14**	**38.50**	**41.77**	**43.15**	**59.83**	**62.79**	**64.74**	**69.77**
SD	**0.04**	**0.05**	**0.14**	**0.03**	**0.28**	**0.03**	**0.01**	**0.43**	**0.02**

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Table 26 The fatty acid composition of *Oroxylum indicum* seed oil from 15 sources

Source	Peak area (%)								
	Palmitic acid	Linoleic acid	Oleic acid	Stearic acid	Beheric acid	Gondoic acid	Arachidic acid	Lignoceric acid	9, 10-dihydroxystearic acid
1	9.43	2.66	75.28	1.95	-	6.27	1.22	-	0.61
2	10.95	2.94	73.88	2.32	-	5.76	1.10	-	0.22
3	10.70	4.84	63.21	2.11	9.05	3.29	0.97	1.34	-
4	10.62	2.76	70.15	2.15	3.13	6.12	1.24	2.40	0.51
5	11.30	2.19	69.93	1.22	6.82	4.44	0.52	0.97	-
6	9.96	2.97	68.11	2.36	3.08	6.66	1.50	3.16	0.30
7	10.30	2.92	63.93	1.78	9.52	5.79	1.22	2.11	0.28
8	10.37	3.12	71.18	2.19	-	6.53	1.43	3.47	0.46
9	8.51	1.63	57.95	1.19	18.84	3.42	0.54	3.38	-
10	10.28	2.65	71.49	1.93	-	6.42	0.93	3.47	0.26
11	11.48	2.69	71.41	1.85	3.94	5.81	1.35	0.44	0.30
12	11.02	1.57	73.73	1.85	1.52	7.72	0.98	0.57	0.12
13	8.62	2.00	54.78	0.94	13.84	4.54	0.61	8.47	0.44
14	9.91	2.40	64.10	2.03	-	4.49	0.74	9.46	0.66
15	9.81	3.00	70.67	1.97	3.05	6.69	0.98	1.18	0.77
Mean	**10.22**	**2.69**	**67.99**	**1.86**	**7.28**	**5.60**	**1.02**	**3.11**	**0.41**
SD	**0.88**	**0.77**	**5.98**	**0.42**	**5.60**	**1.28**	**0.31**	**2.83**	**0.20**

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VITA

Miss Kirana Praserdmek was born on July 25, 1992 in Suratthani, Thailand. She received her Bachelor's degree in Sciences (Oriental Medicine) from Rangsit University in 2014. She attended to study Master of Sciences Program in Public Health Sciences in 2015 at College of Public Health Sciences, Chulalongkorn University, Thailand.

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Proceedings

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