PHARMACOGNOSTIC SPECIFICATION AND QUERCITRIN CONTENT OF *DENDROPHTHOE PENTANDRA* LEAVES

Miss Supattra Prom-in

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Public Health Sciences
College of Public Health Sciences
Chulalongkorn University
Academic Year 2013

ข้อกำหนดทางเภสัชเวทและปริมาณสารเควอซิทรินของใบกาฝาก

นางสาวสุพัตรา พรหมอินทร์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์สาธารณสุข
วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2556

Thesis Title	PHARMACOGNOSTIC SPECIFICATION AND QUERCITRIN CONTENT OF *DENDROPHTHOE PENTANDRA* LEAVES
By	Miss Supattra Prom-in
Field of Study	Public Health Sciences
Thesis Advisor	Chanida Palanuvej, Ph.D.
Thesis Co-advisor	Associate Professor Nijsiri Ruangrungsi, Ph.D.

Accepted by the College of Public Health Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

…… Dean of the College of Public Health Sciences

(Professor Surasak Taneepanichskul, M.D.)

THESIS COMMITTEE

……..Chairman

(Kanchana Rungsihirunrat, Ph.D.)

…….…….Thesis Advisor

(Chanida Palanuvej, Ph.D.)

…….. Thesis Co-advisor

(Associate Professor Nijsiri Ruangrungsi, Ph.D.)

……..External Examiner

(Associate Professor Sompop Prathanturarug, Ph.D.)

สุพัตรา พรหมอินทร์ : ข้อกำหนดทางเภสัชเวทและปริมาณสารเควอซิทรินของใบกาฝาก. (PHARMACOGNOSTIC SPECIFICATION AND QUERCITRIN CONTENT OF *DENDROPHTHOE PENTANDRA* LEAVES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ดร. ชนิดา พลานุเวช, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. ดร. นิจศิริ เรืองรังษี, 121 หน้า.

กาฝากหรือกาฝากมะม่วง มีชื่อทางวิทยาศาสตร์คือ *Dendrophthoe pentandra* (L.) Miq. ใบกาฝากมะม่วงเป็นเครื่องยาสมุนไพรที่ในทางการแพทย์แผนไทยมีสรรพคุณใช้สำหรับเป็นน้ำกระสายยาที่ใช้ในการขับปัสสาวะและรักษาความดันโลหิตสูง ในการศึกษานี้มีวัตถุประสงค์เพื่อจัดทำข้อกำหนดทางเภสัชเวทของใบกาฝากมะม่วงในประเทศไทย รวมทั้งการวิเคราะห์หาปริมาณสารเควอซิทรินซึ่งเป็นสารสำคัญในใบกาฝากมะม่วง โดยวิธีทินเลเยอร์โครมาโทกราฟี เด็นซิโทเมทรี และการวิเคราะห์รูปภาพทางทินเลเยอร์โครมาโทกราฟีด้วยโปรแกรมอิมเมจเจ โดยการศึกษาใบกาฝากมะม่วงของประเทศไทยทั้งหมด 13 แหล่ง วาดภาพลายเส้นแสดงลักษณะทั้งต้นของกาฝากมะม่วง เตรียมเครื่องยาโดยล้างให้สะอาดและอบให้แห้ง ลักษณะมหภาคของเครื่องยาเป็นใบแห้ง หนา ใบรูปไข่หรือรูปรี เนื้อใบหนาเรียบ สีเขียวปนสีน้ำตาล ลักษณะเด่นทางจุลภาคของใบกาฝากมะม่วงคือ ผิวชั้นนอกสุด ปากใบแบบพาราไซติก ขนไม่มีต่อม การศึกษาเอกลักษณ์ทางเคมี-ฟิสิกส์พบว่า ปริมาณเถ้ารวม ปริมาณเถ้าที่ไม่ละลายในกรด น้ำหนักที่หายไปเมื่อทำให้แห้ง และปริมาณน้ำไม่เกินร้อยละ11.94±0.19, 4.17±0.88, 9.16±0.61 และ 9.99±0.70 โดยน้ำหนัก ตามลำดับ ปริมาณสารสกัดด้วยเอทานอลและปริมาณการสกัดด้วยน้ำไม่น้อยกว่าร้อยละ 7.67±0.72 และ 22.24±2.59 โดยน้ำหนัก ตามลำดับ การศึกษาทางทินเลเยอร์โครมาโทกราฟี โดยใช้ตัวทำละลายเอทิลอะซิเทต อะซิติกแอซิด ฟอร์มิกแอซิด และน้ำ ในอัตราส่วน 13: 1: 1: 2.5 เป็นวัฏภาคเคลื่อนที่ ตรวจจับด้วยภายใต้แสงขาวแสงอัลตราไวโอเลต (254 และ 366 นาโนเมตร) และตรวจจับด้วยการชุบด้วยเฟอร์ริกคลอไรด์ในเอทานอล พบแถบที่ชัดเจนมีค่า hRf เท่ากับร้อยละ 73.5 การวิเคราะห์สารเควอซิทรินโดยวิธีทินเลเยอร์โครมาโทกราฟี เด็นซิโทเมทรีมีช่วงเป็นเส้นตรงระหว่าง 0.25-1.0 มิลลิกรัม และมีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.998 มีขีดจำกัดการตรวจพบและขีดจำกัดการหาปริมาณเท่ากับ 0.16 และ 0.49 มิลลิกรัม ตามลำดับ ระดับความเที่ยงของวิธีวิเคราะห์ ประเมินจากค่าสัมประสิทธิ์ของการกระจาย มีค่าระหว่างร้อยละ 2.46-7.39 ค่าเฉลี่ยการคืนกลับร้อยละ 96.41-100.97 การวิเคราะห์สารเควอซิทรินโดยวิธีการวิเคราะห์รูปภาพทางทิเลเยอร์โครมาโทกราฟีมีช่วงเป็นเส้นตรงระหว่าง 0.25-1.0 มิลลิกรัม และมีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.995 มีขีดจำกัดการตรวจพบและขีดจำกัดการหาปริมาณเท่ากับ 0.16 และ 0.50 มิลลิกรัม ตามลำดับ ระดับความเที่ยงของวิธีวิเคราะห์ ประเมินจากค่าสัมประสิทธิ์ของการกระจาย มีค่าระหว่างร้อยละ 3.24-9.02 ค่าเฉลี่ยการคืนกลับร้อยละ 81.57-103.09 ปริมาณสารเควอซิทรินในใบกาฝากมะม่วงโดยวิธีทินเลเยอร์โครมาโทกราฟี เดนซิโทเมทรีและการวิเคราะห์รูปภาพทางทินเลเยอร์โครมาโทกราฟีมีค่าเฉลี่ย 3.71 และ 3.89 กรัม/100 กรัมของใบแห้ง ตามลำดับ โดยสองวิธีถูกเปรียบเทียบโดยใช้สถิติ t-test พบว่าปริมาณสารเควอซิทรินโดยทั้งสองวิธีไม่มีความแตกต่างกันอย่างมีนัยสำคัญ ($P > 0.05$) จากการศึกษาครั้งนี้สามารถจัดทำเป็นข้อกำหนดทางมาตรฐานของสมุนไพรกาฝากมะม่วงในประเทศไทย ซึ่งจะเป็นประโยชน์ต่อการควบคุมคุณภาพและปลอดภัยในการใช้เครื่องยาสมุนไพรนี้ต่อไป

สาขาวิชา วิทยาศาสตร์สาธารณสุข ลายมือชื่อนิสิต.....

ปีการศึกษา 2556 ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5578957453 : MAJOR PUBLIC HEALTH SCIENCES
KEYWORDS : PHARMACOGNOSTIC SPECIFICATION/ *DENDROPHTHOE PENTANDRA*/ QUERCITRIN/ THIN LAYER CHROMATOGRAPHY (TLC)
SUPATTRA PROM-IN : PHARMACOGNOSTIC SPECIFICATION AND QUERCITRIN CONTENT OF *DENDROPHTHOE PENTANDRA* LEAVES : ADVISOR : CHANIDA PALANUVEJ, Ph.D., CO-ADVISOR : ASSOC. PROF. NIJSIRI RUANGRUNGSRI, Ph.D., 121 pp

Dendrophthoe pentandra leaf has been used in traditional Thai medicine as aqueous adjuvant and also used to treat diuretic and hypertension. This study expected to establish the pharmacognostic specification and analyse the chemical complement, quercitrin by thin layer chromatographic densitometry and thin layer chromatographic image analysis. The crude drugs were collected from 13 different locations throughout Thailand. The macroscopic characteristics were shown dried leaves, ovate and elliptic shapes, thickly leaves, green and brown colors and different sizes. Anatomical and histological characteristics demonstrated epidermis, paracitic typed stomata and unicellular trichome. The total ash, acid insoluble ash, loss on drying and water content should be not more than 11.94±0.19, 4.17±0.88, 9.16±0.61 and 9.99±0.70 w/w respectively. Ethanol extractive value and water extractive value should be not less than 7.67±3.48 and 22.24±5.50 w/w respectively. Thin layer chromatographic fingerprint of ethanolic extracts of the leaves was developed using ethyl acetate: formic acid: acetic acid: H_2O (13: 1: 1: 2.5) as mobile phase. Detection under daylight, ultraviolet light (254 and 366 nm) and dipping with ferric (III) chloride reagent indicated the apparent band at hRf 73.5%. Quantitative analysis of quercitrin was performed by TLC densitometry. Linearity range of quercitrin was 0.25-1.0 mg with correlation (r^2) of 0.998. LOD and LOQ were 0.16 and 0.49 mg respectively. The precision determined by the % RSD of repeatability and intermediate precision, were between 2.46-4.30 % RSD and 5.99-7.39% RSD respectively. The recoveries were 96.41-100.97% recoveries. Quantitative analysis of quercitrin was performed by TLC image analysis. Linearity range of quercitrin was 0.25-1.0 mg with correlation (r^2) of 0.995. LOD and LOQ were 0.16 and 0.50 mg respectively. The precision determined by the % RSD of repeatability and intermediate precision, were between 3.24-6.14 % RSD and 4.05-9.02 % RSD respectively. The recoveries were 81.57-103.09 % recoveries. The comparison between TLC densitometric and TLC image analysis of quercitrin content in *D. pentandra* leaves was performed by paired t-test. The averages of quercitrin content by TLC densitometry and TLC analysis using imageJ were 3.71±1.33 g/100 g and 3.89±0.97 g/100 g respectively. The comparison was found that the quercitrin contents by two methods were not significantly different ($P > 0.05$).This result can be used as specification for quality control and standardization of *D. pentandra* leaves in Thailand.

Field of Study: Public Health Sciences Student's Signature:.....
Acedemic Year: 2013 Advisor's Signature:.....
Co-advisor's Signature:.....

ACKNOWLEDGEMENTS

The author wishes to express her deepest gratitude and appreciation to her thesis advisor, Chanida Palanuvej, Ph.D. and her thesis co-advisor, Assoc. Prof. Nijsiri Ruangrungsi, Ph.D. for their advices, guidance, valuable suggestions, encouragements, and supports throughout this study

The author is thankful to express her thanks to the thesis committee and the thesis external examiner for their important and suggestion to improve thesis.

The author is thankful to the Herbal Remedies and Alternative Medicine Task Force of STAR: Special Task Force for Activating Research under 100 years Chulalongkorn University Fund for financial support**.**

The author would like to express his special thanks to Mr. Worathat Thitikornpong from Faculty of Pharmaceutical Sciences, Chulalongkorn University for providing researches facilities and his kind assistance in techniques of TLC-densitometric method throughout this study. His kind assistance will always be remembered.

The author would like to express her appreciation all staff member, companions of College of Public Health Sciences, Chulalongkorn University and other persons whose names have not been mentioned here for helping the author through the difficult times, kindness and friendships.

Finally which is the most important; the author would like to express all her love and gratitude to her family for their understanding, encouragement and supporting for concentration her study.

CONTENTS

Page

CHAPTER

Page

LIST OF TABLES

Page

Table

LIST OF FIGURES

Figure **Page**

Figure **Page**

Figure **Page**

Figure **Page**

Figure **Page**

Figure **Page**

LIST OF ABBREVIATIONS

$^{\circ}C$	=	Degree Celsius
D.	=	*Dendrophthoe*
DDPH	=	1,1-diphenyl-2-picrylhydrazyl
GC	=	Gas chromatography
g	=	Gram
HCl	=	Hydrochloric acid
HPLC	=	High performance liquid chromatography
IC_{50}	=	50% Inhibitory concentration
ICH	=	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IR	=	Infrared radiation
LD	=	Lethal dose
LOD	=	Limit of detection
LOQ	=	Limit of quantitation
mg	=	milligram
ml	=	milliliter
nm	=	namometer
r^2	=	Correlation coefficients
R_f	=	Retention factors
RSD	=	Relative standard deviation
SD	=	Standard deviation
TLC	=	Thin layer chromatography
UV	=	Ultraviolet
WHO	=	World Health Organizations
µg	=	microgram
µl	=	microliter
α	=	Alpha

CHAPTER I

INTRODUCTION

Background and rationale

Herbal medicines are prepared from a variety of plant materials such as leaves, stems, roots, bark. They usually contain many biological active ingredients and are used primarily for treating mild or chronic ailments. The herbal medicines are used at home in many ways, including fresh and dried materials [1].

Quality control for medicinal plant materials is essential. The methods for assessing their quality include modern control techniques, suitable standard markers and following World Health Organization guidelines [2].

One of mistletoe plants, *Dendrophthoe pentandra* (L.) Miq. (From Greek, pente = five and andro = male) is named, in Thai-as "Kafakmamuang or Kafak". This plant is a semi-parasitic plant in Loranthaceae family. People in Thailand call Kafak followed by the host plant such as Kafak-mamuang (Kafak that grow on *Mangifera indica* L.), kafak-khanun (Kafak that grow on *Artocarpus heterophyllus* Lam.), kafak-yangpara (Kafak that grow on *Heveabra siliensis* L.) *etc*. People in Indonesia usually call *Dendrophthoe pentandra* (L.) Miq. "benalu" followed by the host plant which it grows on, such as benalu teh (mistletoe that grow on *Camellia sinensis* L.), benalu belimbing (mistletoe that grows on *Averrhoa carambola* L.) and benalu mangga (mistletoe that grows on *Mangifera indica* L.), and *etc* [3-5].

D. pentandra is one of medicinal plant used in the alternative and traditional medicine. Akha's traditional medicine in China and Thailand are used all part of this plant for rheumatoid arthritis [6]. In Laos, Cambodia and Vietnam, the leaves of *D. pentandra* is used to make a tea-like drink used to treat cough while in Malaysia, Kafak is used as a remedy to recover from childbirth, and to heal sores, wounds and ulcers [3, 7]. Indonesian use leaves of *D. pentandra* to treat cough, diabetes, hypertension, cancer, diuretic, smallpox, ulcer, skin infection and after child-birth [5]. For traditional Thai medicine, Kafakmamuang leaves are used as aqueous adjuvant and also used to treat diuretic and hypertension [8].

D. pentandra from different host plants (from star fruit and mango tree) were investigated for the active antioxidant compound which appeared to be quercitrin (quercetin-3-O-rhamnoside). This compound showed antioxidant activity [4, 5, 9].

D. pentandra leaves were reported by Sukpondma and Jasakul for two flavonol rhamnosides including quercetin 3-rhamnoside and kaempferol 3-rhamnoside. These compounds have the potential to reduce hypertension [9].

The toxicity of *D. pentandra* using brine shrimp lethality test was shown that the methanolic and water extracts of this plant leaves were non-toxic [5]. Preliminary antibacterial study of *D. pentandra* leaves showed no effect [10].

The taxonomic marker of Loranthaceae family is quercetin and quercitrin, which can be isolated from four phyllogenetically linked genera of *Scurrula, Taxillus, Dendrophthoe* and *Helixanthera* [6]. Quercitrin is a glycoside formed from the flavonoid quercetin and the deoxy sugar, rhamnose. It can be founded in Tartary buckwheat *(fagopyrum tataricum)* and in oaks species such as European red oak *(Quercusrobur)*, North American white oak *(Quercusalba)* and citrus fruit. Quercetin and quercitrin are also found in *D. pentandra* leaves [6].

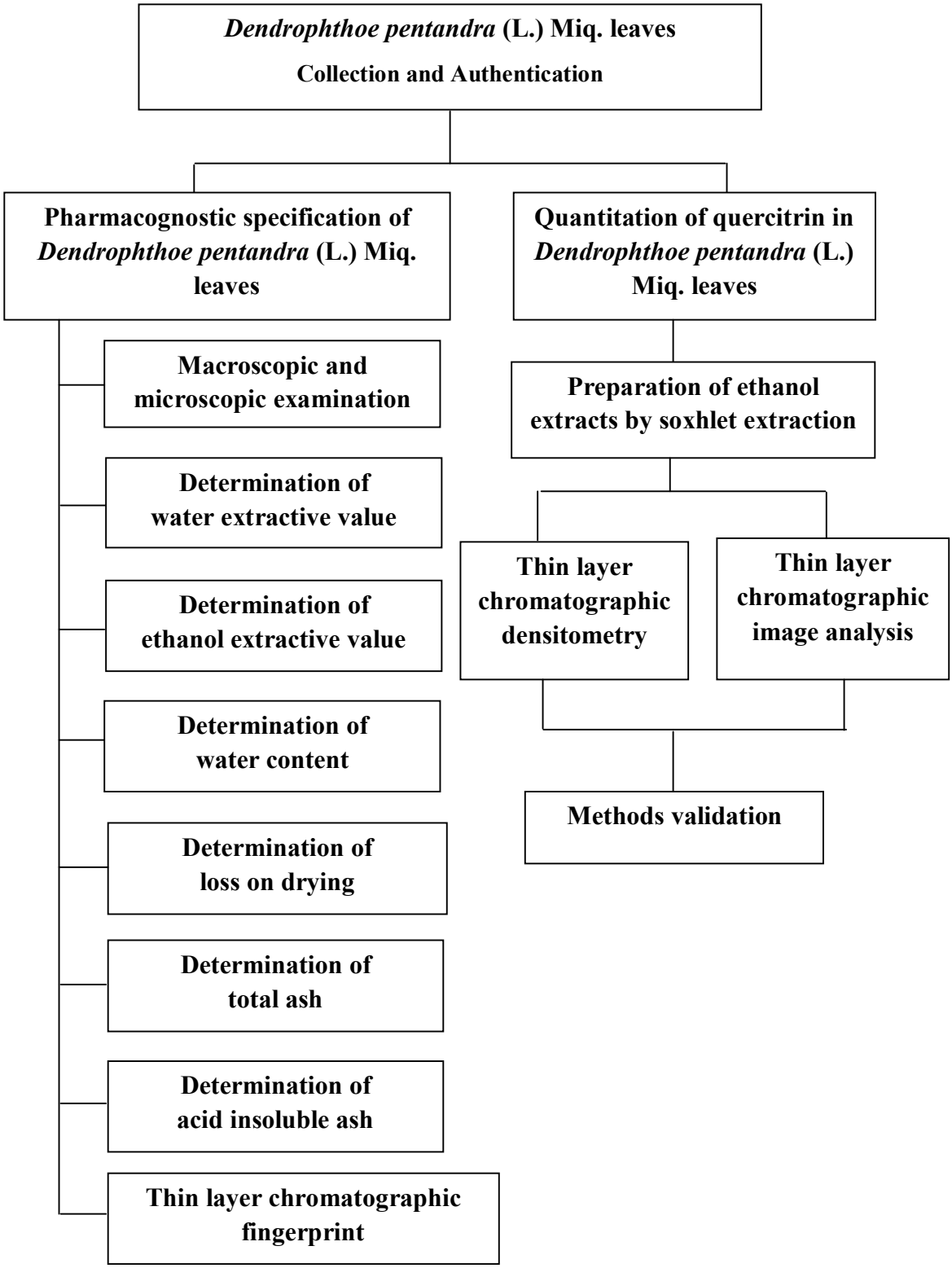
Quercetin is the most abundant bioflavonoid found in vegetables and fruits, and this compound is mainly present in the glycoside form, for example quercitrin [11]. Quercitrin (quercetin-3-rhamnoside) is flavonoid, which found in many plants. Quercitrin is founded in many foods and herbal including apples, tea, onions, nuts, berries, cauliflower, cabbage and many other foods. Quercitrin provides many health promoting benefits, such as lipid peroxidation prevention, improvement of cardiovascular health, eye diseases, anti-inflammatory, allergic disorders, hypertension, arthritis and reducing risk for cancers [5, 11-13].

The quality control of herbal medicines is important. Medicinal plant materials could be adulterated with other parts of plants or substances with low quality. Although, *D. pentandra* leaves are widely used in many countries, there is no pharmacognostic specification available for the standardization of this medicinal plant. This study involves the qualitative and quantitative analyses of *D. pentandra* leaves with the special reference to quercitrin marker.

Objectives of the study

1. To develop the standardization parameters of *D. pentandra* leaves.
2. To investigate the quercitrin contents in *D. pentandra* leaves by ImageJ free software compared to TLC densitometry.
3. To validate TLC image analysis by ImageJ free software and TLC densitometric analysis of quercitrin contents in *D. pentandra* leaves.

Conceptual Framework



CHAPTER II

LITERATURE REVIEWS

Parasitic plants

Parasitism is the relationship between parasitic plants with its host. The parasitic plant uses the nutrients and water of host plant. Root of parasitic plants is haustorium root, which penetrates the host plant and connects to the xylem, phloem or both. Parasitic plants belong to about 15 families of flowering plants. There are many species of mistletoes, belonging to Viscaceae and Loranthaceae, include about three-quarters of all parasitic species. Other families of parasitic plants are also well-known, especially the broomrapes (Orobanchaceae) and dodders (Cuscutaceae) [14-17].

Loranthaceae family is one of parasitic family plant. Loranthaceae is a tropical family with showy red, yellow or orange tubular flowers. The Loranthaceae family is a largest family that belong to the order *Santalales*, it consists of approximately 70 genera and 950 species occurring mainly in tropical regions. This family has epiphytic plants and semi-parasitic plants. It adheres the branches and twigs of host trees. Loranthaceae family was found in temperate habitats of Europe, Asia, South America, Australia and New Zealand [16-18].

Description of Loranthaceae family

Shrubs, usually aerial hemiparasites on other seed plants, often spreading along host by runners (epicortical roots), more rarely terrestrial root-parasitic shrubs or trees, nodes not articulated, glabrous or hairy, hairs often stellate or verticillate. Leaves opposite or alternate, stipules absent; petiole often indistinct; leaf blade simple, usually pinnately veined, margin entire. Inflorescences terminal or axillary, racemes, spikes, or umbels (sometimes condensed into heads); bracts usually inconspicuous, sometimes forming conspicuous involucre (in Tolypanthus). Flowers usually bisexual, rarely unisexual (plants dioecious), 4 6-merous, actinomorphic or zygomorphic, often conspicuous. Calyx adnate to the ovary, limb annular to cupular, entire or shortly toothed, persistent. Petals usually 4 - 6, free or connate, valvate. Disk usually inconspicuous to – absent. Stamens as many as petals, opposite and adnate to them; anthers mostly basifixed, sometimes dorsifixed, 2 4-loculed, dehiscence longitudinal, locules sometimes with many transverse divisions so as to be

multilocellate. Pollen oblate or suboblate, usually trilobate, or triangular. Ovary inferior, 1- or 3- or 4-loculed, without true ovules, embryo sacs originating from a central column or at the ovary base, integument absent. Style simple; stigma small. Fruit a berry (rarely a drupe or capsule), with a viscin layer (sticky mucilaginous tissue) outside the vascular bundles. Seed 1; testa absent; endosperm copious; embryo large [18].

***Dendrophthoe pentandra* (L.) Miq.**

Family : Loranthaceae

Synonym : *Loranthus pentandrus* L.

Vernacular names : Mistletoe (England), Kemadean, Kemlandean, Mangandeuh, Pasilan, Benalu (Indonesia), Day chum go i (Vietnam), Kafakmamuang, Kafak (Thailand)

Description

Shrubs to 2 m tall, youngest parts puberulous. Branches grayish, scattered lenticellate. Petiole 5-20 mm; leaf blade lanceolate to elliptic or suborbicular, 5-15 × 2.5-10 cm, thickly leathery, lateral veins 2-4 pairs, base cuneate or obtuse, apex acute or rounded, glabrous. Racemes solitary or 2 or 3 together, 3-10-flowered; peduncle 7-20 mm, with grayish or white stellate hairs; bracts broadly ovate, 1-1.5 mm. Pedicel 2 mm. Calyx 2-2.5 mm, limb 0.5-1.5 mm, 5-denticulate. Mature bud 1.5-2 cm. Corolla orange, basal 1/2 slightly inflated, lobes lanceolate, 12 mm, reflexed. Filaments 3-4 mm; anthers 3-5 mm. Berry red, 8-10 × 5-6 mm, minutely pilose or glabrous [18].

Figure.1 *Dendrophthoe pentandra* (L.) Miq.

D. pentandra has been widely used in traditional medicines in Thailand and other countries in Asia (Table. 1).

Table.1 *D. pentandra* in alternative medicine and traditional Thai medicine [4, 7, 19, 20, 21]

Country	Parts of used	Application
Indonesia	leaves	To treat cancer , cough, diabetes, hypertension, diuretic, smallpox, ulcer, skin infection and after child-birth treatment
Malaysia	leaves	Remedy ingredient and to recover from childbirth, to heal sores, wounds and ulcers to heal sores, wounds and ulcers
Vietnam	leaves	To make a tea-like drink used to treat cough
China	whole plants	To fractures, rheumatoid arthritis
Thailand	leaves	To treat diuretic and hypertension and diabetes, used as aqueous adjuvant and paralysis remedy ingredient
Laos	leaves	To treat cough
Cambodia	leaves	To treat cough

Ecology

Recorded hosts include *Aleurites moluccana* L., *Canarium album* L., *Canarium pimela* L., *Clausena lansium* L., *Hevea brasiliensis* L., *Mangifera indica* L., *Vernicia Montana* L., and species of *Ficus* [18].

Distribution

This plant can be found in forest and plantations; 100-1600 m. Guangdong, Guangxi, Yunnan, Cambodia, India, Indonesia, Singapore, Vietnam, Laos, Malaysia, Myanmar, Philippines and Thailand [18,19].

Pharmacological activities

In 2006, Artanti *et al* investigated the leaves of *D. pentandra* that grew on star fruit (*Averrhoa carambola* L.). The study were based on antioxidant activity guided isolation of chemical constituent from ethanol extract of this plant using DPPH free radical method. Separation was conducted using vacuum column chromatography and characterization was conducted using TLC, LC-MS, UV-VIS and IR spectrophotometer and melting point apparatus. The results showed that the isolated compound was a flavonol glycoside, quercitrin (quercetin-3-O-rhamnoside) and it had antioxidant activity with value of IC_{50} 5.19 μg/ml [5].

Quercetin is one of the compounds in *D. pentandra*. leaves that has high antioxidant activity. The antioxidant activity was measured using DPPH free radical scavenging assay. The methanolic extract of leaves of *D. pentandra* grew on *Stelechocarpus burahol* L. *(*Annonaceae) showed antioxidant activity with IC_{50} of 21.5 ug/ml. It is suggested that *D. pentandra*. leaves extracts are potential source for natural antioxidant [10].

D. pentandra leaves was also investigated by Sukpondma and Jasakul and found that the leaves had flavonol-rhamnosides, quercetin 3- rhamnoside and kaempferol 3-rhamnoside. The compounds presented hypotensive activity in the range of 16.3-27.0 mmHg [10].

Antidiabetes activity was measured using α-glucosidase inhibiton assay. Both methanolic and water extracts of *D. pentandra* leaves extract from different hosts showed significant α-glucosidase inhibition activity. The highest activity was from water extract of *D. pentandra* leaves grew on *Camellia sinensis* (L.) Kuntzen (IC_{50} = 11.8 mg/ml) [6].

Toxicity was measured using brine shrimp lethality test. The results showed that *D pentandra* extracts from leaves (methanolic and water extracts) were non-toxic. The highest activity was *D. pentandra* leaves grew on *Stelechocarpus burahol* L. extract with IC_{50} >1,000 μg/ml [6].

The acute toxicities of two isolates from n-hexane fraction and ethanol fractions of *D. pentandra* leaves were investigated on male and female mice after giving a single dose of samples and observing their influences on behavioral responses (pharmacological profile), the development of body weight and the

mortality each day for 14 days. The observation of vital organ was done on fifteenth day. The results showed that at a dose of 2000 mg/kg body weight, no animal died and no significant toxic effect was demonstrated. LD_{50} of the two isolates on mice were more than 2000 mg/kg body weight [22].

Dried leaves of *D. pentandra* (Kaphakmamuang) were successively extracted with hexane, dichloromethane and ethanol, respectively. Rats were induced to hypertensive state by the Glodblatte 2 Kidney-1 clip method. The result showed the decreasing of systematic blood pressure obtained from a tail cuff measurement of renal hypertension rats after received all extracts and captopil at high and low doses. The mean arterial pressure that measured directly from the femoral artery of rats was significantly reduced in hexane, dichloromethane extracts and captopil group [23].

Dendrophthoe species was examined in animal model with liver tissue damage. The control group was given with aquademineralisata and treatment group was given with infusion of *Dendrophthoe* spicies. Serum transaminase, specific marker of hepatocellular necrosis and the histology of mouse liver were studied 17 days after the treatment with plant infusion. Data of serum transaminase and histology were compared between treatment and control groups. The result suggested that the infusion of Loranthaceae *Dendrophthoe* did not cause liver diseases in animal model [24].

Quercetin and quercitrin

Quercetin is flavonoids commonly found in many species. The name comes from the Latin-quercetum, which means oak forest or quercus oak. The compound consists of 3 rings and 5 hydroxyl groups (Figure 2). Quercetin is a member of the class of flavonoids called flavonol and forms the backbone for many other flavonoids including the citrus flavonoids like rutin, hesperidins, naringenin and tangeritin. It is widely distributed in the plant kingdom in rinds and barks. Quercetin itself is an aglycon or aglucone that does not possess a carbohydrate moiety in its structure. Quercetin is founded in onions, broccoli, apples and berries.

Quercetin glycone conjugates include rutin and quercitrin. Rutin is also known as quercetin-3-rutinoside. Quercitrin is also known as thujin (quercetin-3-L-rhamnoside, 3- rhamnosyl quercetin) (Figure 3). Quercitrin is a common antioxidant flavonoid found in vegetables and fruites. Quercitrin exhibits a scavenger and antioxidant activity, and these effects probably are mediated via different mechanisms, which may involve the negative modulation of the Fenton reaction and NMDA receptor [13, 25, 26].

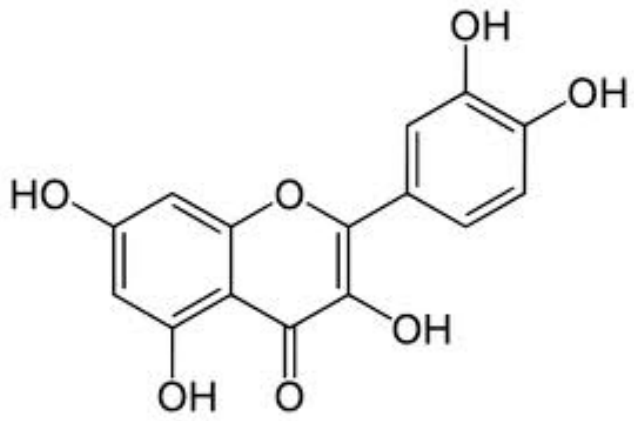


Figure 2. Structure of quercetin

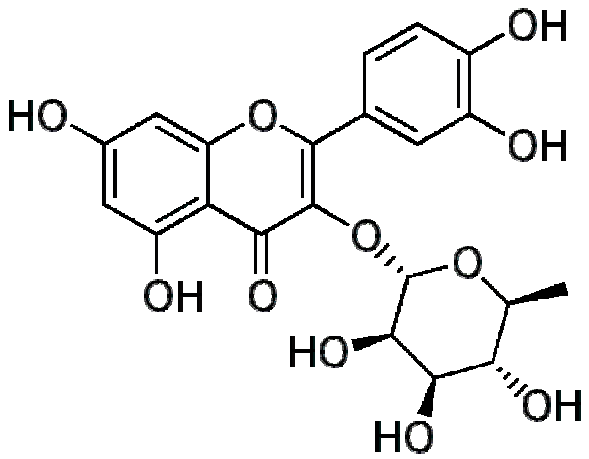


Figure 3. Structure of quercitrin

IUPAC name: 3-O-α-L-Rhamnopyranosyloxy-3', 4', 5, 7-tetrehydroxyflavone

Other names: Quercetin 3-rhamnoside; Quercitronic acid; Quercitroside; Thujin

Formula: $C_{21}H_{20}O_{11}$

Molecular weight: 448.38

Melting point: 182-185 °C

CAS number: 522-12-3

Physical description: Yellow powder [5, 12, 13]

Quercitrin can be dissolved in high polar solvent such as ethanol, methanol and water. It was found in *D. pentandra* leaves. Quercitrin was isolated from mistletoe plants *(D. pentandra*) and Loranthacease family especially mistletoes grew on *Averrhoa carambola* L. and *Mangifera indica* L. It has been reported various benefits such as antioxidant, lipid peroxidation prevention, anti-inflammatory, allergic disorders, hypertension, arthritis and reducing risk for cancers [5, 6, 13, 26].

Quality control method for medicinal plant materials

Quality control of medicinal plant materials is important. The quality of medicinal plant materials has a direct impact on their safety and efficiency. The methods for assessing their quality use modern control technique, applying suitable standard and following World Health Organization (WHO) guidelines. This guideline has explained specification of method information on various analytical methods for determining possible contaminants and residues in medicinal plant materials [3].

Macroscopic and microscopic examinations

An examination to determine these characteristics is the first step towards establishing the identity and the degree of purity of such materials, and should be carried out before any further tests are undertaken. Macroscopic characteristics of medicinal plant materials are based on shape, size, color, surface characteristics, texture, characteristics and appearance of the cut surface**.** Microscopic verification of medicinal plant material is investigation for the identification of broken or powdered material [1, 3].

For the leaf, it has four significantly different types of stoma which are distinguished by their forms and the arrangement of the surrounding cells, especially the subsidiary cells, include *anomocytic type* (the stoma is surrounded by a varying number of cells, generally not different from those of the epidermis), *anisocytic type* (the stoma is usually surrounded by three or four subsidiary cells, one of which is markedly smaller than the others), *diacytic type* (the stoma is accompanied by two subsidiary cells, the common wall of which is at right angles to the stoma) and *paracytic type* (the stoma has two subsidiary cells, of which the long axes are parallel to the axis of the stoma) (Figure 4) [1, 3].

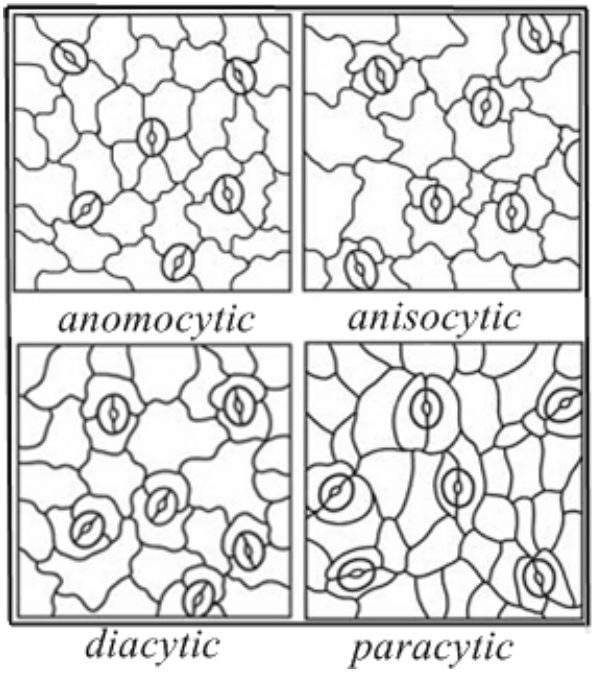


Figure 4. Type of leaf stomata

Determination of loss on drying

The evaluation for loss on drying determines both water and volatile matter. Drying can be carried out either by heating to 100- 105 ˚C or keeping in a desiccator. The desiccation method is especially useful for materials that melt to a sticky mass at elevated temperatures [3].

Determination of total ash and acid insoluble ash

Total ash indicates carbonates, phosphate, silicates and silica from both a physiology of plant tissue and non-physiology, which is the residue of the adhering material to the plant surface such as soil and sand. The material which has a high ash value has contamination, adulteration, or carelessness in the material preparation from several locations [1, 3].

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth [3].

Determination of solvent extraction value

This method determines the amount of active constituents extracted with solvent from a given amount of herbal material. It is employed for materials for which as yet no suitable chemical or biological assay exists [1, 3].

Determination of water content

An excess of water in herbal materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water [3].

The azeotropic method gives a direct measurement of the water presenting in the material being examined (Figure 5). When the sample is distilled together with an immiscible solvent, such as toluene or xylene, the water present in the sample is absorbed by the solvent. The water and the solvent are distilled together and separated in the receiving tube on cooling. If the solvent is anhydrous, water may remain absorbed in it leading to false results. It is therefore advisable to saturate the solvent with water before use [3].

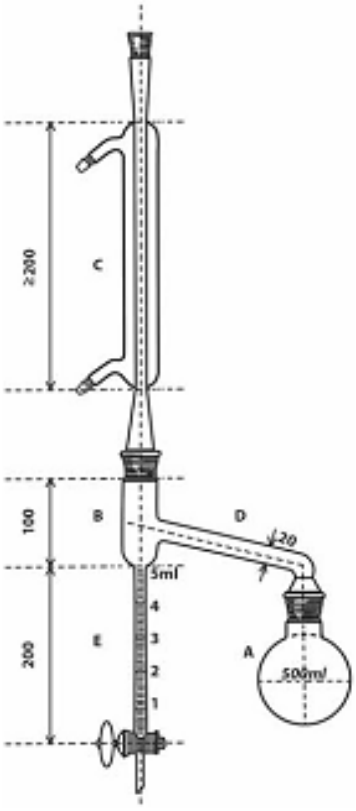


Figure 5. Azeotropic apparatus used for determination of water content

Determination of volatile oil

Volatile oils are characterized by their odour, oil-like appearance and ability to volatilize at room temperature. They consist of a mixture of chemical compounds especially aromatic compounds for example monoterpenes, sesquiterpenes and the oxygenated derivatives. Volatile oils are often biologically active and are considered to be the "essence" of the medicinal plants so they are also known as "essential oils" [3]. Volatile oils in medicinal plant materials can be extracted by hydrodistillation using Clevenger apparatus (Figure 6).

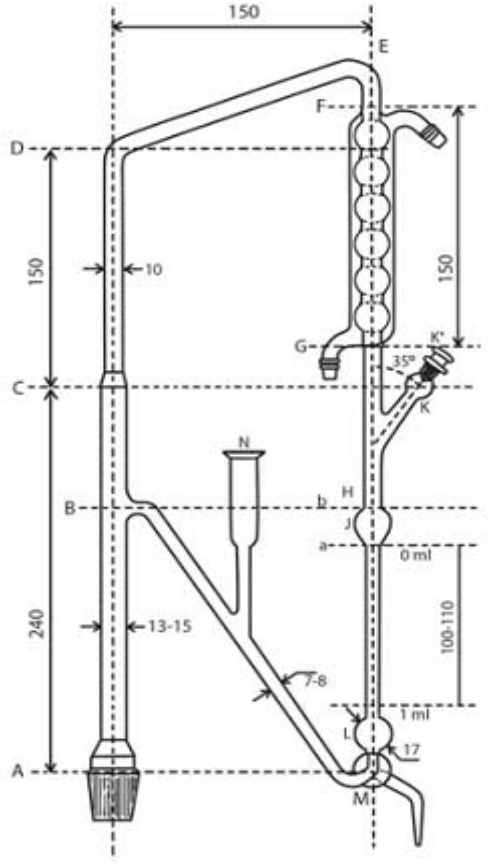


Figure 6. Clevenger apparatus used for determination of volatile oil content

Thin layer chromatography

Chromatography is general technique for separation of mixture compounds. The stationary phase is a solid usually alumina or silica, which is highly polar (normal phase) or non-polar (reverse phase). Chromatographic techniques also have a liquid or gas mobile phase which pushes the mixture through the stationary phase. Substances with a low affinity for the stationary phase will move through the medium quickly and

those with a strong affinity for the stationary phase will move slowly. Common chromatography techniques include column chromatography, gas chromatography (GC), thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) [27-29].

Thin layer chromatography (TLC), also called planar chromatography, is a widely accepted and extensively used separation technique over 65 years. TLC is a solid-liquid form of chromatography, which the stationary phase is normally a polar adsorbent and the mobile phase can be a single solvent or mixture of solvents. Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. It is a widely used method for qualitative analysis to determine the number of components in a mixture, to determine the identity of two substances, or to monitor the progress of a reaction. This technique is simple, cheap, and useable in all laboratories around the world. It can be easily adapted to any given situation of qualitative, quantitative or preparative separation. This technique is most suitable for qualitative analyses of plant extract and is necessary for the standardization of plant materials as the fingerprint profiling or analysis of a marker. The advantages of the technique over other analytical techniques are many when handling plant materials. The preparation of samples is easily and quickly [29-30].

Retention factor

The retention factor or R_f is the distance a compound has moved up the TLC plate divided by the distance that the solvent front moved (Figure 7). R_f value is between 0 and 1, the best is between 0.1 and 0.8 (10–80 for hR_f). The reproducibility of R_f value can be obtained by controlling the parameters such as chamber saturation, constant composition of solvent mixtures and constant temperature. The evaluation depends on the purpose of a chromatographic analysis. For qualitative determination often localisation of substances is sufficient. This can be easily achieved by parallel runs with reference substances. A parameter often used for qualitative evaluation is the R_f value (retention factor) or the 100 fold value, hR_f. The R_f value is defined as follows [29, 31].

$$R_f = \frac{\text{distance starting line} - \text{middle of spot}}{\text{distance starting line} - \text{solvent front}} = \frac{b}{a}$$

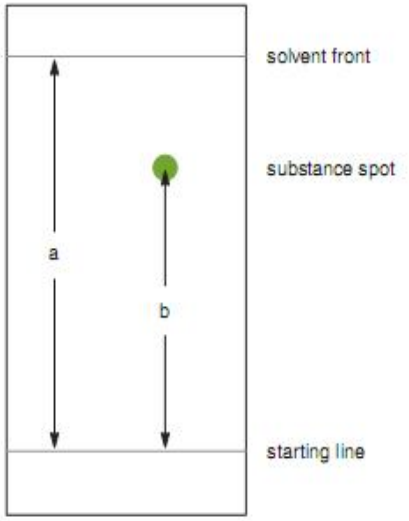


Figure 7. TLC plate; a is solvent front, b is substance spot

Visualization of TLC

The visualization of compounds on TLC plate can be visualized under ultraviolet light at the wavelength of 254 nm and 365 nm or 366 nm (Figure 8) as well as chemical specific reagents. Different chemical reagents are used depending on the functional groups of the molecules that need to be visualized (Table.2) [30, 31].

Figure 8. Ultraviolet analysis cabinet for visualization of TLC

Table 2. Staining reagents for TLC visualization [5, 31-34]

Spray reagents	Preparation	Treatment	Detection
p-Anisaldehyde /sulfuric acid	1 ml p-ansaldehyde, 1 ml conc. Sulfuric acid in 20 ml ethanol	Heat to 105 °C until maximum of spots. Enhance background with water vapor spray. Component give blue, red, violet, grey or green spot.	Sugar, phenol, steroids, and terpenes.
Dragendroff reagent	Solution 1) 1.7 g basic bismuth nitrate and 20 g tartaric acid in 80 ml water Solution 2) 16 g potassium iodide in 40 ml water Stock solution (stable for several weeks in a refrigerator): Mix equal volumes of solutions 1 and 2 Procedure: Spray with a solution of 10 g tartaric acid, 50 ml water and 5 ml stock solution		Nitrogen compounds, alkaloids

Table 2. Staining reagents for TLC visualization (cont.) [5, 31-34]

Spray reagents	Preparation	Treatment	Detection
Ferric (III) chloride	Dissolve ferric (III) chloride 2 g in ethanol	Heat at 110 C until appear blue or red	Phenol and phenolic compounds
Gentian Violet	0.1 g gentian violet (crystal violet) in 100 ml methanol onto plate and place in a tank containing bromine vapor.	Look for blue spots on a yellow background	For detection of lipids
Fuchsin reagent	Dissolve 0.05 g Fuchsin (4-rosaline hydrochloride) in 50 ml water. Add 2 ml saturated sodium bisulphite solution, leave on bench for 1 hour. Add 1 ml conc. HCl and allow to stand overnight to give a colorless liquid.	Gives violet to purple spots.	For detection of aldehydes
Ninhydrin	Dissolve 0.2 g ninhydrin in 100 ml ethanol, or in 94 water and 6 ml acetone	Spray and heat to 110 C until reddish spot appear	Amino acid, amine amino sugar (amphetamines)

Table 2. Staining reagents for TLC visualization (cont.) [5, 31-34]

Spray reagents	Preparation	Treatment	Detection
Sulphuric acid	5% w/v of the acid in ethanol		For organic compounds, bile acids
Phosphomolydbic acid.	0.25 g molybdatophosphoric acid in 50ml ethanol	Heat to 120°C until spots appear (oven or heat gun) If necessary, treat with ammonia vapors to remove some background coloration.	For detection of reducing substances, e.g, alcohols, bile acids, lipids, fatty acids, steroids
Phosphoric acid	50 ml conc. phosphoric acid 50 ml water.	heat 10-15minutes at 120°C until the layer appears	For detection of sterols, steroids, and bile acids
Vanillin/ sulfuric acid	Dissolve 1 g of vanillin in 100 ml conc. Sulfuric acid or 0.5 g vanillin in 80 ml sulfuric acid and 20 ml ethanol	Heat at 120 C until maximum color development. Components give red and blue colors.	A universal spray, terpenoids, steroids saponins

Quantitative detection of TLC

An evaluation is possible by suitable calibration measurements. For this purpose, either the area of a substance spot is measured or a photometric evaluation is performed directly on the layer. The latter procedure, however, requires a higher instrumental expense [29]. TLC is considered to be a semi-quantitative analytical procedure if only visual evaluation of spots is performed. Since the eyes can only compare but not measure absolute values. However, if a direct optical evaluation as a TLC scanner is performed on the TLC plate, the exact quantitative results are possible. TLC scanner is an instrument used for the densitometric evaluation of objects from the fields of planar chromatography and electrophoresis. The scanner has three light sources include a deuterium lamp, a halogen-tungsten lamp and a high-pressure mercury lamp so, it offers many features such as evaluation in absorption and fluorescence, unattended programmed scanning of lanes, multi-wavelength measurement, background correction, selectable base line for integration, recording of spectra, evaluation of circular or anti-circular chromatograms with very high ease of operation. In addition to manual, operation control by a computer is possible with respective data collection and storage. The evaluation of TLC scanner is depended on reflectance or transmittance mode by absorbance or fluorescence range of 190-800 nm. All chromatographic visualization can be evaluated under the proper wavelength for more reliability then recorded as peaks on the chromatogram. Quantitative evaluation is based on the comparison of peak heights or peak areas of the unknowns with those of calibration standards on the same plate [29, 35, 36].

Besides TLC scanner, quantitative TLC can be performed by digital camera and image analysis system which separates red, green, and blue brightness values in an image of a TLC plate to create multi-spectral scans [37]. TLC chromatogram are created by taking brightness values from a multi-spectral scan, transforming them in terms of density, then plotting the density values against their distance from the origin on the plate. Black and white on TLC chromatogram helps analyze the pixel values of a TLC plate. The spots on TLC plate and the background showed different color. A digital camera captures these brightness values using an array of light-sensitive pixels on a semiconductor chip. The lens of camera focuses on the semiconductor chip when taking a picture. Since pixels are monochrome, color filters are used to record a

picture's color. The filter array has an alternating pattern of red and green filter rows for each brightness value. While intensity measures the brightness of a pixel, density measures the darkness [35]. This technique is so called digitally enhanced TLC. ImageJ is a public domain, Java-based image processing program developed by the National Institutes of Health in Bethesda, Maryland, USA. This software is freely available program and can be used by any computers [38]. Moreover, it is an open source that each developer or user has freedom to fix the problem and develop the program to ultimate processing [39]. Analyzer can find the maximum of pixel on TLC plate. TLC analyzer is an inexpensive and easy tool for the analysis of the images. TLC image analysis can be used to quantify the chemical constituents in herbal materials [34].

CHAPTER III

METERIALS AND METHODOLOGY

Chemicals

Acetic acid	BDH, Chemical, LTD, England
Ethanol	RCI Labscan Limited, Bangkok, Thailand
Ethyl acetate	Malinckrodt® Inc., USA
Formic acid	Fisher Scientific, LTD, UK
Hydrochloric acid	RCI Labscan Limited, Bangkok, Thailand
Toluene	RCI Labscan Limited, Bangkok, Thailand
Ferric(III) chloride	SIGMA-ALDRICH., St.Louis, USA
Quercitrin	ChromaDex, USA

The chemicals used were of analytical grade.

Materials

Cover slide	Menzel. Glazer
Filter paper No.40 ashless	Whatman ^{TM} Paper, UK
Filter paper No.4	Whatman ^{TM} Paper, UK
TLC silica 60 F_{254}	MERCK, LTD, USA

Instruments and equipments

Ashing furnace	Carbolite, UK
Balnce readability 0.01 g	Pioneer^{TM} Ohaus Crop. Pine Brook, NJ, USA
Balance readability 0.0001 g	Becthai Bangkok Equipment and Chemical Co., LTD, Bangkok
Clevenger apparatus	
Desiccator	
Digital camera	Canon, PowerShot A650 IS camera Canon Marketing (Thailand) Co., LTD, Bangkok
Hot air oven	WTC binder tuttlingen, Germany
ImageJ softwere	The National Institute of Mental Health, USA
Microscope	Carl Zeiss model Axio Lab, Germany
Rotary vacuum evaporator	Buchi, Switzerland
Soxhlet apparatus	
Shaker	Adolf Kuhner AG, Switzerland
Syringe	Hamilton Company, USA
TLC chamber	Camag, Switzerland
TLC densitometry instrument - Linomat III - TLC scanner 3 - TLC visualizer	Camag, Switzerland with winCATS software
Ultrasonic bath	Analytical Lab Science Co., LTD, Bangkok
Ultraviolet fluorescence analysis cabinet	Theeratrading Co.,LTD, Bangkok
Water bath	Brinkmann, USA

Methods

Plant materials

Dendrophthoe pentandra (L.) Miq leaves were collected from 13 different locations in Thailand. Plant specimens were authenticated by Ruangrungsi N. The voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. The leaves were cleaned and air-dried in the shade then kept in a well-closed container in a dark place. For quality assessment, the leaves were ground and weighed using 0.0001 g readability balance.

Macroscopic and microscopic examinations

Visual characteristics of *D. pentandra* were examined. *D. pentandra* whole plant was illustrated botanically by line drawing. Microscopic evaluation of *D. pentandra* leaf was carried out under the appropriate magnitude using a photomicroscope attached with digital camera. The leaf was cross sectioned as well as pulverized and examined under microscope by wet mounting in water. Photographs were taken by camera. The anatomical and histological characters were drawn related to the original size.

Determination of loss on drying

Placed 3 g of ground air-dried *D. pentandra* leaves in a pre-weighed dried crucible. Dried the sample at 105 °C for 6 hour and weighed. Calculated the loss of weight in a percentage of air-dried material. Each sample was performed in triplicate.

Determination of total ash

Placed 3 g of ground air-dried *D. pentandra* leaves in a pre-weighed crucible and incinerated at 500°C until it was white. Allowed to cool in a desiccator and quickly weighed and calculated the content of total ash in a percentage of air-dried *D. pentandra* leaves. Each sample was performed in triplicate.

Determination of acid insoluble ash

For the crucible containing the total ash, added 25 ml of hydrochloric acid (70 g/l), covered with a watch-glass and boiled weakly for 5 minutes. Rinsed the watch-glass with 5 ml of hot water and added the liquid to the crucible. Collected the insoluble ash with an ashless filter-paper and washed with hot water until the filtrate was neutral. Removed the filter-paper containing the insoluble matter to the previous crucible, dried on hot-plate and incinerated at 500 °C to constant weight. Allowed the

residue to cool in a desiccator, weighed without delay and calculated the content of acid-insoluble ash in a percentage of air-dried material.

Determination of water-soluble extractive value

Placed 5 g of ground air-dried *D. pentandra* leaves in a pre-weighed glass stoppered conical flask. Macerated with 70 ml of water for 6 hours in shaking bath then allowed to stand for 18 hours. Filtered, rinsed the marc and adjust to 100 ml of final volume. Transferred 20 ml of the filtrate in pre-weighed small beaker and evaporated to dryness on water-bath. Dried at 105 $^{\circ}$C in an oven to constant weight, calculated the content of extractable value in a percentage of weight. Each sample was performed in triplicate.

Determination of ethanol-soluble extractive value

Placed 5 g of ground air-dried *D. pentandra* leaves in a pre-weighed glass stoppered conical flask. Macerated with 70 ml of ethanol for 6 hours in shaking bath then allowed to stand for 18 hours. Filtered quickly to avoid loss of ethanol, rinsed the marc and adjust to 100 ml of final volume. Transferred 20 ml of the filtrate in pre-weighed small beaker and evaporated to dryness on water-bath. Dried at 105 $^{\circ}$C in an oven to constant weight, calculated the content of extractable value in a percentage of weight. Each sample was performed in triplicate.

Determination of water content

Thirty grams of ground air-dried *D. pentandra* leaves were distilled with 200 ml of water-saturated toluene using azeotropic apparatus. Water distilled from plant material was in receiving tube of azeotropic apparatus then was recorded in percentage. Each sample was performed in triplicate.

Determination of volatile oil content

One hundred–grams of ground air-dried *D. pentandra* leaves were distilled with 600 ml of water using Clevenger apparatus. Volatile oil distilled from plant material was in receiving tube of the apparatus then was recorded in percentage.

Thin layer chromatographic fingerprinting

Twenty milliliters of the filtrate from determination of ethanol-soluble extractive value were evaporated to dryness and re-dissolved in 1 ml of ethanol. Applied 3 µl to TLC plate coated with silica gel 60 F_{254} and developed the TLC plate in the chamber with ethyl acetate: formic acid: acetic acid: H_2O (13: 1: 1: 2.5 v/v).

Observed the spot in daylight, under short wavelength UV (254 nm), long wavelength UV (365 nm) and dipped the TLC plate in 2% w/v $FeCl_3$ in ethanol.

Preparation of quercitrin standard solutions

The stock solution of standard quercitrin (1 mg/ml) was prepared in 95% ethanol. The stock solution was appropriately diluted to obtain the standard solutions of 0.25, 0.325, 0.5, 0.75 and 1 mg/ml. These solutions were stored in refrigerator.

Preparation of ethanol extracts of *D. pentandra* leaves

Five grams of the powdered air-dried of *D. pentandra* leaves were exhaustively extracted with 95% ethanol by soxhlet apparatus. The extract was filtered and evaporated to dryness. The extract was dissolved in 95% ethanol to get the concentration of 1.0 mg/ml. This extract was used for TLC densitometry and TLC image analysis.

TLC densitometry

Three microliters of the ethanol extract solution of *D. pentandra* leaves and standard quercitrin solutions were applied on 20 x 10 cm silica gel 60 F_{254} TLC plate by Camag Linomat 5 (length band 5.0 mm, distance between band 10 mm). The plate was developed using a solvent of ethyl acetate: formic acid: acetic acid: H_2O, 13: 1: 1: 2.5 v/v. The length of each chromatogram run was 8.0 cm. Each sample was performed in triplicate.

The TLC plate was scanned by Camag TLC scanner 3 under maximum absorption wavelength (265 nm) of quercitrin. The intensity of band was transformed to the chromatographic peak by winCATS software. The calibration curve of quercitrin was prepared by plotting peak areas *vs.* concentrations of quercitrin applied.

TLC image analysis by ImageJ software

The developed TLC plate was examined under UV 254 nm by ultraviolet fluorescence analysis cabinet. The photo was taken using Canon, PowerShot A650 IS camera and stored as JPEG files with C mode ISO 80, fluorescent, largest and superfine image. The ImageJ software was used to analyze and quantitate the quercitrin spot on TLC plate. The calibration curve of quercitrin was prepared by plotting peak areas *vs.* concentrations of quercitrin applied.

Method validation

Linearity

Regression line, linearity and correlation coefficient of calibration curve were estimated using Excel 2007.

Accuracy

The accuracy of the method was performed by spiking the known amount of quercitrin standard (0.15, 0.30 and 0.60 mg/spot) into the sample extract. At each concentration, three determinations were performed. The accuracy was calculated as % recovery by using following formula:

$$\% \text{ Recovery} = (C_s/C + C_a) \times 100$$

Where, C_s = the amount of quercitrin tested in spiked sample extract

C = the amount of quercitrin tested in un-spiked sample extract

C_a = the amount of quercitrin standard actually added to the sample extract

Repeatability and intermediate precision

The precision of the method was assessed by repeatability (intra-day) and intermediate precision (inter-day) examination. Intra-day and inter day precision were operated by analyzing quercitrin in sample extract in triplicate on the same day and three different days respectively [40]. The precision of quercitrin content analysis was determined in term of percentage of relative standard deviation by using following formula:

$$\% \text{ RSD} = \text{SD} \times 100/\text{mean}$$

Limit of detection (LOD)

The detection limit was the lowest amount of quercitrin which could be detected but not necessarily quantitated as an exact value [40]. LOD was calculated from the calibration curve using following formula:

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

Where, SD = standard deviation of y-intercept

S = slope of regression line

Limit of quantitation (LOQ)

The limit of quantitation was the lowest amount of quercitrin which could be detected as an exact value [40]. LOQ was calculated from the calibration curve using following formula.

$$LOQ = 10\ (SD)/S$$

Where, SD = standard deviation of y-intercept

S = slope of regression line

Robustness

Robustness of the TLC method was performed by introducing small changes in the mobile phase complements including ethyl acetate: formic acid: acetic acid: H_2O (12.9: 1: 1: 2.5), (13: 0.9: 1: 2.5), (13: 1: 1: 2.6), (13: 1.1: 1.1: 2.5). Each variation was determined in triplicate. The results of robustness were shown by the peak area value. The %RSD values of the peak area were calculated for all variations [41].

Specificity

Specificity of TLC quantitative analysis was operated by identification method. The identification method was performed by comparison of absorption spectra of quercitrin standard and all samples using CAMAG TLC Scanner [26].

Data analysis

The pharmacognostic specification was calculated as grand mean and pooled standard deviation. The quercitrin contents between TLC image analysis and TLC-densitometry were compared by paired t-test statistical analysis.

CHAPTER IV

RESULTS

Pharmacognostic specification of *Dendrophthoe pentandra* (L.) Miq.

Common name	KA-FAK
Other names	KA-FAK-MA-MUANG
Vernacular names	MISTLETHOE (England), KEMADEAN, KEMLANDEN, MANGANDEUH, BENELU, PASILAN (Indonesia), DAY CHUM GO I (Vietnam), KAFAKMAMUANG, KAFAK (Thailand)
Scientific name	*Dendrophthoe pentandra* (L.) Miq.
Synonyms	*Loranthus pentandrus* L.
Family	LORANTHACEAE
Distribution	Throughout tropics
Used part	Leaves
Ethnomedical use	Used as aqueous adjuvant and also used to treat diuretic and hypertension

Characteristic of plant

Shrubs to 2 m tall, youngest parts puberulous. Branches grayish, scattered lenticellate. Petiole 5-20 mm; leaf blade lanceolate to elliptic or suborbicular, 5-15 × 2.5-10 cm, thickly leathery, lateral veins 2-4 pairs, base cuneate or obtuse, apex acute or rounded, glabrous. Racemes solitary or 2 or 3 together, 3-10-flowered; peduncle 7-20 mm, with grayish or white stellate hairs; bracts broadly ovate, 1-1.5 mm. Pedicel 2 mm. Calyx 2-2.5 mm, limb 0.5-1.5 mm, 5-denticulate. Mature bud 1.5-2 cm. Corolla orange, basal 1/2 slightly inflated, lobes lanceolate, ca. 12 mm, reflexed. Filaments 3-4 mm; anthers 3-5 mm. Berry red, 8-10 × 5-6 mm, minutely pilose or glabrous [18].

Phamacognostic specification

The drawing of whole plant of *Dendrophthoe pentandra* was indicated in Figure 8. *D. pentandra* dried leaves was used as crude drug. The crude drugs were green and brown leaves with various sizes (Figure 9-11).

The anatomical character showed upper epidermis, unicellular trichrome, palisade cell, vascular tissue, parenchyma, collenchyma, crystal of calcium oxalate and lower epidermis (Figure 12). The stomata was in lower epidermis and stomata type was paracytic (Figure 14).

The histological character was performed of rosette aggregate crystals of calcium oxalate; annular vessel; fiber cell; multicellular trichome; unicellular trichome; prism crystals of calcium oxalate; epidermal cell; epidermal cell containing stomata; surface view of lower epidermis (Figure 13, 14).

A pharmacognostic constant numbers due to the quality of *D. pentandra* leaves were presented in table 3.

Thin layer chromatography fingerprint of ethanol extract of *D. pentandra* leaves were indicated in Figure 15.

Figure 9. *Dendrophthoe pentandra* (L.) Miq.

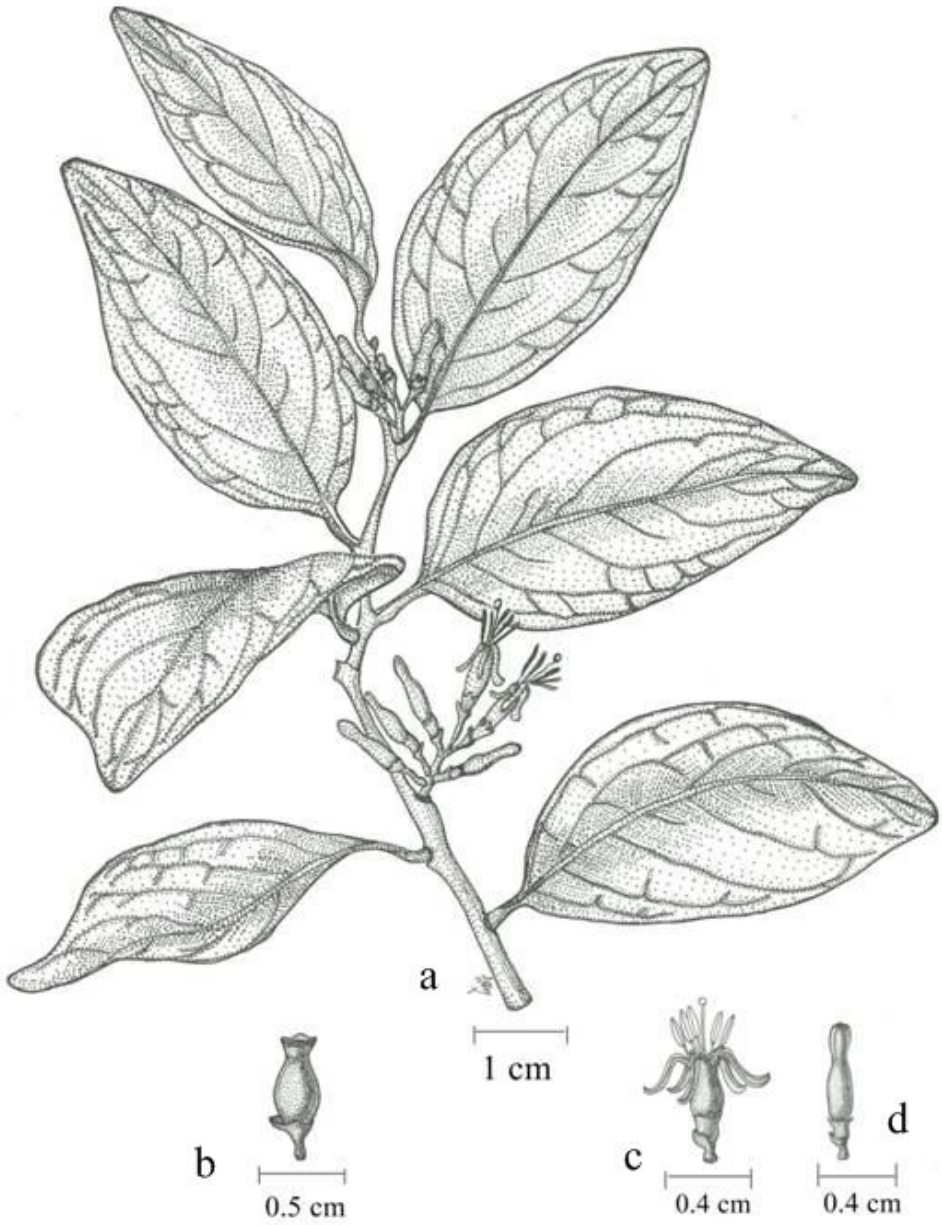


Figure 10. Drawing of *Dendrophthoe pentandra* (L.) Miq.;

a. Whole plant

b. Fruit

c. Blooming flower

d. Budding flower

Figure 11. ***Dendrophthoe pentandra*** **dried leaves crude drug**

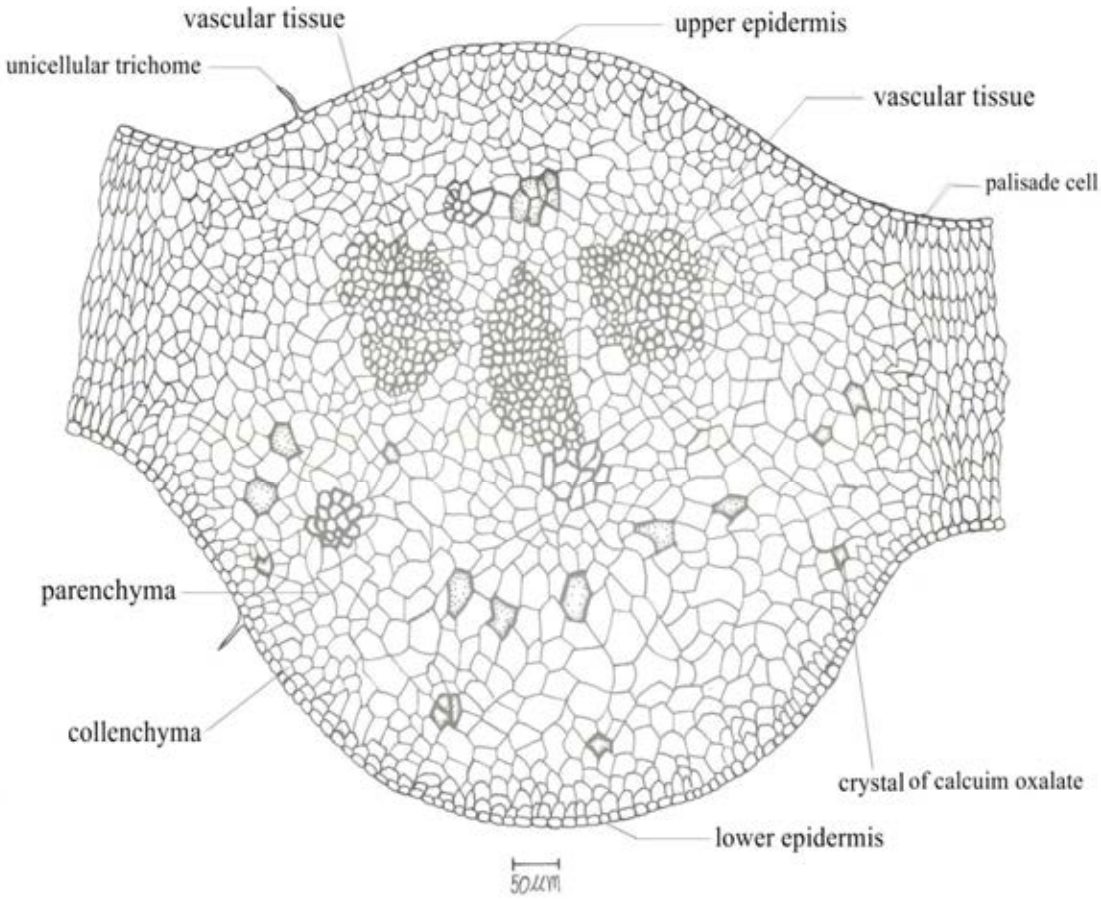


Figure 12. Transverse section of *Dendrophthoe pentandra* leaves

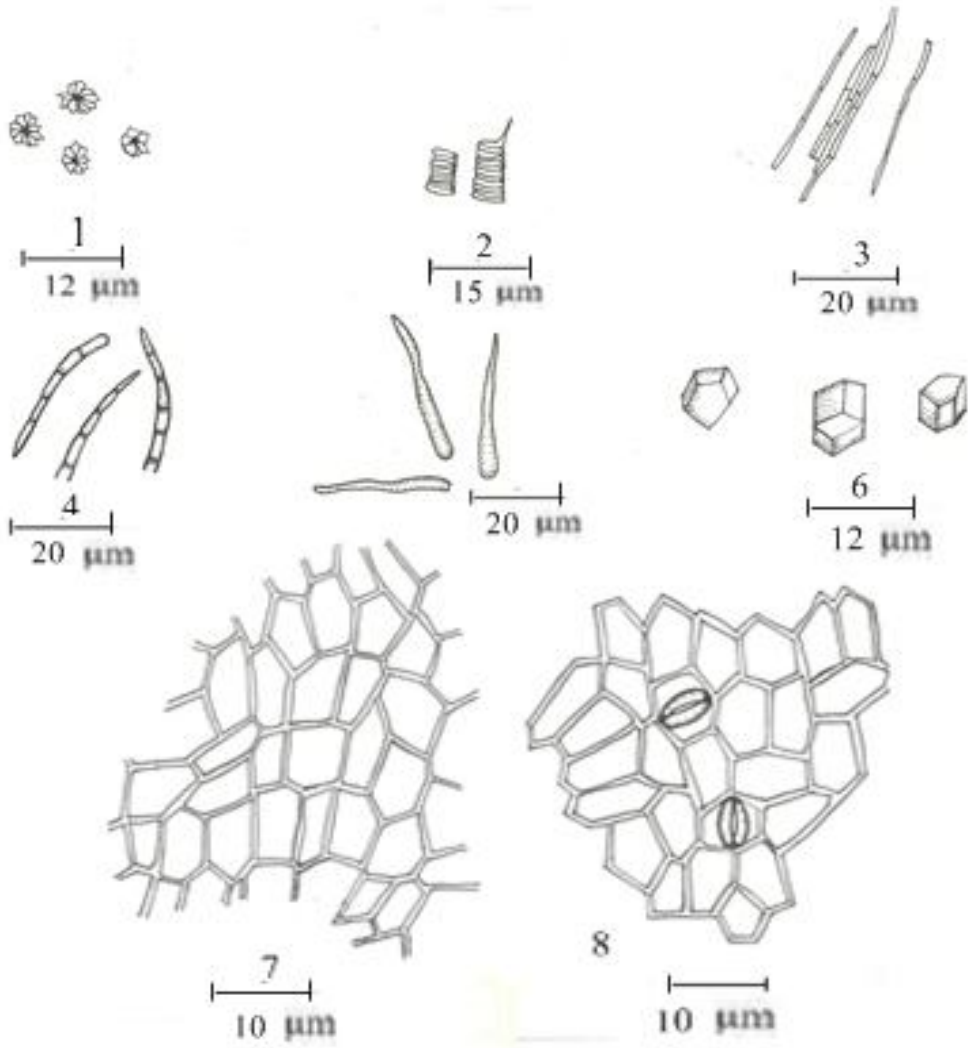


Figure 13. Powder of *Dendrophthoe pentandra* leaf: (1) Rosette aggregate crystals of calcium oxalate; (2) Annular vessel; (3) Fiber cell; (4) Multicellular trichome; (5) Unicellular trichome; (6) Prism crystals of calcium oxalate; (7) Epidermal cell; (8) Epidermal cell containing stomata

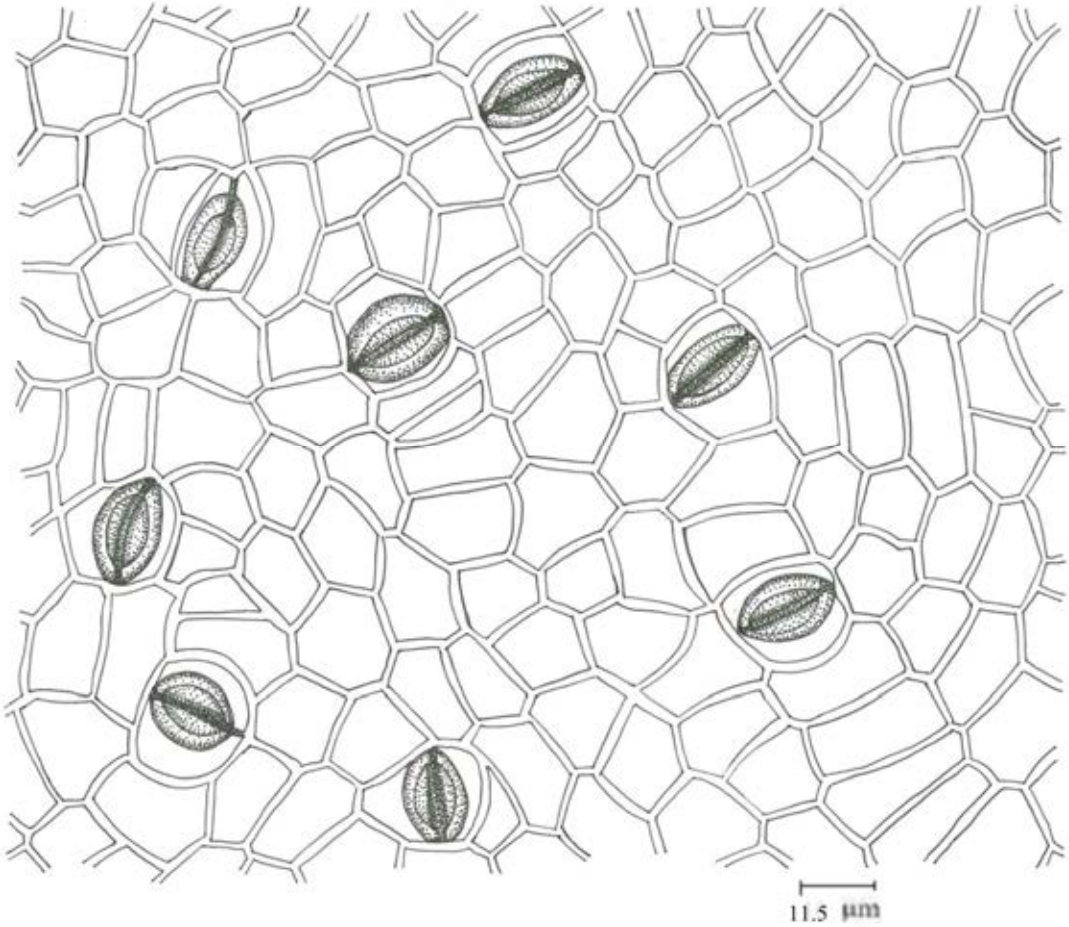


Figure 14. Surface view of lower epidermis of *Dendrophthoe pentandra* leaves

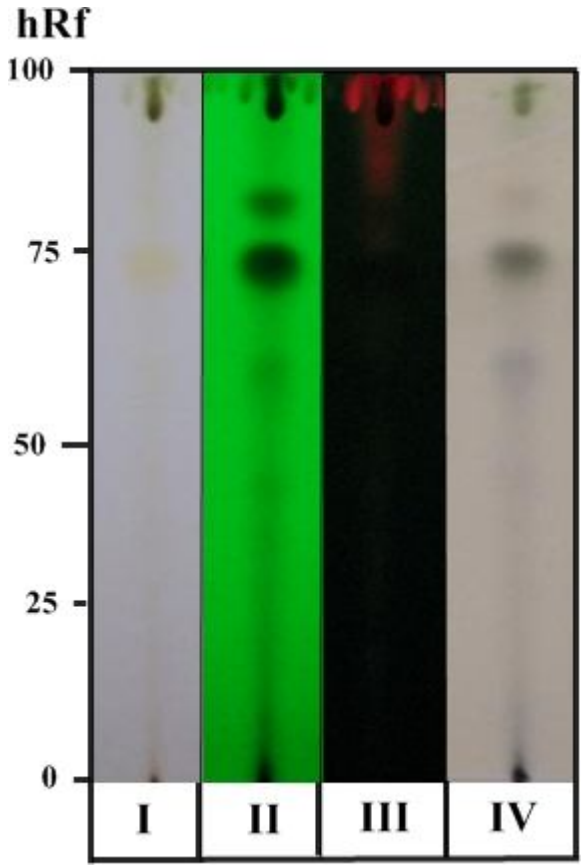


Figure 15. TLC fingerprint of ethanol extract *Dendrophthoe pentandra* leaves

Solvent system	ethyl acetate: formic acid: acetic acid: H_2O (13: 1: 1: 2.5)	
Detection	I	= detection under daylight
	II	= detection under UV light 254 nm
	III	= detection under UV light 366 nm
	IV	= detection with ferric chloride staining reagent

Table 3. The pharmacognostic constant numbers due to the quality of *Dendrophthoe pentandra* leaves

Content (% by weight)	Mean±SD [a]	Range (Mean ± 3SD)
Water	9.99 ± 0.70	7.88- 12.09
Loss on drying	9.16 ± 0.61	7.32 – 10.99
Total ash	11.94 ± 0.19	11.36 – 12.51
Acid insoluble ash	4.17 ± 0.88	1.53 - 6.82
Ethanol-soluble extractive	7.67 ± 0.72	5.51 – 9.83
Water-soluble extractive	22.24 ± 2.59	14.48 - 29.99
Volatile oil	0	0

[a] The parameters were shown as grand mean ± pooled SD. Samples were from 13 different locations throughout Thailand. Each sample was performed in triplicate.

Ethanol extracts of dried *Dendrophthoe pentandra* leaves

The percent yield of ethanol extracts of dried *Dendrophthoe pentandra* leaves were presented in table 4 with the average of 22.81±5.88% by dried weight.

Table 4. The percent yield of ethanol extract of dried *Dendrophthoe pentandra* leaves

Source	Crude drug	Ethanolic extract	
	(g)	(g)	%
1	5.0038	1.7189	34.35
2	5.0028	1.3121	26.23
3	5.0018	0.9313	18.62
4	5.0005	1.0763	21.52
5	5.0016	0.7388	14.77
6	5.0028	1.1348	22.68
7	5.0026	0.9795	19.58
8	5.0021	1.1554	23.10
9	5.0038	1.1336	22.67
10	5.0016	1.6717	33.42
11	5.0026	1.4268	28.52
12	5.0034	0.9711	19.41
13	5.0003	0.9313	18.62
Average	5.0018	1.1409	22.81

The amount of quercitrin in dried *Dendrophthoe pentandra* leaves by TLC densitometry

The quercitrin content in *Dendrophthoe* p*entandra* leaves from 13 different locations were determined in triplicate by TLC densitometry. The quercitrin content in crude drugs were calculated and the average content was 3.71 ± 1.33% (Table 5).

Table 5. The amount of quercitrin in *Dendrophthoe pentandra* leaves by TLC-densitometry (% by dried weight)

Source	Quercitrin in ethanol extract (mg/mg)		Yield of ethanol extract (g/100g of dried leaves)	Quercitrin in *D. pentandra* dried leave (g/100 g)	
	Mean	SD		Mean	SD
1	0.3730	0.1319	34.3519	12.8126	4.5301
2	0.1707	0.0761	19.2445	3.2858	1.4638
3	0.1874	0.0685	18.6193	3.4890	1.2750
4	0.1103	0.0438	21.5238	2.3733	0.9434
5	0.1842	0.0890	14.7713	2.7204	1.3150
6	0.2175	0.0835	22.6833	4.9347	1.8931
7	0.2006	0.0768	19.5798	3.9268	1.5030
8	0.0565	0.0220	23.0983	1.3055	0.5079
9	0.0531	0.0165	22.6697	1.2048	0.3742
10	0.1925	0.0523	33.4233	6.4329	1.7484
11	0.0873	0.0393	28.5212	2.4906	1.1210
12	0.0745	0.0087	19.4088	1.4465	0.1696
13	0.0942	0.0285	18.6249	1.7552	0.5306
Average	0.1539	0.0567	22.8092	3.7060	1.3345

Method validation of TLC densitometry

Calibration curve

The calibration curve of quercitrin by TLC densitometric method was linear. The regression equation was y = 17034x+2492 and correlation coefficients (r^2) was 0.998. The linearity range of quercitrin was 0.25 - 1.00 mg (Figure 16).

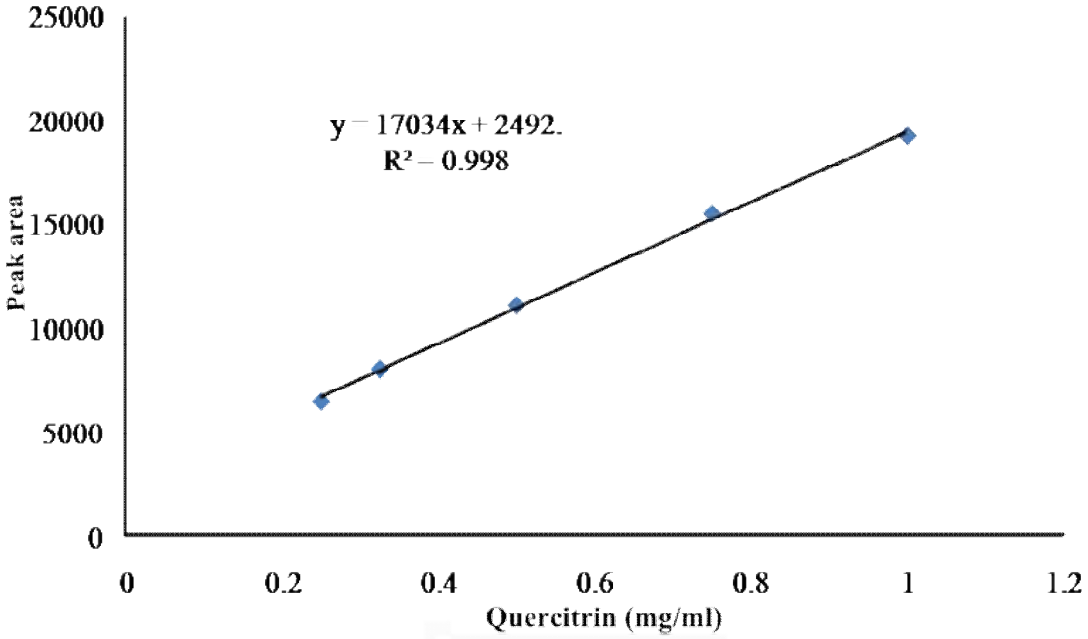


Figure 16. The calibration curve of TLC densitometry method

Accuracy

The accuracy of quercitrin quantitation by TLC densitometric method was evaluated in percentage of recovery. The recovery of quercitrin was performed on sample spiked with three different concentrations of quercitrin (0.15, 0.30, 0.60 mg/ml). The recovery method was done in triplicate. The results were between 98.69 - 100.97% (Table 6).

Table 6. Recovery of quercitrin (n=3) by TLC densitometric method

Quercitrin added (mg)	Quercitrin found (mg)	% Recovery
0.00	0.03	
0.15	0.19	100.97
0.30	0.32	96.41
0.60	0.63	98.69

Precision

The precision of quercitrin quantitation by TLC dencitometric method was evaluated in triplicate of each concentration group (0.15, 0.30, 0.60 mg/ml). The result was presented as the percentage of relative standard deviation which represented the error of the method. The repeatability was performed by three different concentrations on the same day. The intermediate precision was determined by three different concentrations on the different days. The repeatability and intermediate precision were between 2.46-4.30% and 5.99-7.39% respectively (Table 7).

Table 7. Repeatability and intermediate precision of quercitrin by TLC densitometry (n=3)

Sample conc. (mg/spot)	Repeatability (% RSD)	Intermediate precision (% RSD)
0.19	4.30	7.39
0.32	3.28	6.99
0.63	2.46	5.99

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

For this study, limit of detection and limit of quantitation in TLC densitometry were measured based on the standard deviation of y-intercept. The values of slope of regression line and the y-intercept standard deviation were 17034.00 and 834.67. The LOD and LOQ were found to be 0.16 and 0.49 mg/ml respectively.

Robustness

Robustness of the TLC densitometry method was performed by introducing small changes in the mobile phase complements (ethyl acetate: formic acid: acetic acid: H_2O). Each variation was determined in triplicate. The robustness value was 3.43%RSD. The peak area of quercitrin were between 13472.42 - 17362.27 (Table 8).

Table 8. **Robustness of the TLC densitometry method (n=3)**

Mobile phase (v/v)	Peak area of quercitrin ($Pixel^2$)
12.9: 1: 1: 2.5	16741.77
13: 0.9: 1: 2.5	15500.47
13: 1: 1: 2.5	13737.30
13: 1: 1: 2.6	17362.27
13: 1.1: 1.1: 2.5	13472.42
Mean ± SD	15362.85±1741.71
% RSD	3.43

Specificity

The absorption spectra of quercitrin in all samples and standard were identical with the maximum absorption at 265 nm which represented the method specificity.

The amount of quercitrin in *Dendrophthoe pentandra* leaves by TLC-image analysis

The quercitrin content in *Dendrophthoe* p*entandra* leaves from 13 different locations were determined in triplicate by TLC image analysis. The quercitrin content in crude drugs were calculated and the average content was 3.89 ± 0.97% (Table 9).

Table 9. The amount of quercitrin in *Dendrophthoe pentandra* leaves by TLC image analysis (% by dried weight)

Source	Quercitrin in ethanol extract (mg/mg)		Yield of ethanol extract (g/100g of dried leaves)	Quercitrin in *D. pentandra leaves* (g/100 g)	
	Mean	SD		Mean	SD
1	0.3804	0.0841	34.3519	13.2052	2.8882
2	0.1738	0.0548	19.2445	3.4083	1.0544
3	0.1685	0.0593	18.6193	3.5100	1.1036
4	0.1151	0.0366	21.5238	3.0523	0.7878
5	0.1933	0.0672	14.7713	3.1014	0.9922
6	0.2231	0.0471	22.6833	5.0615	1.0675
7	0.2212	0.0810	19.5798	4.8531	1.5855
8	0.0679	0.0159	23.0983	1.3647	0.3667
9	0.0530	0.0143	22.6697	1.2250	0.3237
10	0.1897	0.0369	33.4233	6.3420	1.2334
11	0.0873	0.0215	28.5212	2.1100	0.6145
12	0.0852	0.0177	19.4088	1.5240	0.3432
13	0.0990	0.0105	18.6249	1.8085	0.1947
Average	0.1583	0.0347	22.8092	3.8896	0.9658

Method validation of TLC image analysis by ImageJ softwere with CCD camera

Calibration curve

The calibration curve of quercitrin by TLC image analysis method was linear. The regression equation was y = 23539x+1727 and correlation coefficients (r^2) was 0.995. The linearity range of quercitrin was 0.25 - 1.00 mg (Figure 17)

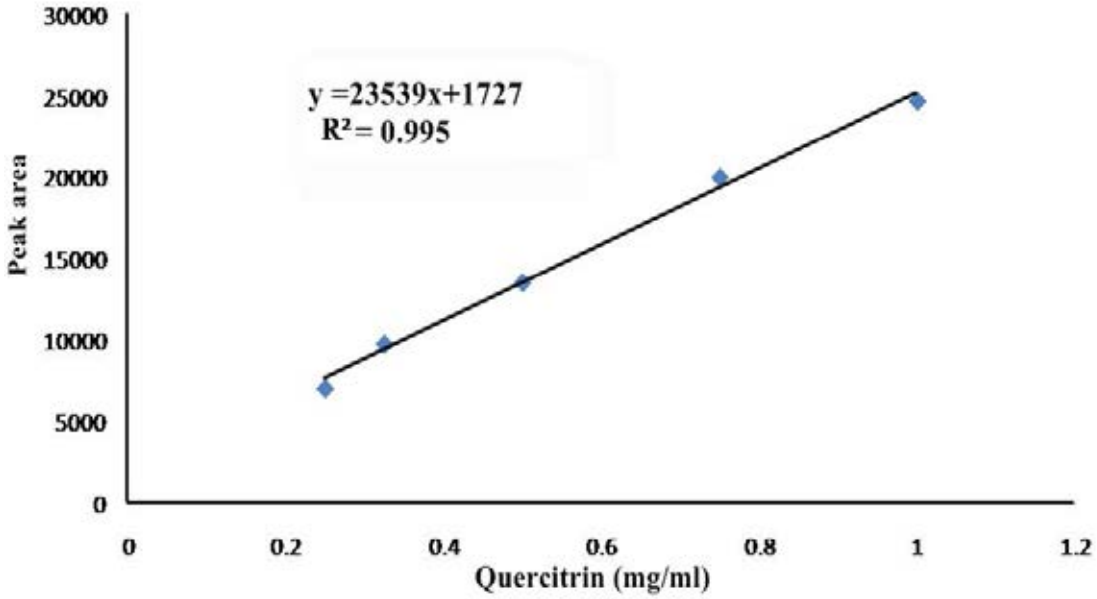


Figure 17. The calibration curve of TLC image analysis method

Accuracy

The accuracy of quercitrin quantitation by TLC image analysis method was evaluated in percentage of recovery. The recovery of quercitrin was performed on sample spiked with three different concentrations of quercitrin (0.15, 0.30, 0.60 mg/ml). The recovery method was done in triplicate. The results were between 81.57-103.09% (Table 10).

Table 10. Recovery of quercitrin (n=3) by TLC image analysis method

Quercitrin added (mg)	Quercitrin found (mg)	% Recovery
0.00	0.04	
0.15	0.19	103.09
0.30	0.28	81.57
0.60	0.60	94.71

Precision

The precision of quercitrin quantitation by TLC image analysis method was evaluated in triplicate of each concentration group (0.15, 0.30, 0.60 mg/ml). The results were presented as the percentage of relative standard deviation which represented the error of the method. The repeatability was performed by three different concentrations on the same day. The intermediate precision was determined by three different concentrations on the different days. The repeatability and intermediate precision were between 3.24-7.75% and 6.81 -9.02% respectively (Table 11).

Table 11. Repeatability and intermediate precision of quercitrin by TLC image analysis method (n=3)

Sample conc. (mg/spot)	Repeatability (% RSD)	Intermediate precision (% RSD)
0.19	3.24	6.81
0.28	7.75	4.05
0.60	6.14	9.02

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

For this study, limit of detection and limit of quantitation in TLC image analysis method were measured based on the standard deviation of y-intercept. The values of slope of regression line and the y-intercept standard deviation were 23539.00 and 508.74. The LOD and LOQ were found to be 0.16 and 0.50 mg/ml respectively.

Robustness

Robustness of the TLC image analysis was performed by introducing small changes in the mobile phase complements (ethyl acetate: formic acid: acetic acid: H_2O). Each variation was determined in triplicate. The robustness value was 6.26%RSD. The peak area of quercitrin were between 17825.77 - 33233.13 (Table 12).

Table 12. Robustness of the TLC image analysis method (n=3)

Mobile phase (v/v)	Peak area of quercitrin ($Pixel^2$)
12.9: 1: 1: 2.5	30480.60
13: 0.9: 1: 2.5	33119.36
13: 1: 1: 2.5	28629.61
13: 1: 1: 2.6	33233.13
13: 1.1: 1.1: 2.5	17825.77
Mean±SD	28657.69±6354.04
%RSD	6.26

Comparison of quercitrin contents between TLC densitometry and TLC image analysis by imageJ

The quercitrin between TLC densitometry and TLC image analysis by imageJ were compared by paired t-test statistical analysis. The comparison was found that the quercitrin by two methods were not significantly different ($P > 0.05$). The correlation coefficients (r^2) were 0.994. The mean and standard deviation of TLC densitometry method was 3.71±1.33. The mean and standard deviation of TLC image analysis by imageJ 3.89±0.97. The result of comparison of quercitrin contents between TLC densitometry and TLC image analysis by imageJ were shown in table 13.

Table 13. The comparison of quercitrin contents between TLC densitometry and TLC image analysis by imageJ

Source	Quercitrin content (%)	
	TLC densitometry	TLC imageJ
1	12.8126	13.2052
2	3.2858	3.4083
3	3.4890	3.5100
4	2.3733	3.0523
5	2.7204	3.1014
6	4.9347	5.0615
7	3.9268	4.8531
8	1.3055	1.3647
9	1.2048	1.2250
10	6.4329	6.3420
11	2.4906	2.1100
12	1.4465	1.5240
13	1.7552	1.8085
Average	3.7060	3.8897

CHAPTER V

DISCUSSION AND CONCLUSION

Quality control of herbal medicines is important for their safety and efficiency. The determination of these parameters allows an idea related to the specific characteristics of crude drug with examination. These diagnostic features enable the analyst to know the nature and characteristics, including determination of various parameters indicate their acceptability by standard. The procedures give the qualitative data about purity and standard of the crude drug [1, 3].

This study provides the information of pharmacognostic specification and quercitrin content of *D. pentandra* leaves**.** This plant is one of medicinal plant in many countries [4, 6-8]. For traditional Thai medicine, Kafakmamuang leaves are used as aqueous adjuvant and also used to treat diuretic and hypertension [9].

The macroscopic and microscopic examinations are first methods for authentication of the characteristics of plant materials [2]. Their characteristics are used for identification of plant material to assure its purity or adulteration [2, 42]. The leaf was illustrated for its macroscopic structures as its size, shape, color, margin of leaf, leaf apex, base leaf, texture, surface of upper and lower leaf, leaf stalk, and the length and wide of leaf [43]. The leaf was transverse sectioned as well as pulverized and examined under microscope for microscopic structures which was necessary for identification of plant material in powder [1, 3, 43, 44]. These diagnostic characters enable the authenticated characteristics of crude drug [1].

The characteristic specification of *D. pentandra* leaves were shown in terms of anatomical and histological structures. The transverse section of *D. pentandra* leaf is similar in general aspects of leaf structure previously reported for *Loranthus* which showed paracytic stomata on the lower surface of leaves, prismatic calcium oxalate crystal and fiber cell [44].

TLC is usually used to obtain a fingerprint profile of the compounds in the medicinal plants. The TLC fingerprint analysis was used for identification and authentication the medicinal plants. The medicinal plant compounds were separated by TLC fingerprinting. The fingerprint characteristic specification was shown by TLC chromatogram. The TLC fingerprint of each medicinal plant presented pattern of zone

with different colors, and different R_f value which was used as markers for quality assurance and standardization of medicinal plant material [31, 33, 45]. This study indicated ethyl acetate: formic acid: acetic acid: water (13: 1: 1: 2.5) as mobile phase. Visualization was performed by daylight, UV 254 nm, UV 366 nm and dipped with ferric chloride (2% w/v) in ethanol. The spot of quercitrin of *D. pantendra* leaves on TLC plate showed yellow brown as the same previous study in Indonesia [5]. The R_f values was 0.735 which is similar the R_f of quercitrin of isolation from *D. pantendra* leaves in previous study [5].

The physico-chemical parameters are important for identity, purity and quality of crude drug. The procedures normally adopted to get the qualitative data about the purity and standardization of a crude drug consist the determination of different parameters [1] The water content indicated only moisture of plant material. A residue of water in medicinal plants may encourage microorganism growth, the presence of insects and fungi. Addition the water and volatile oil in herbal plant were presented by the determination of loss on drying. The ash of plant material is composed of its non-volatile inorganic components. The material which has a high ash value has contamination, adulteration, or carelessness in the material preparation from several locals market. The total ash indicates carbonates, phosphate, silicates and silica from both a physiology of plant tissue and non- physiology, which is the residue of the adhering material to the plant surface such as soil and sand. The acid-insoluble ash is obtained from boiling the total ash with about 2N hydrochloric acid and ignite residue in soluble matter. This assessment indicates the amount of silica, especially sand siliceous earth [1, 3].

The determination of ethanol and water extractive values is performed as a mean of assessing the crude drug that is not any solvent suitable more than both solvents [1]. These methods evaluates the amount of active complements and various phyto-consituents in a given amount of plant material when extracted with solvents [1, 3].These determinations were presented the upper limit values for unwanted qualifications of *D. pantendra* leaves such as water contents, loss on drying, total ash and acid insoluble ash, but the determination of ethanol and water soluble extractive values were presented lower limit values (Table 5).

For the determination of quercitrin contents, TLC densitometry and TLC image analysis by imageJ software were suitable methods for quantitative analysis.

Validation method of TLC densitometry and TLC image analysis by imageJ software of quercitrin of *D. pantendra* leaves demonstrated the coefficient (r^2) 0.99 in range 0.25-1.0 mg/ml which is good linearity. The accuracy of TLC densitometry were between 96.41-100.97 % recoveries and TLC image analysis by imageJ software were between 81.71-103.09 % recoveries. The accuracy of these methods was shown in appropriate result (80-120%) [41]. Additionally, robustness of the TLC densitometry and TLC image analysis by imageJ software were demonstrated by introducing small changes in the mobile phase complements [46].

Limit of detection and Limit of quantitation of both methods were presented in similar values. The LOD and LOQ of TLC densitometry were 0.16 and 0.49 mg/ml respectively. The LOD and LOQ of TLC analysis by imageJ were 0.16 and 0.50 mg/ml respectively.

For the amount of quercitrin in *D. pentandra* leaves from 13 different locations in Thailand, the result by TLC densitometry method was 3.71±1.33 (1.20 - 12.8) g/100g of dried material. The result by TLC analysis using imageJ was 3.89±0.97 (1.22-13.21) g/100g of dried material. The results of both methods were shown in Table 13.

The comparison between of TLC densitometric and TLC image analysis of quercitrin content in *D. pentandra* leaves was performed by pair t-test as well as correlation analyses. The quercitrin content by TLC densitometry and TLC analysis using imageJ were 3.71±1.33 g/100g and 3.89±0.97 g/100g respectively. The correlation coefficients (r^2, N=13) were 0.994. The comparison was found that the quercitrin by two methods were not significantly different ($P > 0.05$).

The time of collection refers to the season and exact time for collection of medicinal plant with certain growing years. Different times or season may be give to varied quality and chemical constituent of plant material [43].

TLC densitometry is a standard technique with usefulness, convenience, cost effectiveness for simultaneous screening and quantitative determination [47]. TLC image analysis by ImageJ free software is demonstrated to be an alternative method which is easy to use, inexpensive and also has promising analytical potential.

Although it had been previously reported for much more error by manually spot fixing [48]. TLC image analysis can be used to quantify chemical markers in many medicinal plants [49].

Quercitrin provides many health promoting benefits, such as lipid peroxidation prevention, improvement of cardiovascular health, eye diseases treatment, anti-inflammatory, allergic disorders, hypertension, arthritis and reducing risk for cancers [6, 42]. The phamacognostic specification and quercitrin content of *D. pentandra* leaves established in this study can be used as guide marker for quality control in standardization of *D. pentandra* leaves in Thailand.

REFERENCES

[1] Mukherjee, P.K. Quality control herbal drugs an approach to evaluation of botanicals. New delhi: Galaxy printer; 2007.

[2] Mahady, G.B. Are medicinal plants a potential alternative for conventional antibiotics in animal husbandry?. Thai Journal of Phytopharmacy 9, 1 (2002): 50-62.

[3] World Health Organization. WHO Guidelines, Quality control methods for medicinal plant materials. Geneva: World Health Organization, 2011.

[4] Wiart, C. Medicinal plants of the asia-pacific drug for the future. Toh Tuck Link: World Scientific Publishing, 2006.

[5] Artanti, N., Arifa, Y.M., and Hanafi, M. Isolation and Identification of Active Antioxidant Compound from Star Fruit (*Averrhoa carambola*) Mistletoe *Dendrophthoe pentandra* (L.) Miq.) Ethanol Extract. Journal Applied Sciences 6, 8 (2006): 1659-1663.

[6] Artanti, N., Firmansyah. T., and Darmawan, A. Bioactivities evaluation of Indonesian mistletoes *Dendrophthoe pentandra* (L.) Miq. leaves extracts. Journal of Applied Pharmaceutical Science 2, 1 (2012): 24-27.

[7] Tilney, P., and Lubke, R.A. chemotaxonomic study of twelve species of the family Loranthaceae. Journal south African Botany 40 (1974): 315-322.

[8] Inta, A., Shengji, P., Balslev, P., Wangpakapattanawong, P., and Trisonthi, C.A. Comparative study on medicinal plants used in Akha's traditional medicine in China and Thailand, cultural coherence or ecological divergence. Journal of Ethnopharmacology 116 (2008): 508–517.

[9] วุฒิ ธรรมเวช. กาฝากมะม่วง. สารานุกรมสมุนไพร. กรุงเทพฯ: โอเอสพริ้นติ้งเฮ้าส์, 2540.

[10] Sukpondma, Y., Rukachaisirikul, V., and Jasakul, C. Chemical constituents from leaves of *Dendrophthoe pentandra* (L.) Miq: 26th congress on science and technology of Thailand. King Mongkut's University of Technology Thonburi 2000 Oct. Report No.: 43001655.

[11] Laorpaksa, A., *et al.* A primilinary study of antmicrobial activity of Thai medicinal plants against pathogen of gastro-intestinal tract. Jounal Infectious Disease Antimicrobial Agents 8, 4 (1991): 199-208.

[12] Mnica, C., *et al*. In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-jB pathway. European journal of immunology 35 (2005): 584-592.

[13] Lakhanpal, P., and Rai, D.K. Quercetin: A Versatile Flavonoid. Journal of Medical Internet Research 2, 2 (2007): 22-37.

[14] Nickrent, D. Parasitic plants. [Online]. 2013. Avaliable from: http://www.botgard.ucla.edu/html/botanytextbooks/lifeforms/parasiticplants/fulltextonly.html [2013, August]

[15] Mony, R., Dibong, S.D., Ondoua, J.M., and BilongBilong, C.F. Study of Host-Parasite Relationship among Loranthaceae Flowering Shrubs-Myrmecophytic Fruit Trees-Ants in Logbessou District, Cameroon. Annual Review and Research in Biology 1, 3 (2011): 68-78.

[16] Sodde, V., Dashora, N., Prabhu, K., Jaykumar, B., and Lobo, R. Histological and physico-chemical studies of *Macrosolen parasiticus* (L.) Danser Stem - A common parasitic medicinal plant. Der Pharmacia Sinica 2, 1 (2011): 217-221.

[17] Rafael, A. R. and Carvalho, L.N., Del-Claro K. Host specificity of a Brazilian mistletoe, *Struthanthus aff. polyanthus* (Loranthaceae), in cerrado tropical savanna. Flora 201 (2006): 127–134.

[18] Harvard University Herbaria. Flora of China Vol. 5. [Online]. Available from:http://www.efloras.org/florataxon.aspxflora_id=2&taxon_id=200006524 [2013, June]

[19] Giesen, W., *et al*. Mangrove guidebook for Southeast Asia. Bangkok: RAP Publication, 2006.

[20] Chuakul, W., and Sappakun, S. Medicinal plants used in Kungkrabaen Royal Development Study Center, Chanthaburi province.Herbal. Thai Journal of Phytopharmacy 13, 1 (2006): 27-42.

[21] Chuakul, W. Indigenous Medicinal Plants Used as Paralysis Remedy. Thai Pharmaceutical and Health Science Journal 5 (2010): 193-201.

[22] Katrin, S., Soemardji, A.A., Gana, S.A. and Soediro, I.D. The acute toxicity of isolates from n-hexane and ethanolic fraction of *Dendrophthoe pentandra* (L.) Miq. which have immunostimulatory activity. Majalah Farmasi Indonesia 6, 4 (2005): 227-231.

[23] คิดคม เสลสานนท์. ฤทธิ์ลดความดันของกาฝากมะม่วง *Dendrophthoe pentandra* (L.) Miq. ในหนูขาวความดันเลือดสูง. วิทยานิพนธ์ปริญญามหาบัณฑิต. คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น, 2541.

[24] Mustika, A. and Munir, R.S. Effect of Loranthaceae *Dendropthoe* species infusion serum transaminase level and hepatic necrosis in rats. Media Folia Medica Indonesian 43, 1 (2005): 227-231.

[25] Bose, S., Maji, S. and Chakraborty, P. Quercitrin from *Ixora coccinea* Leaves and its Anti-oxidant Activity. Journal of PharmaSciTech 2, 2 (2013): 72-74.

[26] Wagner, C. Quercitrin, a glycoside form of quercetin, prevents lipid peroxidation in vitro. Brain Research 1107, (2006): 192-198.

[27] Chemistry Laboratory Techniques. Thin Layer Chromatography (TLC) Guide [Online]. Available from: http:// http://ocw.mit.edu/courses/chemistry/5-301-chemistry-laboratory-techniques-january-iap-2012/labs/MIT5_301IAP12_TLC_Handout.pdf. [2013, July]

[28] Gocan, S. Stationary Phases for Thin-Layer Chromatography. Journal of Chromatographic Science 40, (2002): 1-12.

[29] Thin Layer Chromatography. Basic principles of TLC. [Online]. Available from: http://www.watrex.cz/watrex/cz/dwn_ds. analysis. Journal of Chemical Education 84, 5 (2007): 842-847.

[30] Komsta, L. Chemometrics in Fingerprinting by Means of Thin Layer Chromatography. Chromatography Research International (2012).

[31] Rafi, M., Rohaeti, E., Miftahudin, A. and Darusman, L.K. Differentiation of *Curcuma longa*, *Curcuma xanthorrhiza* and *Zingiber cassumunar* by Thin Layer Chromatography fingerprint analysis. Indonesia. Journal Chemistry 11, 1 (2011): 71-74.

[32] Dittmer, J.C., and Lester, R.L. A Simple, Specific Spray for the Detection of Phospholipids on Thin-Layer Chromatography. Journal of Lipid Research 15 (1964): 126-127.

[33] Pothitirat, W. and Gritsanapan, W. Quantitative analysis of total mangostin in Garcinia mangostana fruit rind. Journal Health Research 22, 4(2008): 161-166.

[34] Jork, H. Thin-layer chromatography: reagent and detection methods. Weinheim (Federal Republic of Geramany). New York, USA: VCH, 1990.

[35] Hess, A.V.I. Digitally enhanced thin-layer chromatography: An inexpensive, new techniqe for qualitative and quantitative analysis. Journal of Chemical Education 84, 5 (2007): 842.

[36] CAMAG. TLC Scanner3 [Online]. Available from: *www*.kobis.si/material/ Scanner3 brochure.pdf [2013, June]

[37] CMAG. Thin layer chromatography scanner3 [Online]. Available from: http://www.pharmaceutical–business–review.com/suppliers/camag thin layer chromatography/products/tlc scanner 3 [2013, June]

[38] Burger, W., and Burge, M.J. ImaeJ Digital Image Processing: An Algorithmic Introduction Using Java [Online]. 2008. Available from: http:// rsbweb. nih gov/ ij/ index. html [2013, June]

[39] Schneider, C A., Rasband, W. S., and Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9, 7 (2012): 671-675.

[40] International Conference on Harmonisation. ICH Guidelines Validation of analytical procedures: Text and Methodology. Geneva: ICH, 2005.

[41] Srivastava, M. High-Performance ThinLayer Chromatography (HPTLC). Germany, deblik, 2011.

[42] Thitikornpong, W., Phadungchareon. T., and Sukrong S. Pharmacognostic evaluations of Lagerstroemia speciosa leaves Journal of Medicinal Plants Research 5, 8 (2011): 1330-1337.

[43] Willow, J.H.L. Traditional Herbal Medicine research method identification, analysis, bioassay, and pharmaceutical and clinical studies. Singapore. John Willey & Sons, Inc., Publication, 2011.

[44] Shavvon, R.S., Mehrvarz, S.S., and Golmohamadi, N. Evidence from micromorphology and gross morphology of the genus Loranthus (Loranthaceae) in Iran. Turkish Journal of Botany 36 (2012): 655-666.

[45] Pothitirat, W., Chomnawang, M.T., and Gritsanapan, W. Anti-acne inducing bacteria activity and -mangostin content of *Garcinia mangostana* fruit rind extracts from different provenience. Songklanakarin Journal Technology 31, 1 (2009): 41-47.

[46] Phattanawasin, P., Sotanaphun, U., Sukwattanasinit, T., Akkarawaranthorn, J., and Kitchaiya, S. Quantitative determination of sibutramine in adulterated herbal slimming formulations by TLC-image analysis method. Forensic Science International 219 (2012): 96-100.

[47] Starek, M., and browska, M. D. Development and Validation of a TLC Densitometry Method for Quantitative Analysis of Nefopam Hydrochloride Beside. Journal of Analytical Chemistry 67 (2012): 733–739.

[48] Anil, G. M., Narayan. P. F., and Shirish, S. P. TLC analyzer: an inexpensive tool for analysis of astronomical images. Elixir Apply Chemistry 39 (2011): 5087-5089.

[49] Yukongphan, P., Palanuvej, C., and Ruangrungsi, N. pharmacognostic specification of *Ardisia elliptica* fruit their embilin contents by TLC Image analysis. Congress on Science and Technology of Thailand. Chiang Mai, pp 1-6, 2012.

APPENDIX

Table 14 . Determination of water content (% by weight) of *Dendrophthoe pentandra* leaves from 13 different locations

Source	Location name	Crude drug Sample extracts no.	Amount (% by weight)	Mean	SD
1	**Phuket**	1	14.50		
		2	15.00		
		3	15.00	14.83	0.29
2	**Chai Nat**	1	12.99		
		2	12.99		
		3	12.97	12.98	0.01
3	**Phetchaburi**	1	9.00		
		2	9.00		
		3	8.67	8.89	0.19
4	**Bangkok 1**	1	10.00		
		2	10.67		
		3	9.33	10.00	0.67
5	**Nakhon Ratchasima**	1	8.67		
		2	8.00		
		3	8.67	8.44	0.38
6	**Bangkok 2**	1	8.67		
		2	12.0		
		3	12.00	10.89	1.92
7	**Bangkok 3**	1	10.00		
		2	9.33		
		3	10.00	9.78	0.38
8	**Songkhla 1**	1	9.33		
		2	10.67		
		3	9.33	9.78	0.77
9	**Bangkok 4**	1	8.00		
		2	7.50		
		3	8.00	7.83	0.29
10	**Nonthaburi 1**	1	9.00		
		2	8.00		
		3	9.00	8.67	0.58
11	**Nonthaburi 2**	1	7.50		
		2	8.00		
		3	7.00	7.5	0.50
12	**Songkhla 2**	1	10.67		
		2	10.00		
		3	10.00	10.22	0.38
13	**Songkhla 3**	1	10.00		
		2	10.67		
		3	9.33	10.00	0.67
	Grand average			9.99	0.70

Table 15 . Determination of loss on drying (% by weight) of *Dendrophthoe pentandra* leaves from 13 different locations

Source	Location name	Crude drug Sample extracts no.	Amount (% by weight)	Mean	SD
1	**Phuket**	1	12.67		
		2	12.47		
		3	12.77	12.63	0.15
2	**Chai Nat**	1	10.04		
		2	10.07		
		3	10.01	10.04	0.03
3	**Phetchaburi**	1	8.67		
		2	8.50		
		3	8.69	8.62	0.10
4	**Bangkok 1**	1	8.49		
		2	8.38		
		3	8.51	8.40	0.07
5	**Nakhon Ratchasima**	1	9.17		
		2	9.16		
		3	9.18	9.17	0.01
6	**Bangkok 2**	1	9.80		
		2	9.59		
		3	9.74	9.71	0.11
7	**Bangkok 3**	1	12.28		
		2	11.18		
		3	12.36	11.94	0.66
8	**Songkhla 1**	1	8.29		
		2	8.49		
		3	8.41	8.40	0.11
9	**Bangkok 4**	1	5.82		
		2	5.90		
		3	6.08	5.93	0.14
10	**Nonthaburi 1**	1	6.45		
		2	6.51		
		3	6.50	6.49	0.03
11	**Nonthaburi 2**	1	7.31		
		2	7.09		
		3	7.28	7.23	0.12
12	**Songkhla 2**	1	12.58		
		2	10.44		
		3	13.58	12.20	1.60
13	**Songkhla 3**	1	7.30		
		2	7.62		
		3	9.74	8.22	1.32
	Grand average			9.16	0.61

Table 16 . Determination of total ash (% by weight) of *Dendrophthoe pentandra* leaves from 13 different locations

Source	Location name	Crude drug Sample extracts no.	Amount (% by weight)	Mean	SD
1	**Phuket**	1	6.69		
		2	6.70		
		3	6.60	6.66	0.06
2	**Chai Nat**	1	12.83		
		2	12.85		
		3	12.79	12.82	0.03
3	**Phetchaburi**	1	11.76		
		2	11.93		
		3	11.90	11.87	0.09
4	**Bangkok 1**	1	14.45		
		2	14.39		
		3	14.40	14.40	0.03
5	**Nakhon Ratchasima**	1	12.25		
		2	12.30		
		3	12.31	12.83	0.03
6	**Bangkok 2**	1	17.17		
		2	17.12		
		3	17.21	17.16	0.05
7	**Bangkok 3**	1	16.19		
		2	17.34		
		3	16.25	16.59	0.65
8	**Songkhla 1**	1	10.34		
		2	10.45		
		3	10.51	10.43	0.08
9	**Bangkok 4**	1	10.39		
		2	10.32		
		3	10.28	10.33	0.06
10	**Nonthaburi 1**	1	10.30		
		2	10.31		
		3	10.33	10.31	0.01
11	**Nonthaburi 2**	1	14.48		
		2	14.53		
		3	14.44	14.48	0.04
12	**Songkhla 2**	1	8.79		
		2	9.07		
		3	8.75	8.87	0.17
13	**Songkhla 3**	1	9.00		
		2	8.90		
		3	9.00	8.97	0.06
	Grand average			11.94	0.19

Table 17 . Determination of acid insoluble ash (% by weight) of *Dendrophthoe pentandra* leaves from 13 different locations

Source	Location name	Crude drug Sample extracts no.	Amount (% by weight)	Mean	SD
1	**Phuket**	1	0.76		
		2	0.84		
		3	0.83	0.81	0.04
2	**Chai Nat**	1	4.89		
		2	7.78		
		3	4.91	5.86	1.66
3	**Phetchaburi**	1	4.91		
		2	5.28		
		3	5.13	5.11	0.19
4	**Bangkok 1**	1	8.70		
		2	9.26		
		3	5.96	7.97	1.77
5	**Nakhon Ratchasima**	1	8.21		
		2	8.04		
		3	8.30	8.18	0.13
6	**Bangkok 2**	1	8.77		
		2	8.24		
		3	8.45	8.46	0.27
7	**Bangkok 3**	1	4.75		
		2	4.99		
		3	8.32	6.02	1.99
8	**Songkhla 1**	1	3.93		
		2	3.69		
		3	3.54	3.72	0.20
9	**Bangkok 4**	1	1.16		
		2	1.28		
		3	1.19	1.21	0.06
10	**Nonthaburi 1**	1	2.80		
		2	2.48		
		3	2.50	2.59	0.18
11	**Nonthaburi 2**	1	1.31		
		2	1.63		
		3	1.57	1.50	0.17
12	**Songkhla 2**	1	1.45		
		2	1.33		
		3	1.44	1.40	0.07
13	**Songkhla 3**	1	1.38		
		2	1.32		
		3	1.37	1.35	0.03
	Grand average			4.17	0.88

Table 18. Determination of ethanol-soluble extractive (% by weight) of *Dendrophthoe pentandra* leaves from 13 different locations

Source	Location name	Crude drug Sample extracts no.	Amount (% by weight)	Mean	SD
1	**Phuket**	1	11.38		
		2	11.61		
		3	11.74	11.58	0.17
2	**Chai Nat**	1	4.39		
		2	4.36		
		3	4.35	4.37	0.03
3	**Phetchaburi**	1	3.22		
		2	2.89		
		3	2.80	2.97	0.22
4	**Bangkok 1**	1	7.04		
		2	7.14		
		3	7.82	7.33	0.43
5	**Nakhon Ratchasima**	1	3.61		
		2	3.43		
		3	3.58	3.54	0.10
6	**Bangkok 2**	1	8.71		
		2	7.76		
		3	6.83	7.76	0.94
7	**Bangkok 3**	1	10.22		
		2	13.27		
		3	11.37	10.62	1.54
8	**Songkhla 1**	1	6.859		
		2	6.03		
		3	8.55	7.14	1.28
9	**Bangkok 4**	1	15.85		
		2	15.04		
		3	13.64	14.85	1.11
10	**Nonthaburi 1**	1	10.14		
		2	10.22		
		3	9.51	9.96	0.39
11	**Nonthaburi 2**	1	5.30		
		2	5.48		
		3	5.93	5.57	0.33
12	**Songkhla 2**	1	7.12		
		2	7.28		
		3	7.21	7.20	0.08
13	**Songkhla 3**	1	6.01		
		2	5.84		
		3	5.59	5.81	0.21
	Grand average			6.67	0.72

Table 19 . Determination of water-soluble extractive (% by weight) of *Dendrophthoe pentandra* leaves from 13 different locations

Source	Location name	Crude drug Sample extracts no.	Amount (% by weight)	Mean	SD
1	**Phuket**	1	14.75		
		2	17.21		
		3	15.05	15.67	1.34
2	**Chai Nat**	1	23.10		
		2	23.91		
		3	23.29	23.43	0.43
3	**Phetchaburi**	1	20.39		
		2	16.90		
		3	15.86	17.72	2.37
4	**Bangkok 1**	1	31.45		
		2	31.15		
		3	27.36	29.99	2.28
5	**Nakhon Ratchasima**	1	30.47		
		2	18.86		
		3	26.64	25.32	5.92
6	**Bangkok 2**	1	22.61		
		2	23.44		
		3	20.40	22.14	1.57
7	**Bangkok 3**	1	21.88		
		2	22.18		
		3	20.74	21.60	0.76
8	**Songkhla 1**	1	9.00		
		2	11.35		
		3	17.80	12.71	4.55
9	**Bangkok 4**	1	27.73		
		2	30.09		
		3	28.97	28.93	1.18
10	**Nonthaburi 1**	1	24.04		
		2	26.96		
		3	22.54	24.51	2.25
11	**Nonthaburi 2**	1	22.87		
		2	27.36		
		3	22.83	24.36	2.6
12	**Songkhla 2**	1	16.35		
		2	17.51		
		3	16.07	16.64	0.76
13	**Songkhla 3**	1	25.48		
		2	27.43		
		3	25.20	26.34	1.21
	Grand average			22.24	2.59

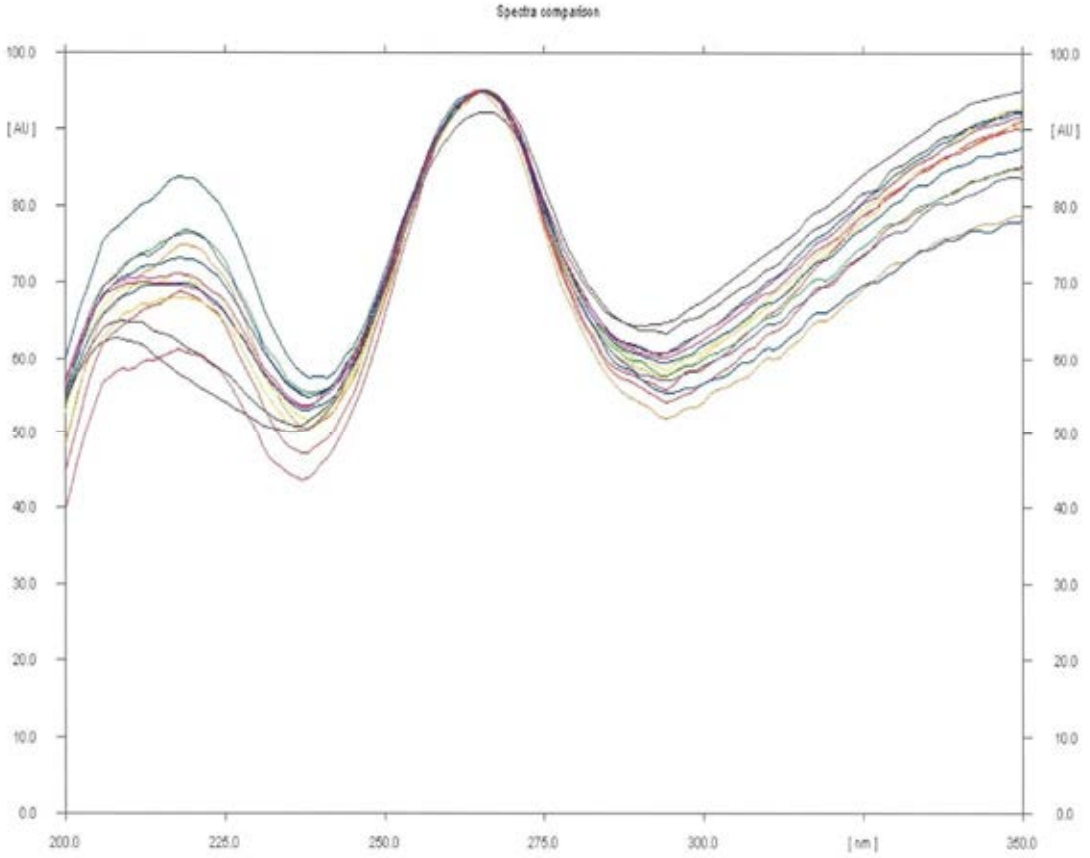


Figure 18. The maximum wavelength of standard quercitrin and all samples

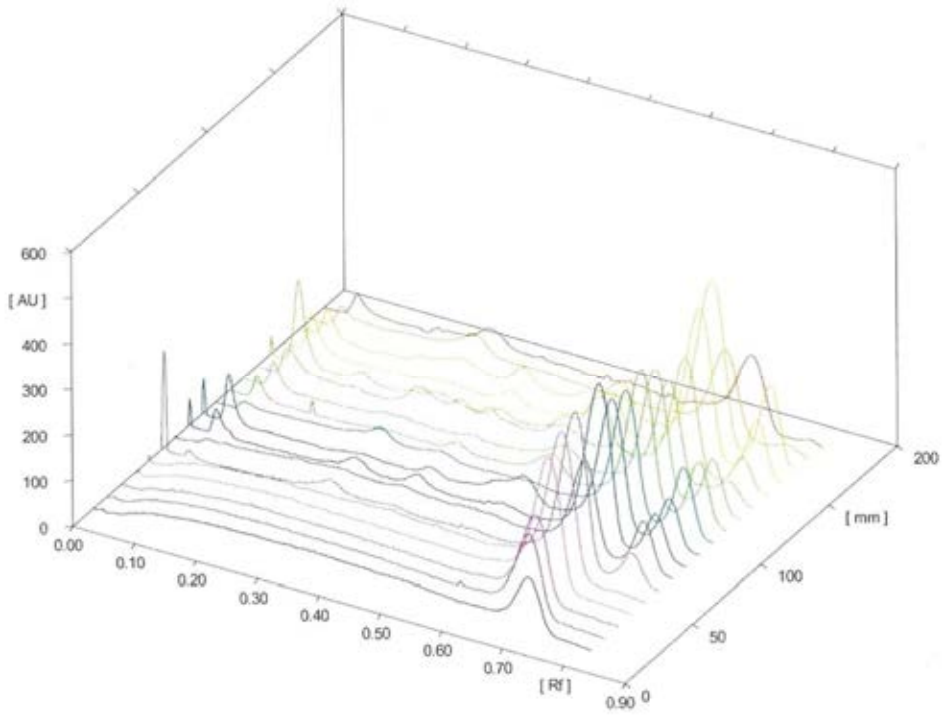


Figure 19. 3D TLC densitometric chromatogram of quercitrin standard and sample extracts (Plate 1)

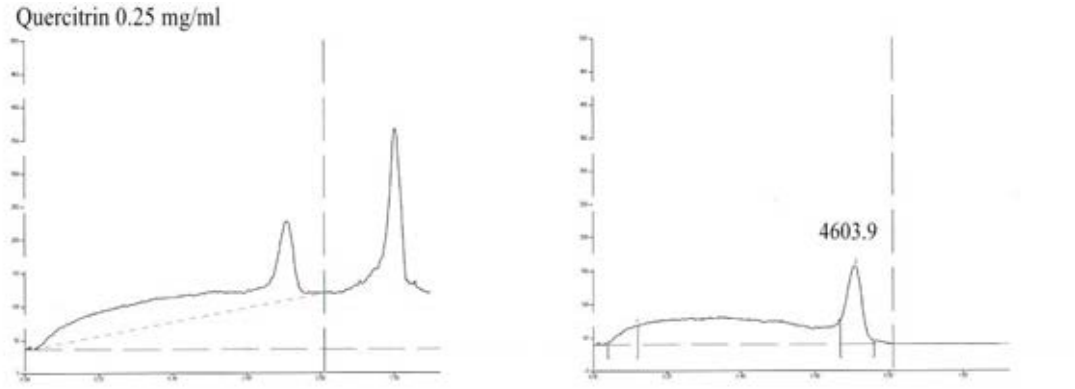


Figure 20. TLC densitometric chromatogram of standard quercitrin 0.25 mg/ml (Plate 1-quercitrin standard and sample extracts from 13 different locations)

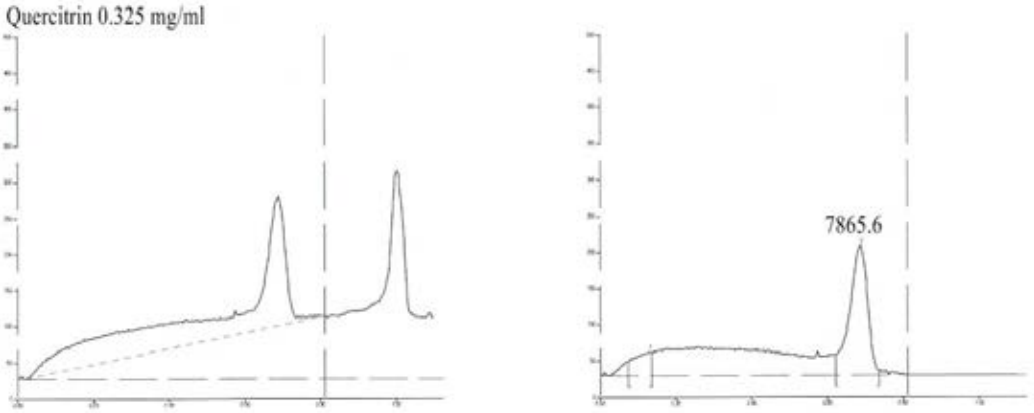


Figure 21. TLC densitometric chromatogram of standard quercitrin 0.325 mg/ml (Plate 1-quercitrin standard and sample extracts from 13 different locations)

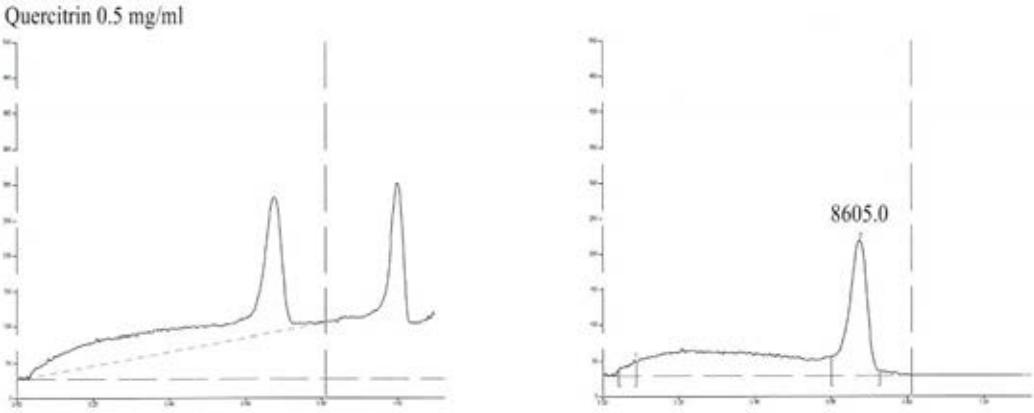


Figure 22. TLC densitometric chromatogram of standard quercitrin 0.50 mg/ml (Plate 1-quercitrin standard and sample extracts from 13 different locations)

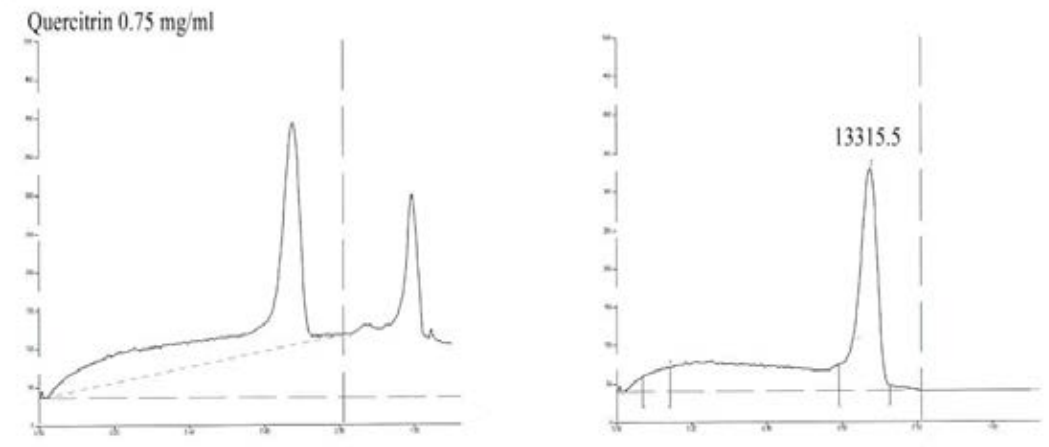


Figure 23. TLC densitometric chromatogram of standard quercitrin 0.75 mg/ml (Plate 1-quercitrin standard and sample extracts from 13 different locations)

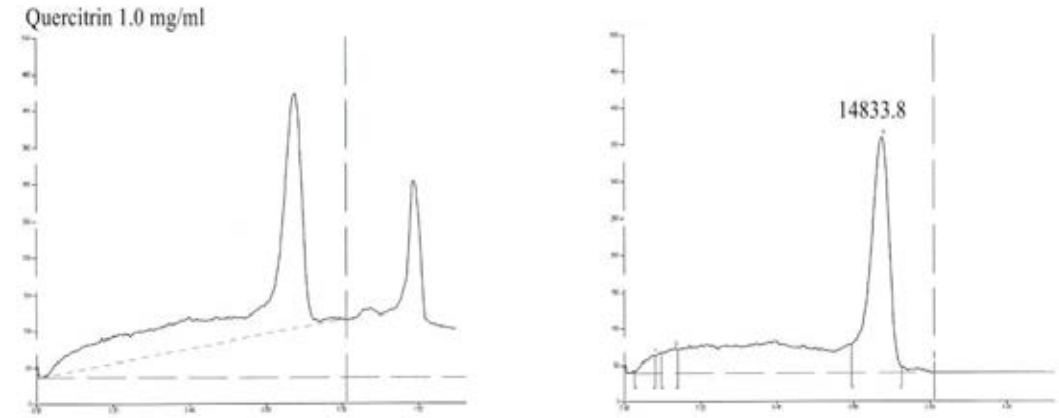


Figure 24. TLC densitometric chromatogram of standard quercitrin 1.00 mg/spot (Plate 1-quercitrin standard and sample extracts from 13 different locations)

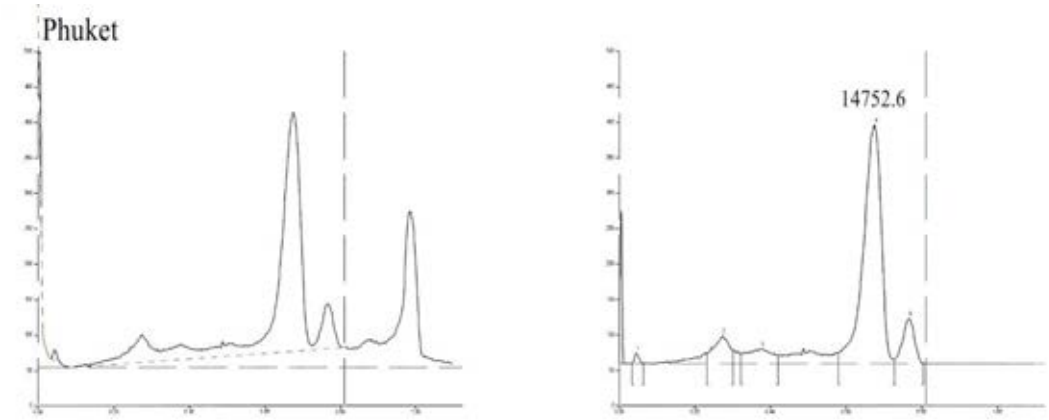


Figure 25. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Phuket (2 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

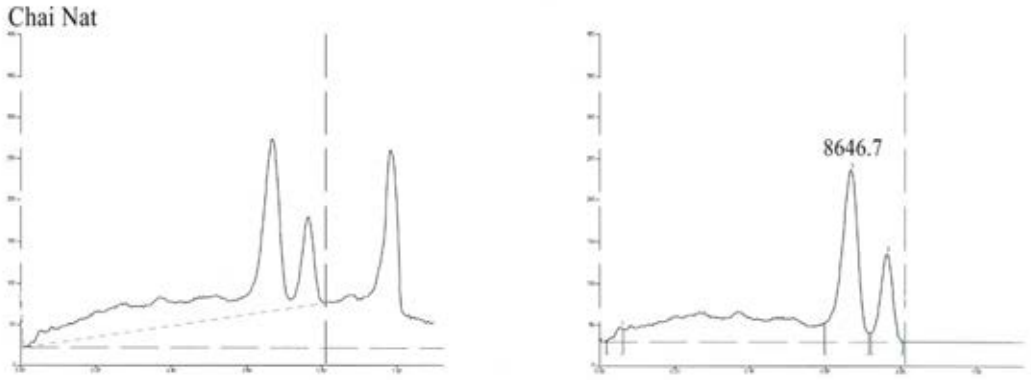


Figure 26. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Chai Nat (2 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

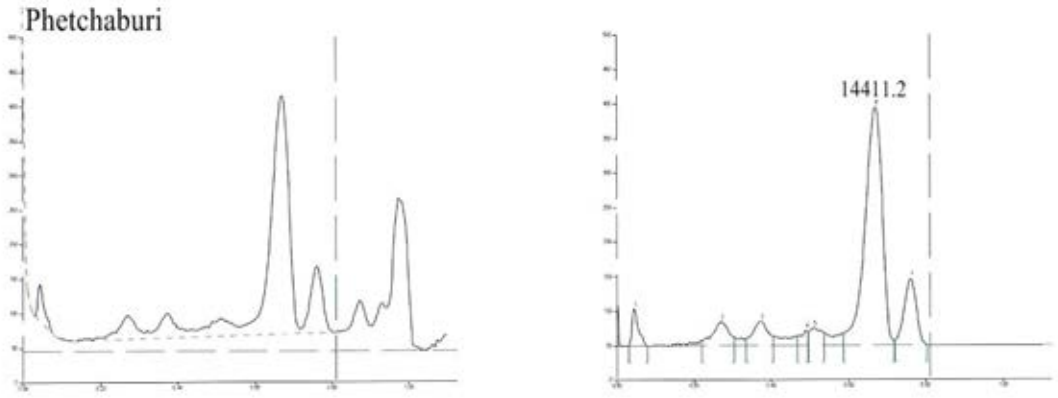


Figure 27. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Phetchaburi (4 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

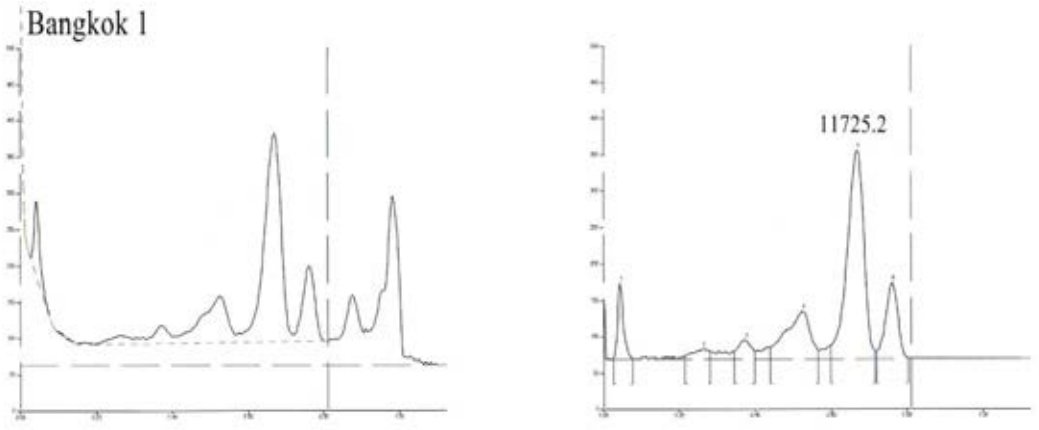


Figure 28. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 1 (5 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

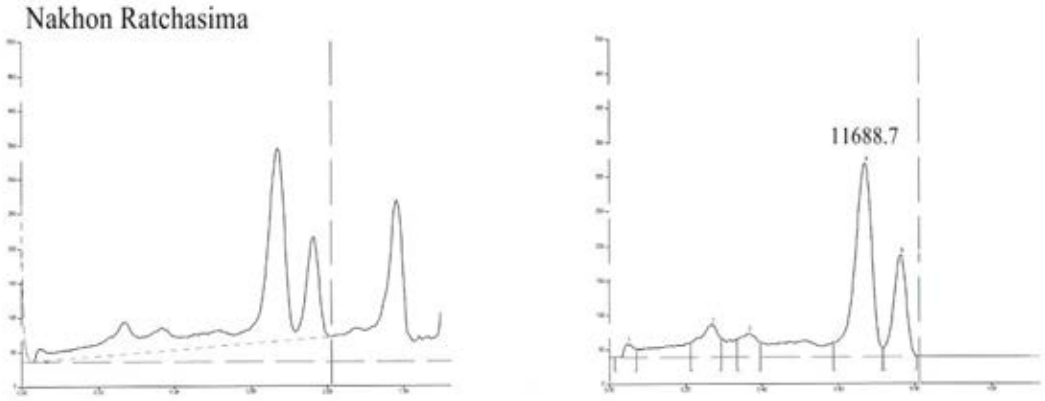


Figure 29. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Nakorn Ratchasima (3 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

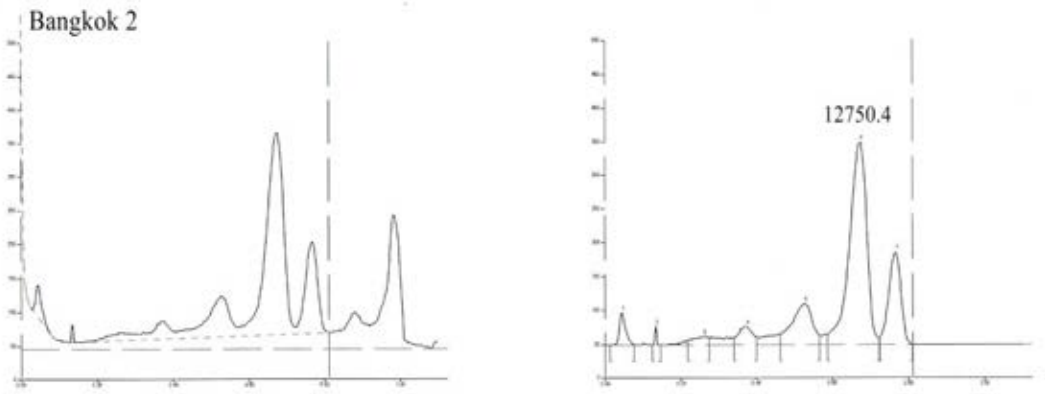


Figure 30. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 2 (3 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

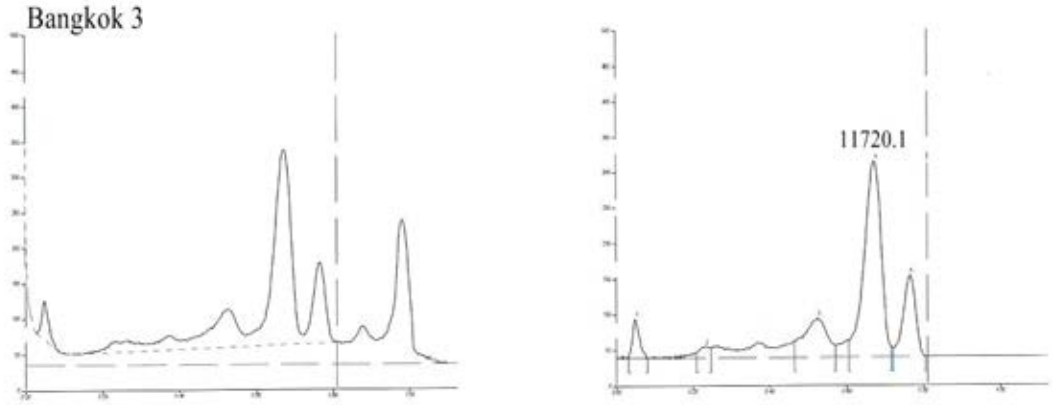


Figure31. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 3 (3 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

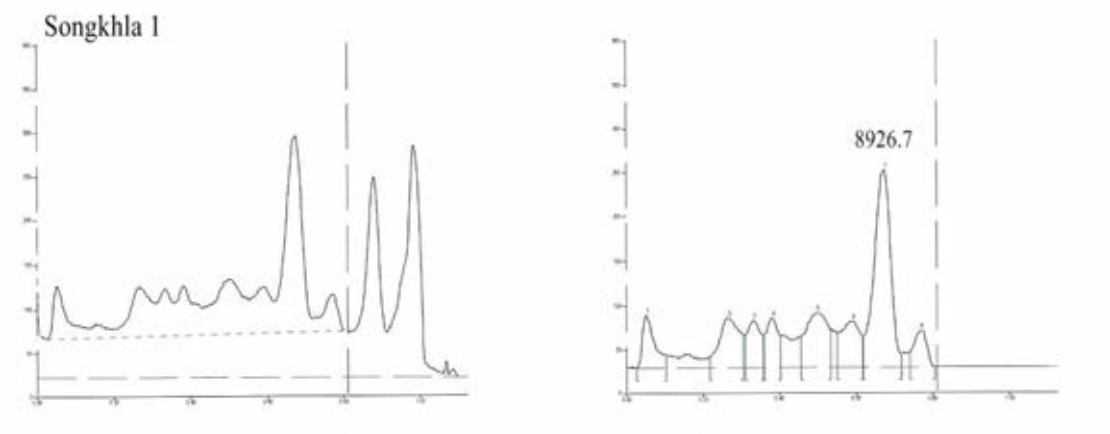


Figure 32. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Songkhla 1 (7 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

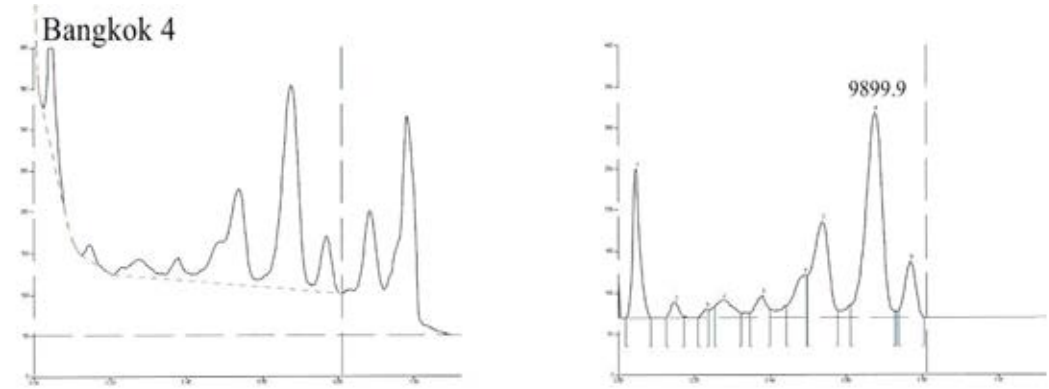


Figure 33. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 4 (9 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

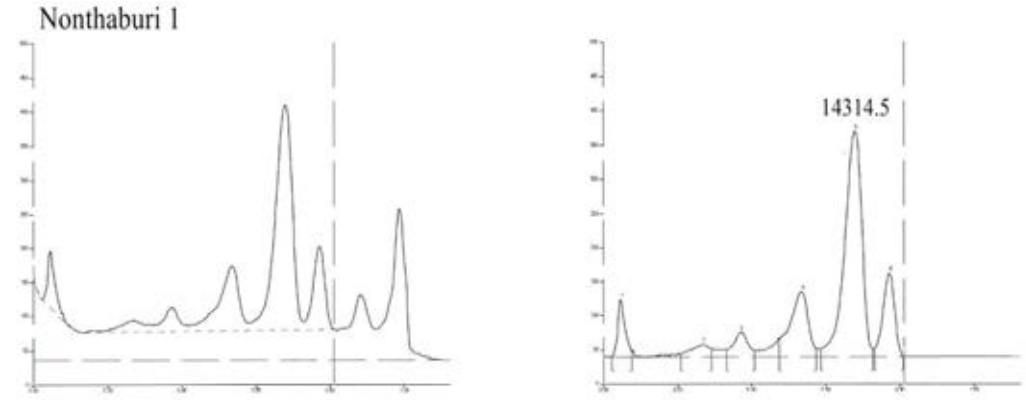


Figure 34. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Nonthaburi 1 (4 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

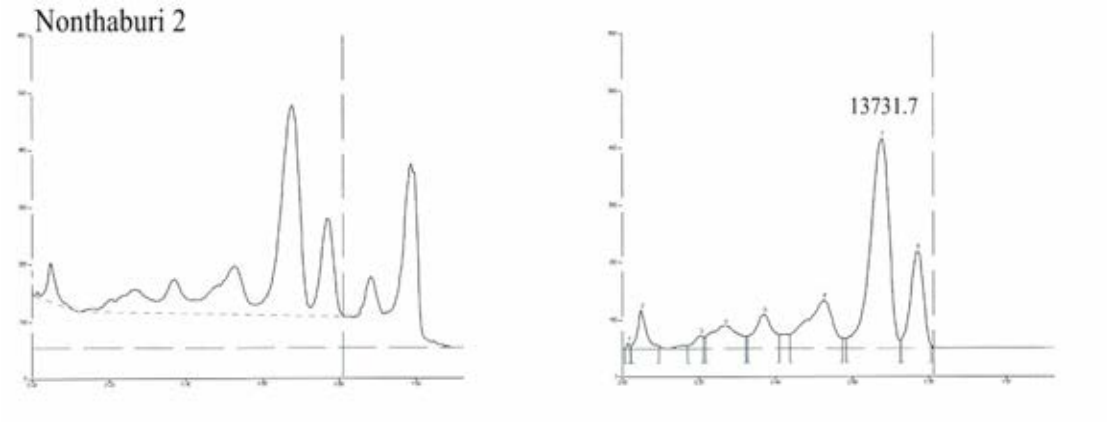


Figure 35. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Nonthaburi 2 (10 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

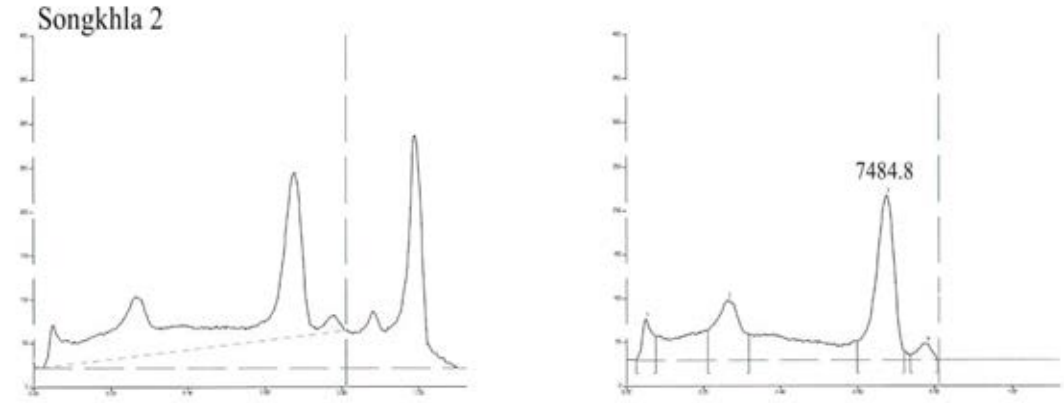


Figure 36. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Songkhla 2 (5.5 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

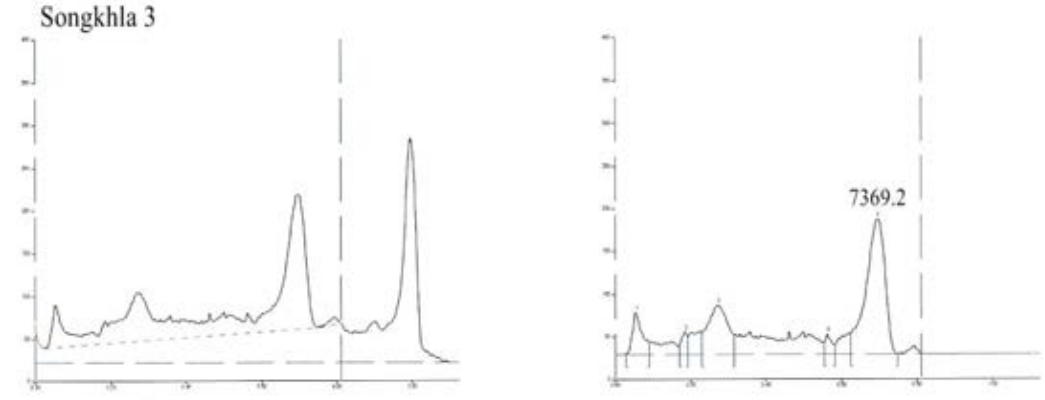


Figure 37. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Songkhla 3 (5 mg/ml) (Plate 1-quercitrin standard and sample extracts from 13 different locations)

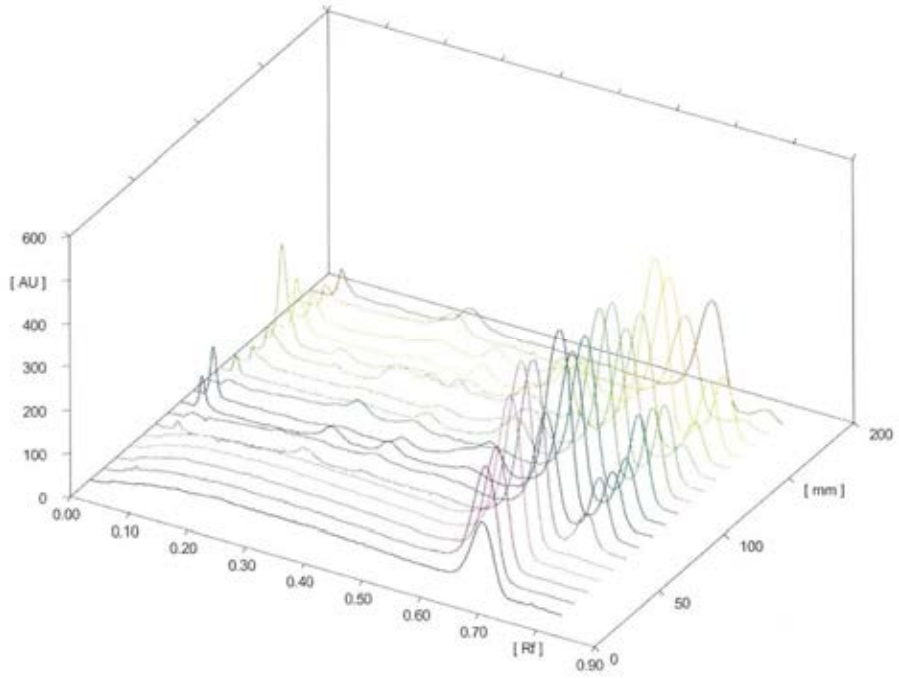


Figure 38. 3D TLC densitometric chromatogram of quercitrin standard and sample extracts (Plate 2)

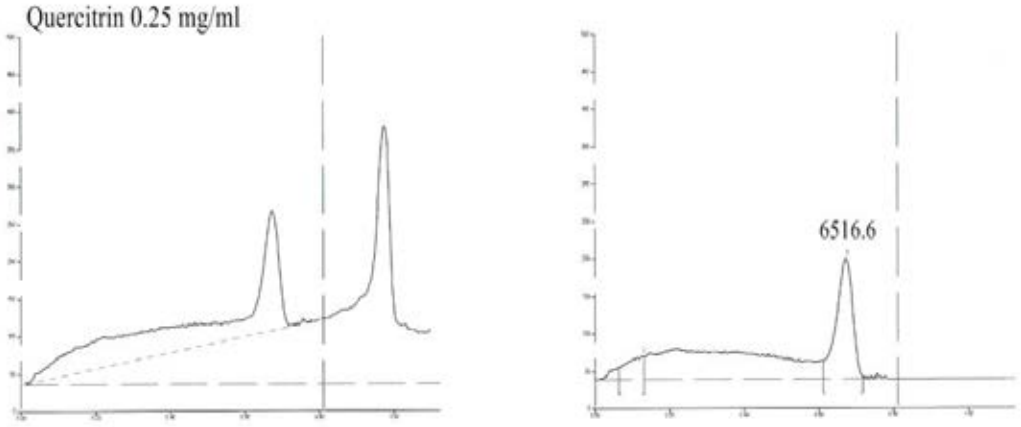


Figure 39. TLC densitometric chromatogram of standard quercitrin 0.25 mg/ml (Plate 2-quercitrin standard and sample extracts from 13 different locations)

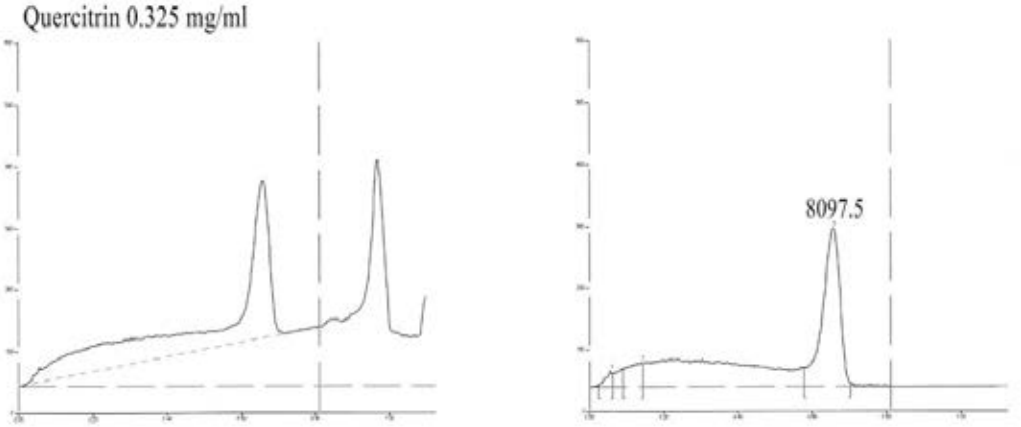


Figure 40. TLC densitometric chromatogram of standard quercitrin 0.325 mg/ml (Plate 2-quercitrin standard and sample extracts from 13 different locations)

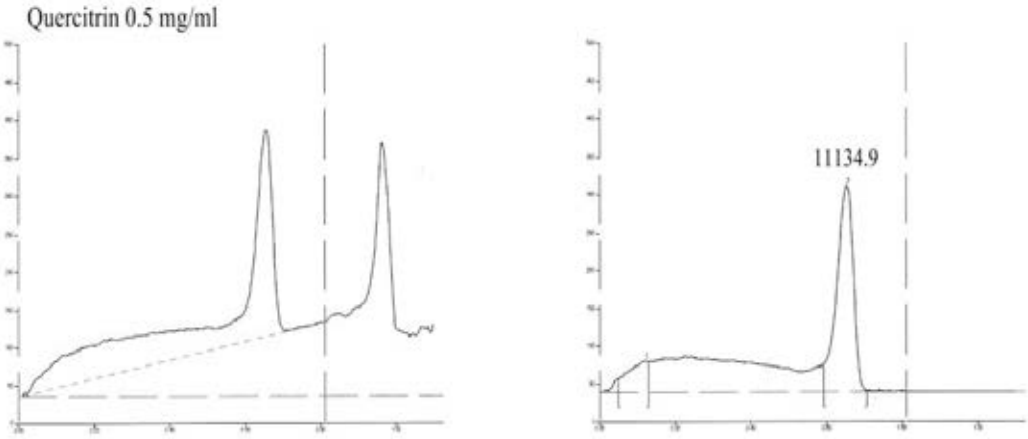


Figure 41. TLC densitometric chromatogram of standard quercitrin 0.50 mg/ml (Plate 2-quercitrin standard and sample extracts from 13 different locations)

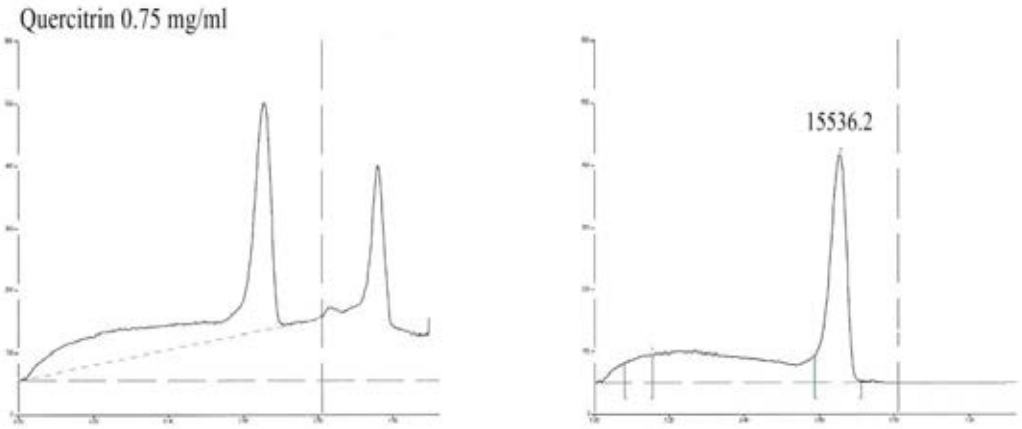


Figure 42. TLC densitometric chromatogram of standard quercitrin 0.75 mg/ml (Plate 2-quercitrin standard and sample extracts from 13 different locations)

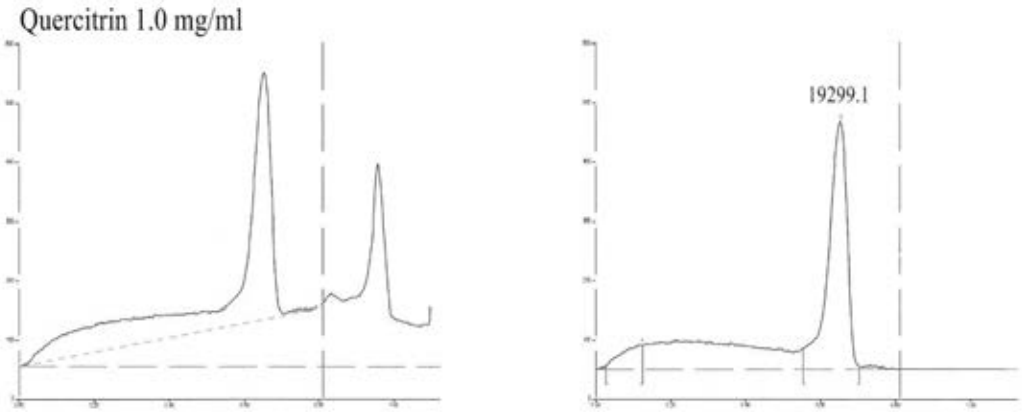


Figure 43. TLC densitometric chromatogram of standard quercitrin 1.0 mg/ml (Plate 2-quercitrin standard and sample extracts from 13 different locations)

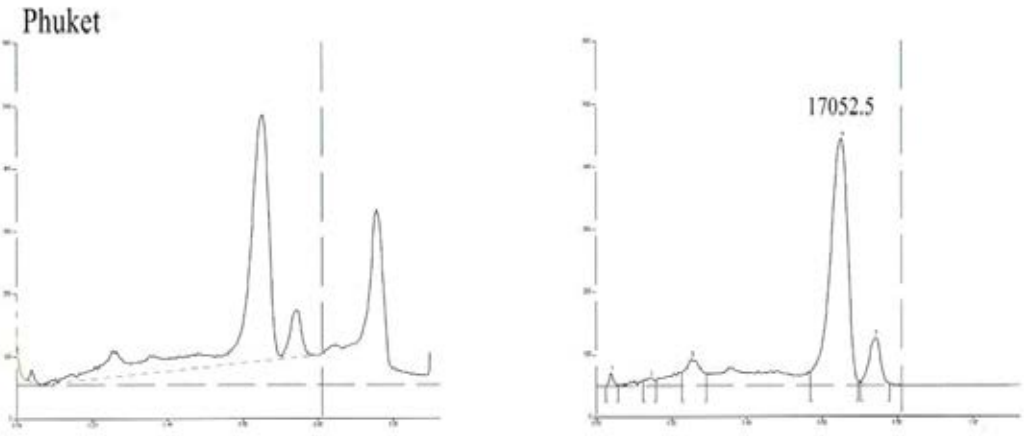


Figure 44. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Phuket (2 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

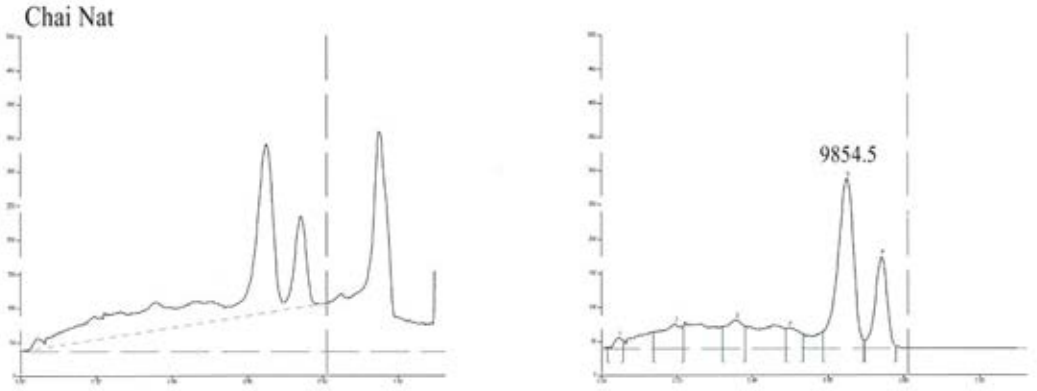


Figure 45. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Chai Nat (2 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

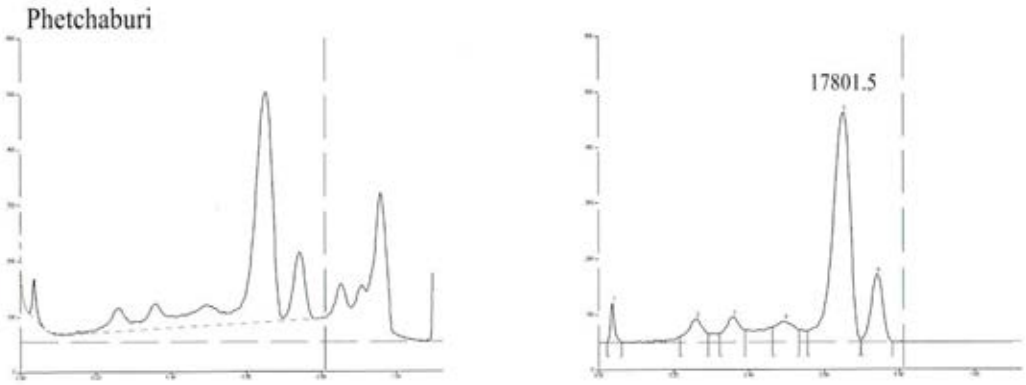


Figure 46. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Phetchaburi (4 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

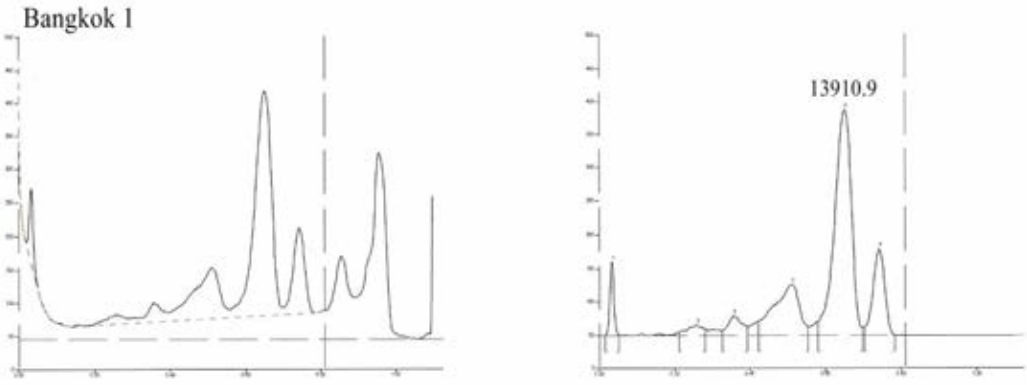


Figure 47. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 1 (5 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

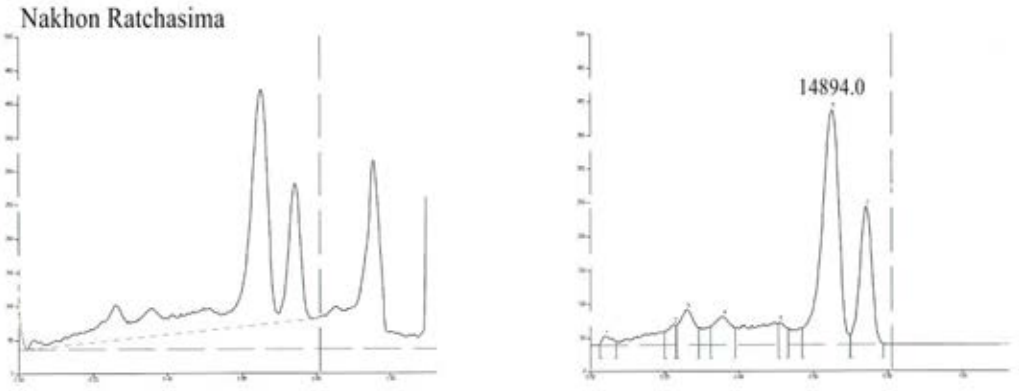


Figure 48. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Nakhon Ratchasima (3 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

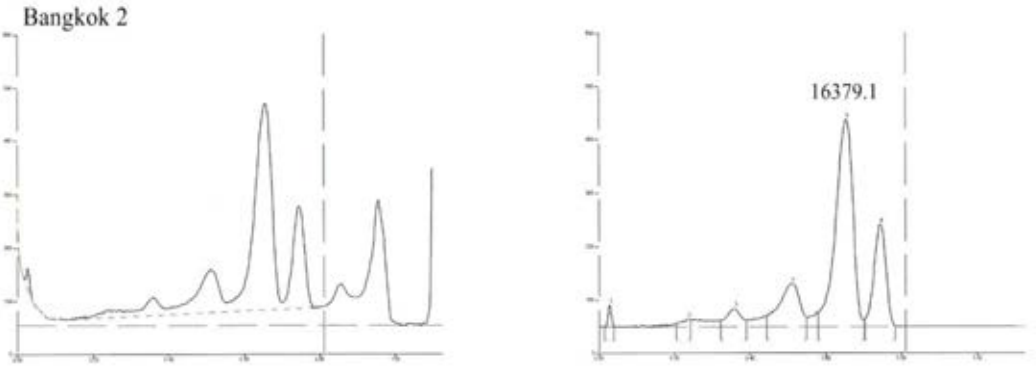


Figure 49. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 2 (3 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

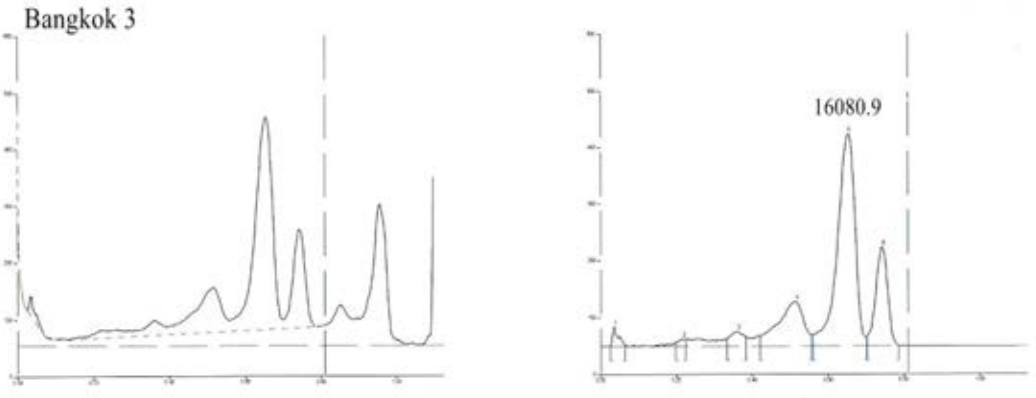


Figure 50. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 3 (3 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

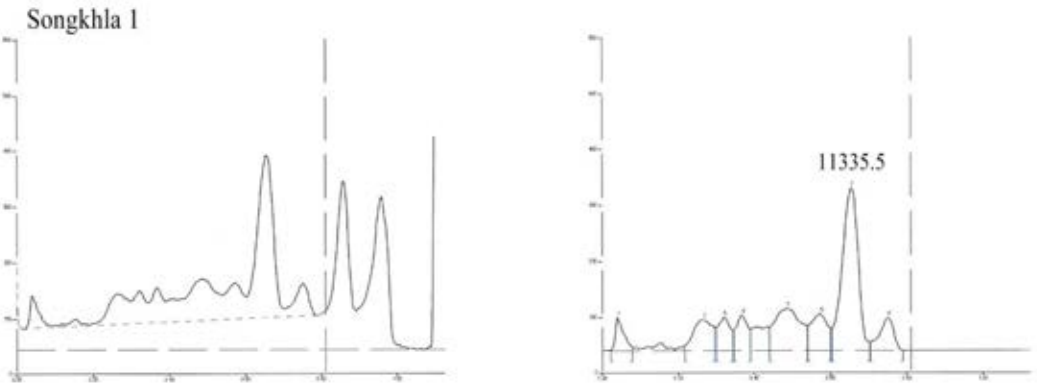


Figure 51. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Songkhla 1 (7 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

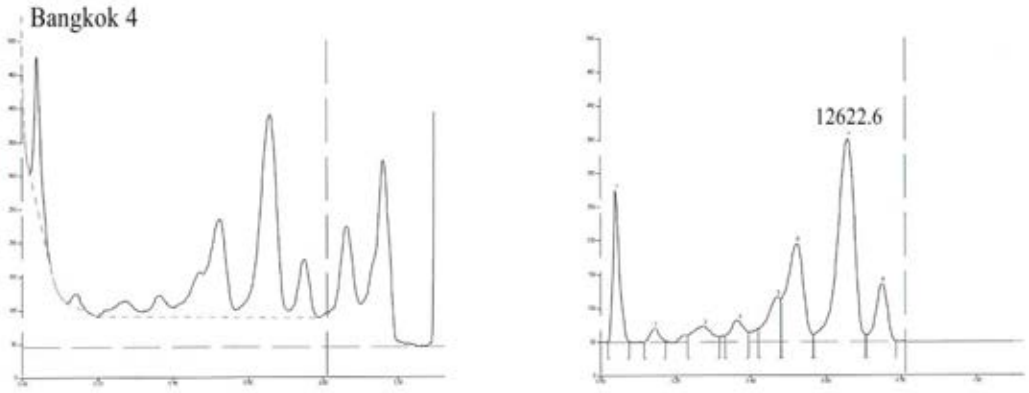


Figure 52. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 4 (9 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

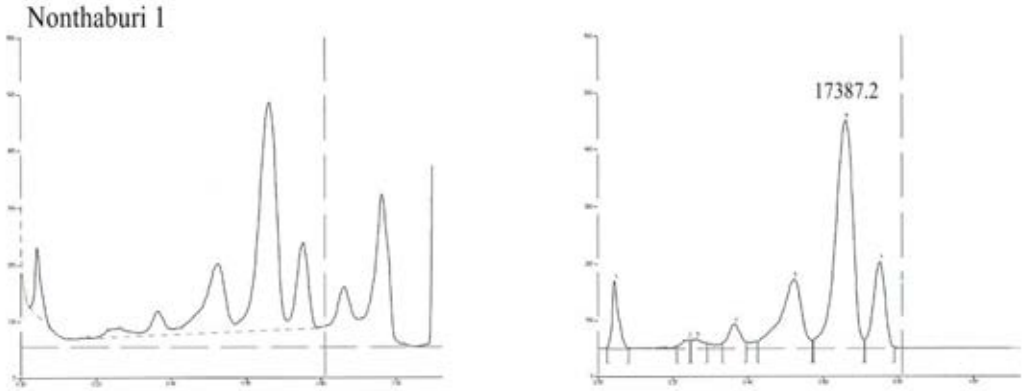


Figure 53. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Nonthaburi 1 (4 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

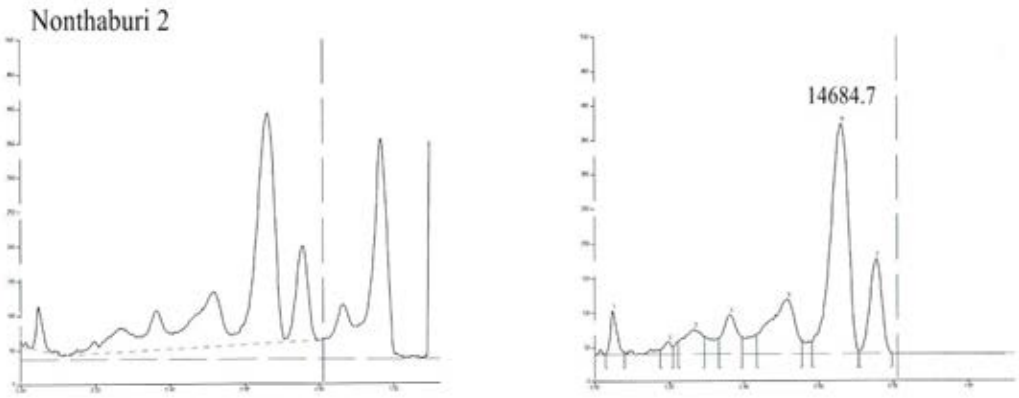


Figure 54. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Nonthaburi 2 (10 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

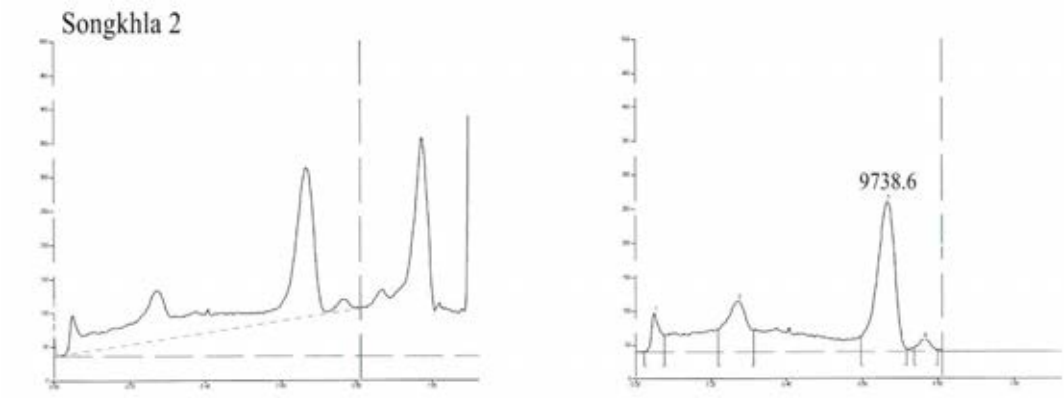


Figure 55. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Songkhla 2 (5.5 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

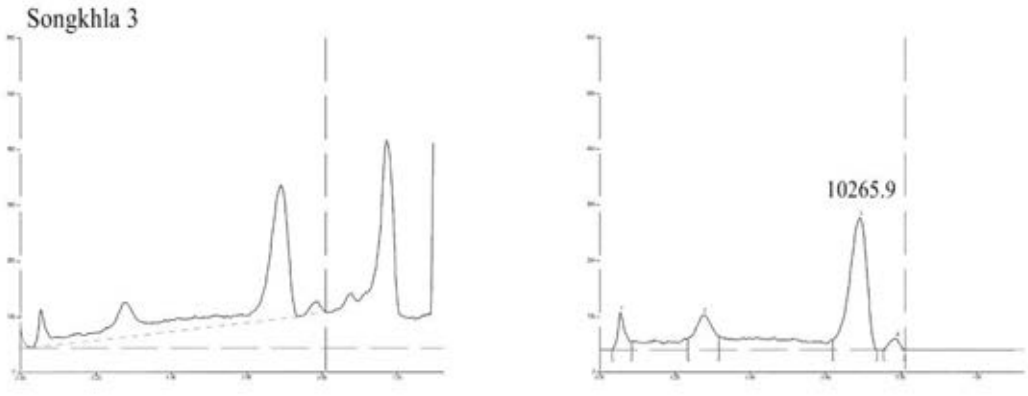


Figure 56. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Songkhla 3 (5 mg/ml) (Plate 2-quercitrin standard and sample extracts from 13 different locations)

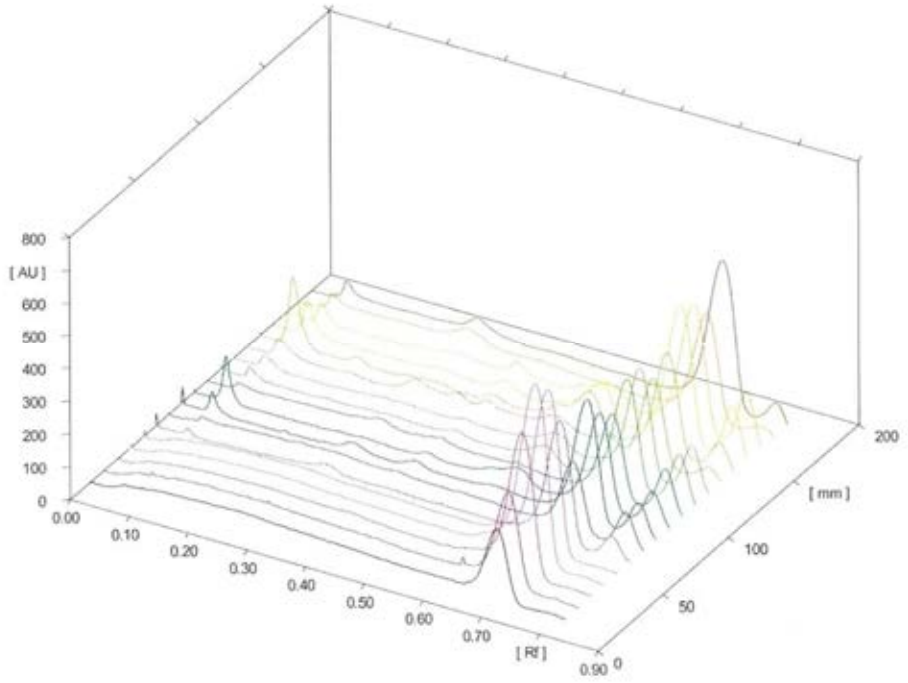


Figure 57. 3D TLC densitometric chromatogram of quercitrin standard and sample extracts (Plate 3)

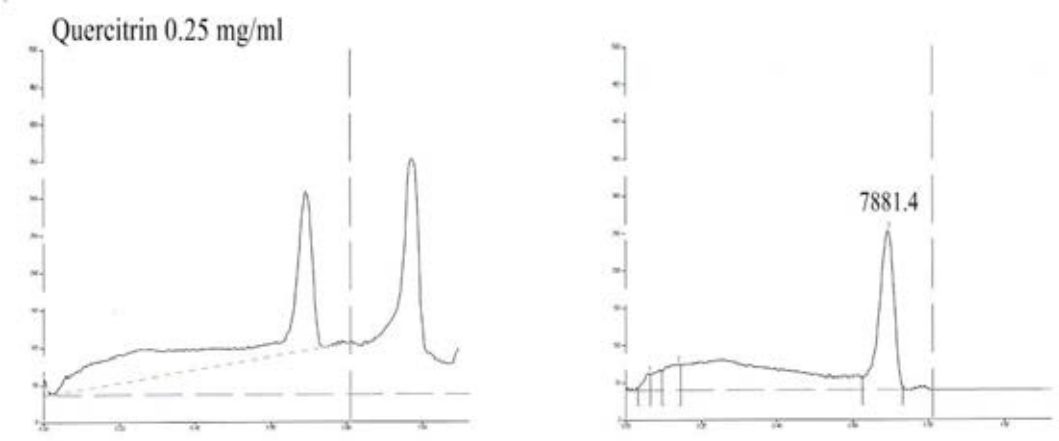


Figure 58. TLC densitometric chromatogram of standard quercitrin 0.25 mg/ml (Plate 3-quercitrin standard and sample extracts from 13 different locations)

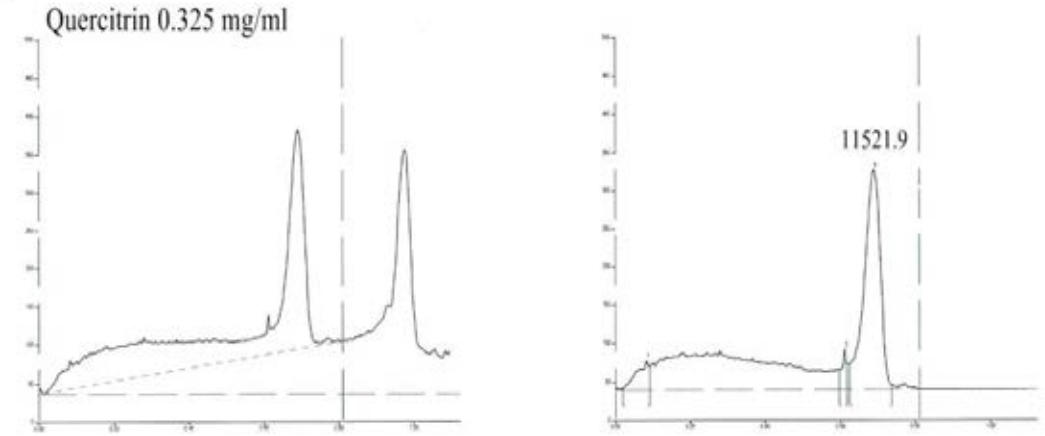


Figure 59. TLC densitometric chromatogram of standard quercitrin 0.325 mg/ml (Plate 3-quercitrin standard and sample extracts from 13 different locations)

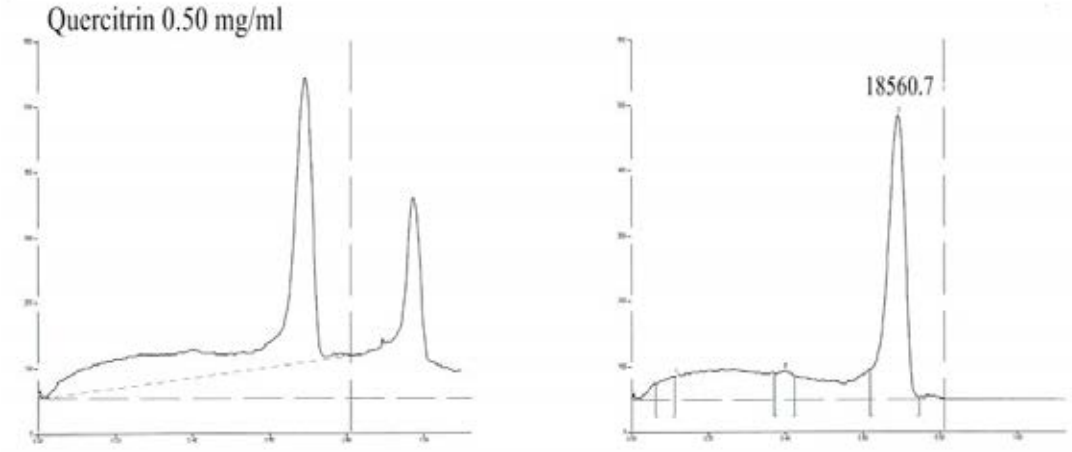


Figure 60. TLC densitometric chromatogram of standard quercitrin 0.50 mg/ml (Plate 3-quercitrin standard and sample extracts from 13 different locations)

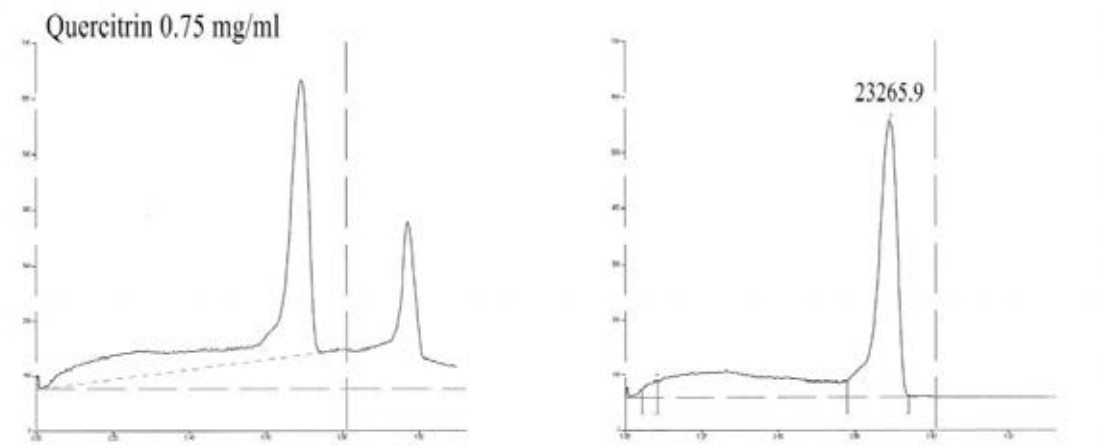


Figure 61. TLC densitometric chromatogram of standard quercitrin 0.75 mg/ml (Plate 3-quercitrin standard and sample extracts from 13 different locations)

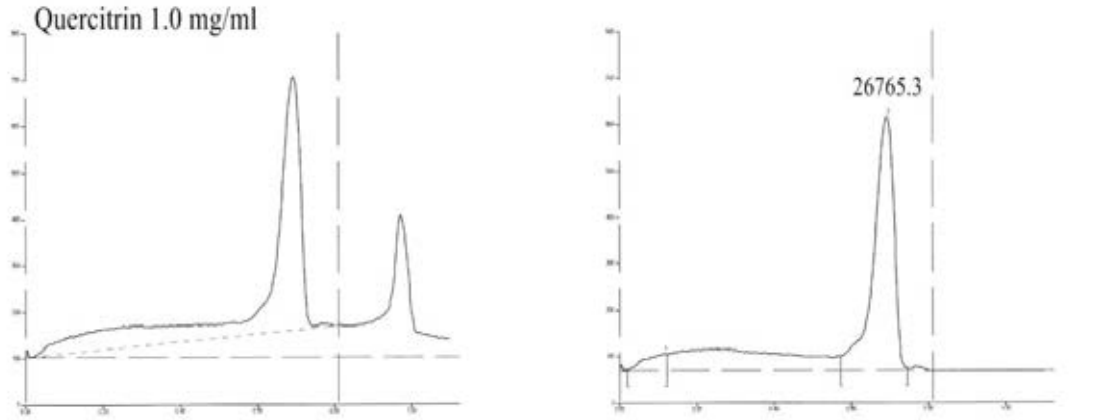


Figure 62. TLC densitometric chromatogram of standard quercitrin 1.0 mg/ml (Plate 3-quercitrin standard and sample extracts from 13 different locations)

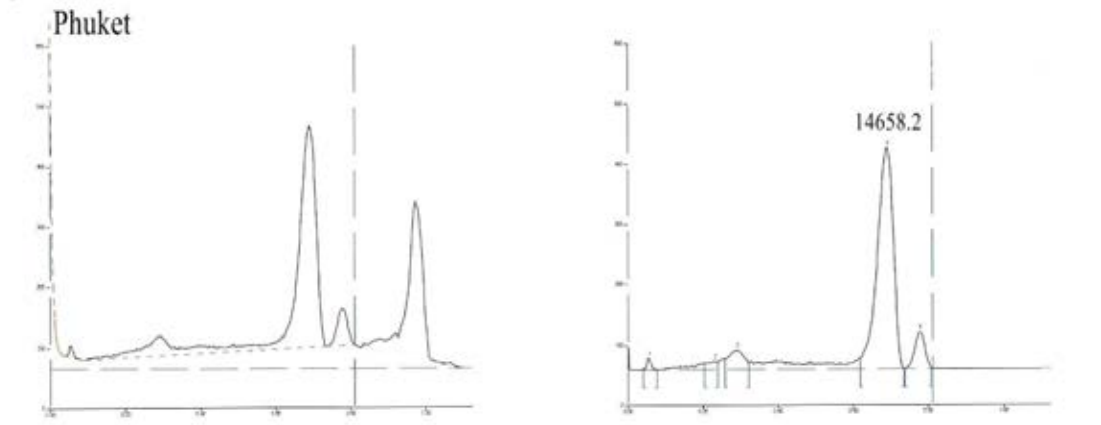


Figure 63. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Phuket (2 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

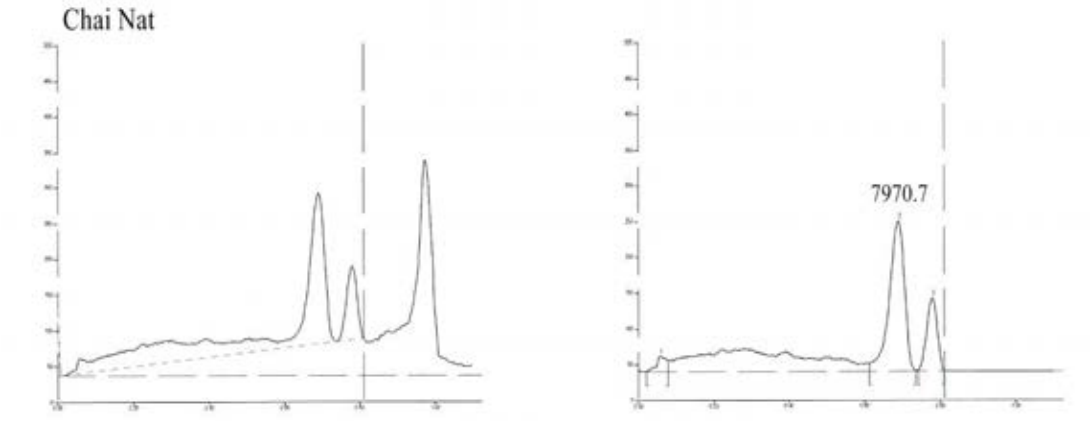


Figure 64. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Chai Nat (2 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

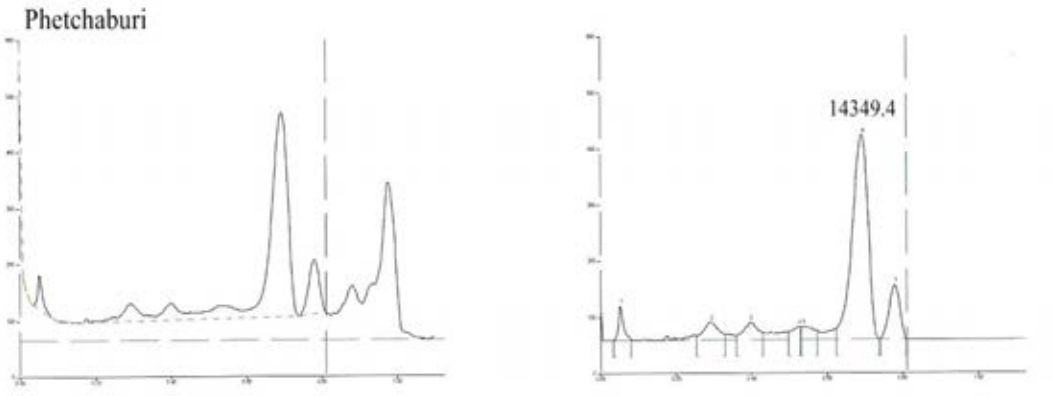


Figure 65. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Phetchaburi (4 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

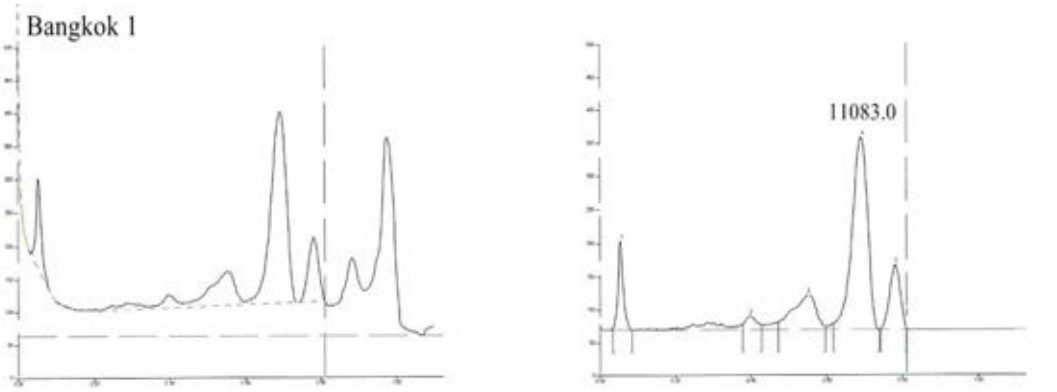


Figure 66. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 1 (5 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

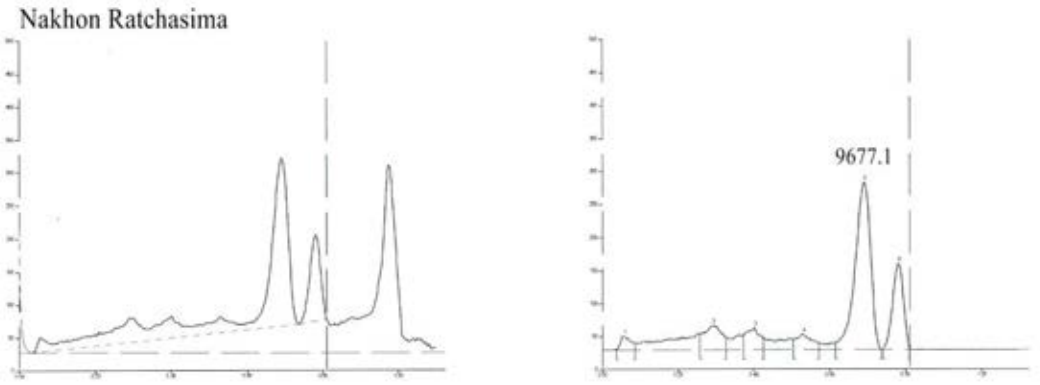


Figure 67. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Nakhon Ratchasima (3 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

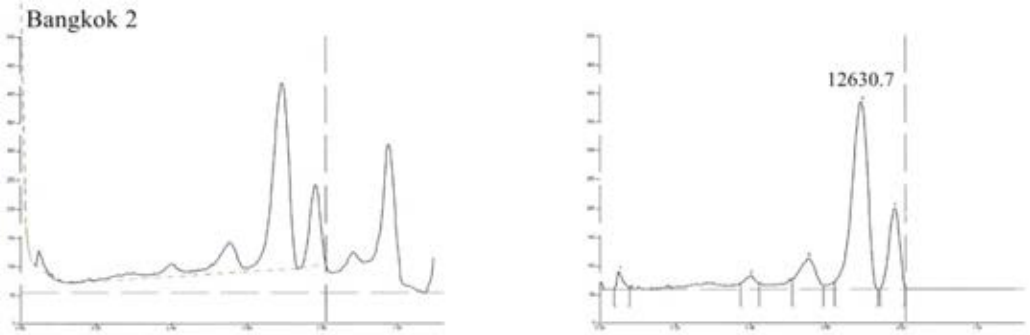


Figure 68. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 2 (3 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

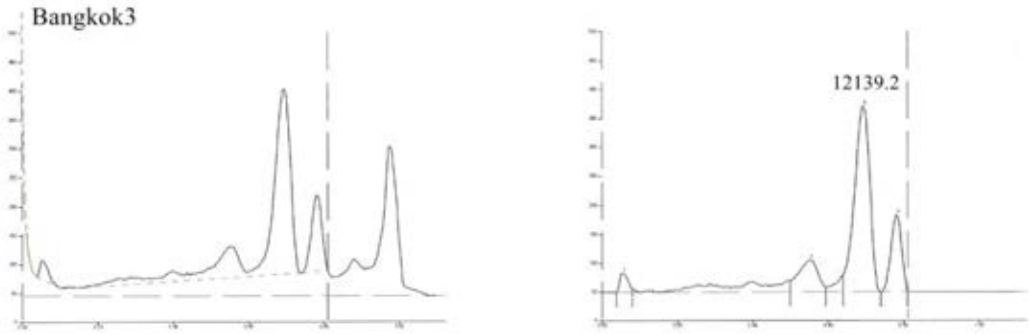


Figure 69. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 3 (3 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

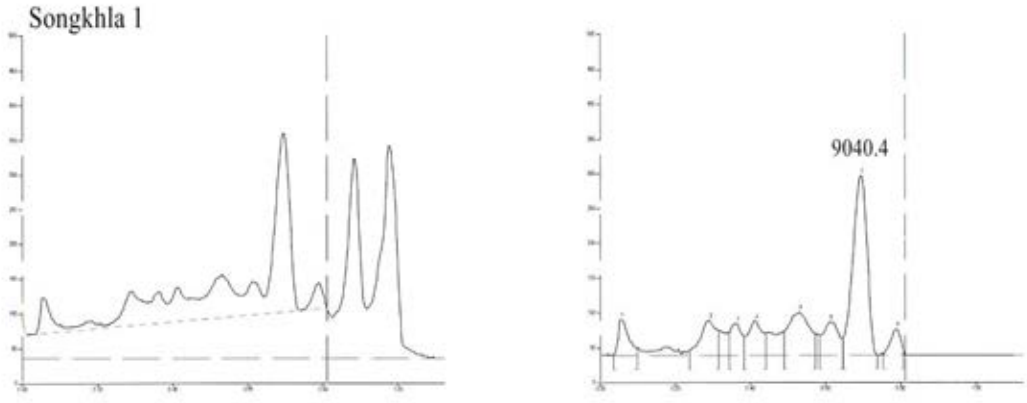


Figure 70. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Songkhla 1 (7 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

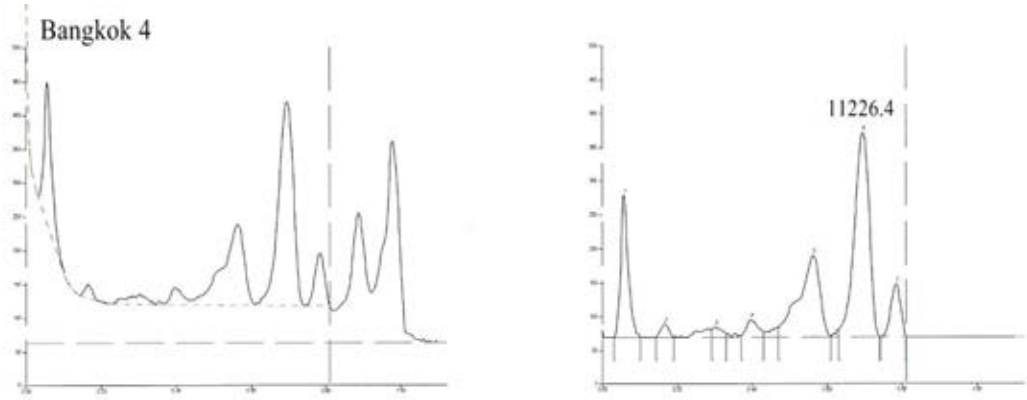


Figure 71. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Bangkok 4 (7 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

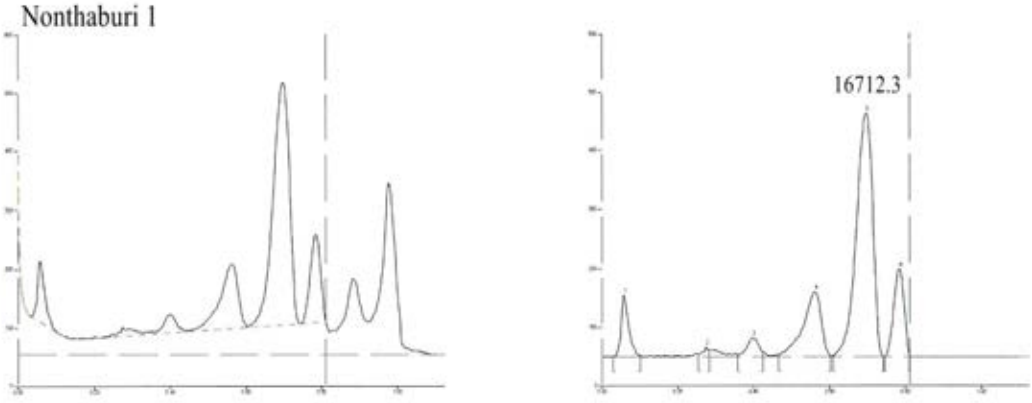


Figure 72. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Nonthaburi 1 (4 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

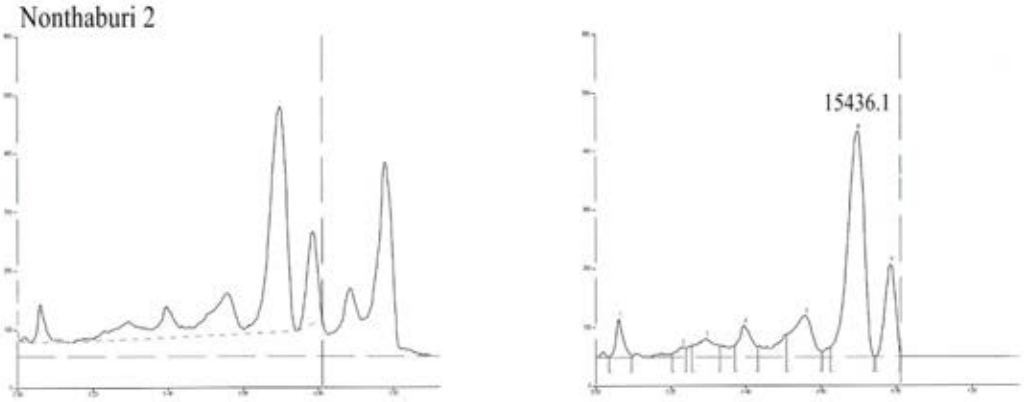


Figure 73. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Nonthaburi 2 (10 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

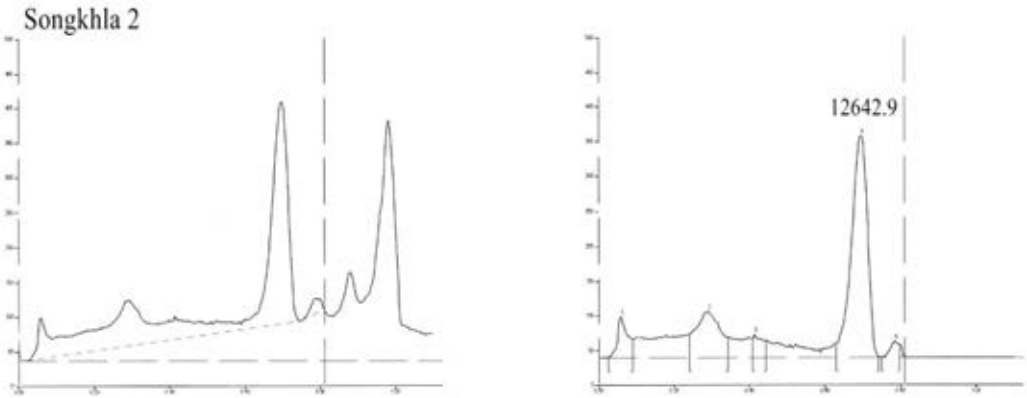


Figure 74. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Songkhla 2 (5.5 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

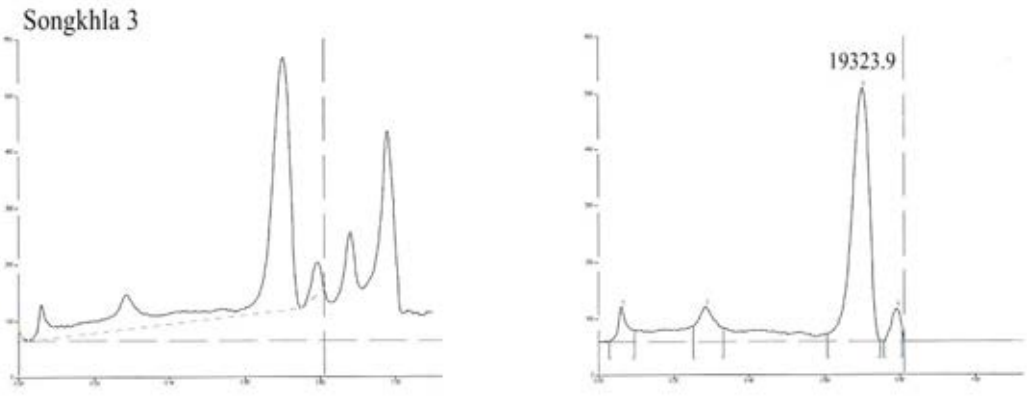


Figure 75. TLC densitometric chromatogram of quercitrin content in *Dendrophthoe pentandra* leaves from Songkhla 3 (5 mg/ml) (Plate 3-quercitrin standard and sample extracts from 13 different locations)

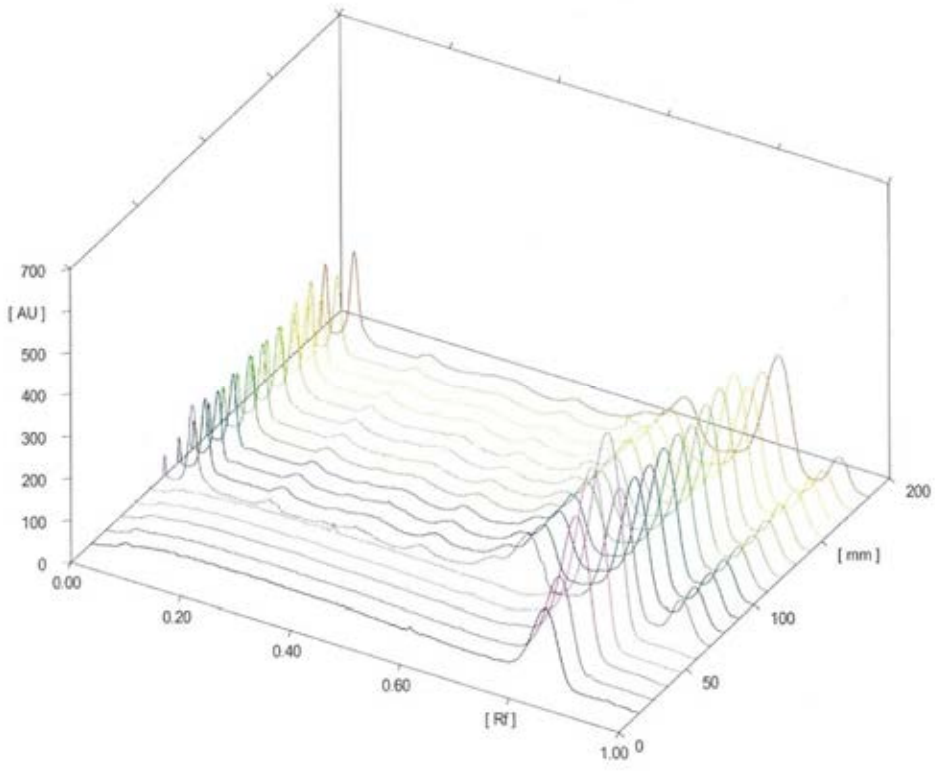


Figure 76. 3D TLC densitometric chromatogram of accuracy method (Plate 1- accuracy and sample extracts No.9)

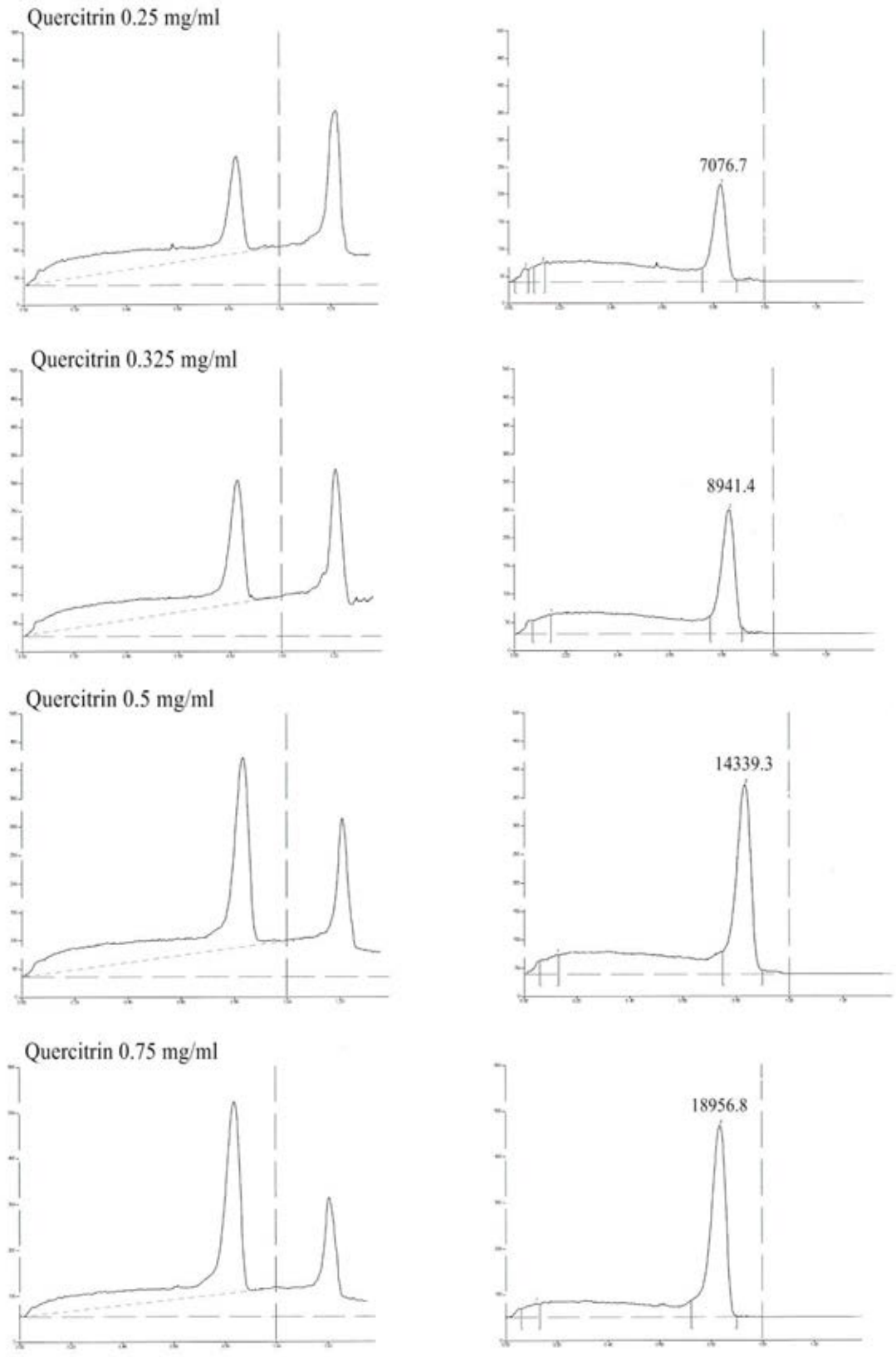


Figure 77. TLC densitometric chromatogram of accuracy method (Plate 1- accuracy and sample extracts No.9)

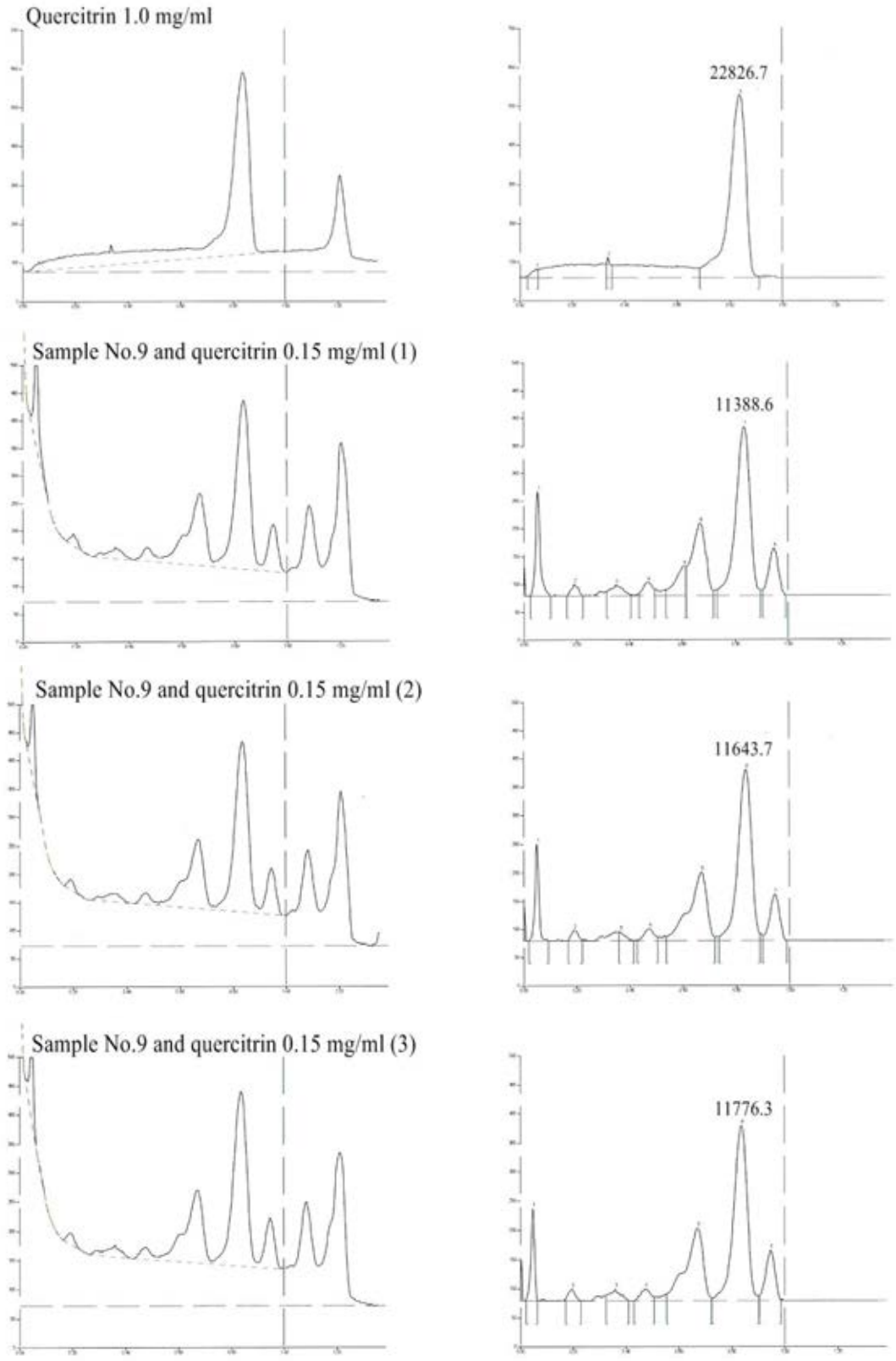


Figure 78. (Cont.) TLC densitometric chromatogram of accuracy method (Plate 1-accuracy and sample extracts No.9)

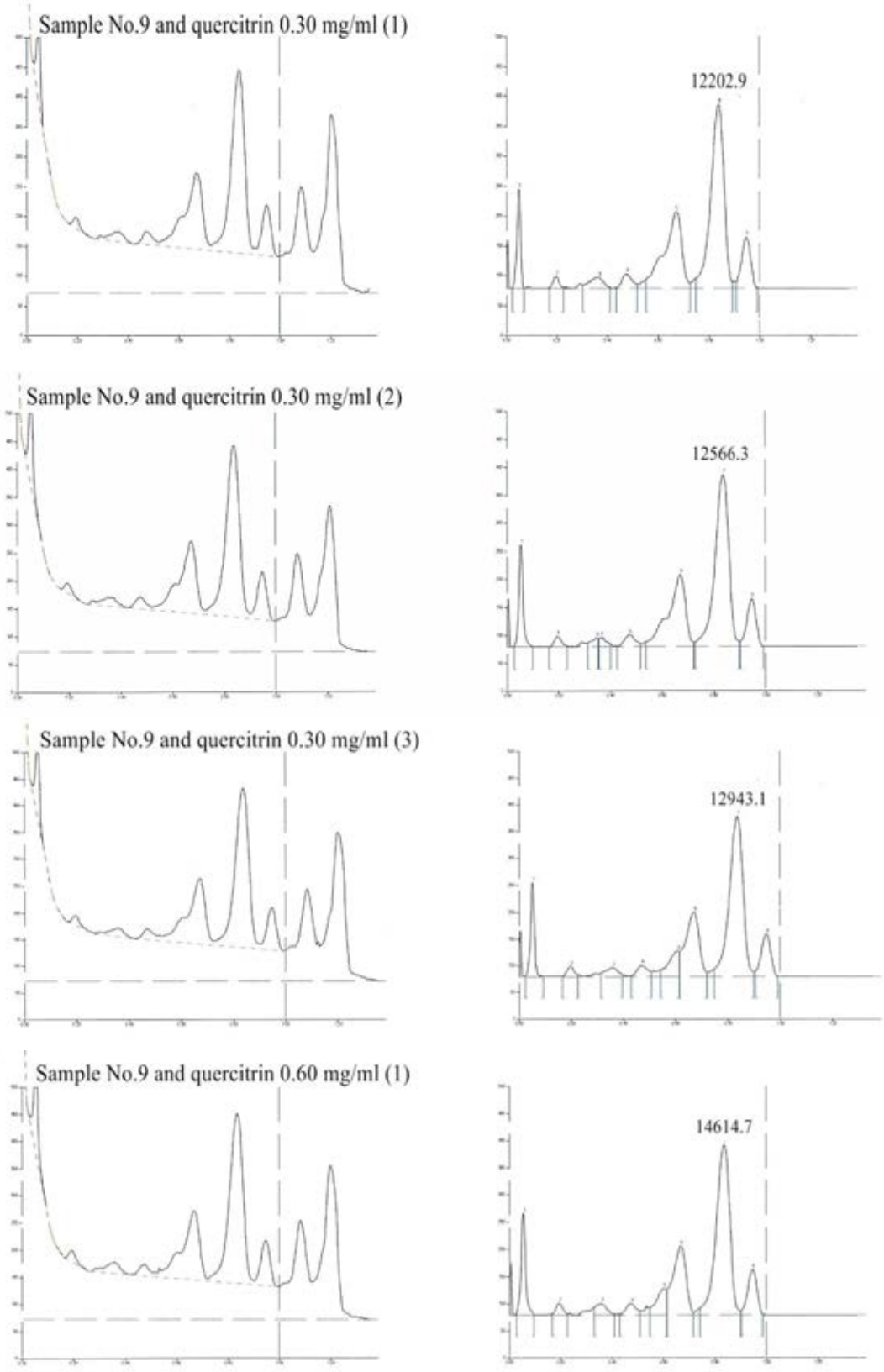


Figure 79. (Cont.) TLC densitometric chromatogram of accuracy method (Plate 1-accuracy and sample extracts No.9)

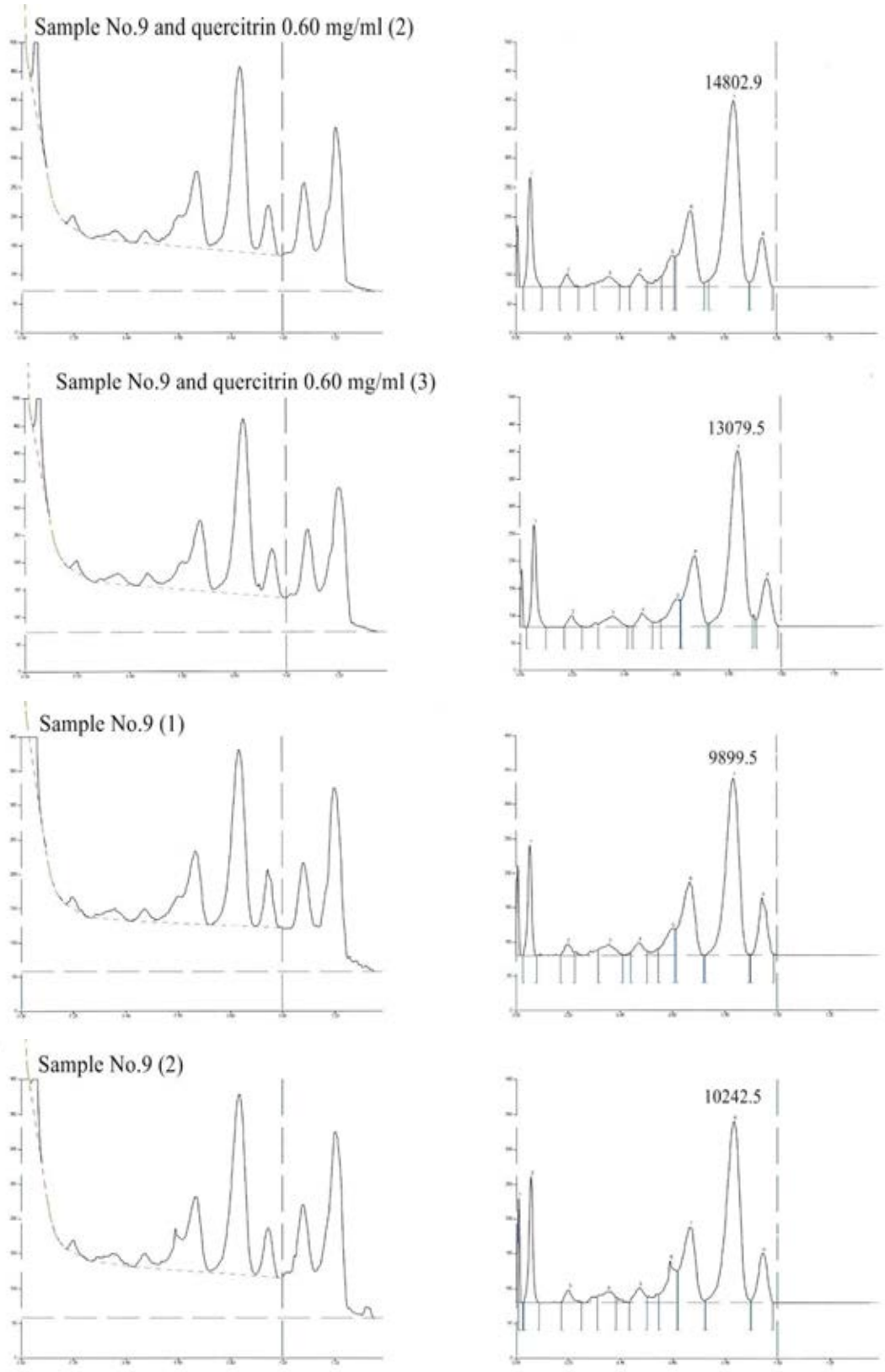


Figure 80. (Cont.) TLC densitometric chromatogram of accuracy method (Plate 1-accuracy and sample extracts No.9)

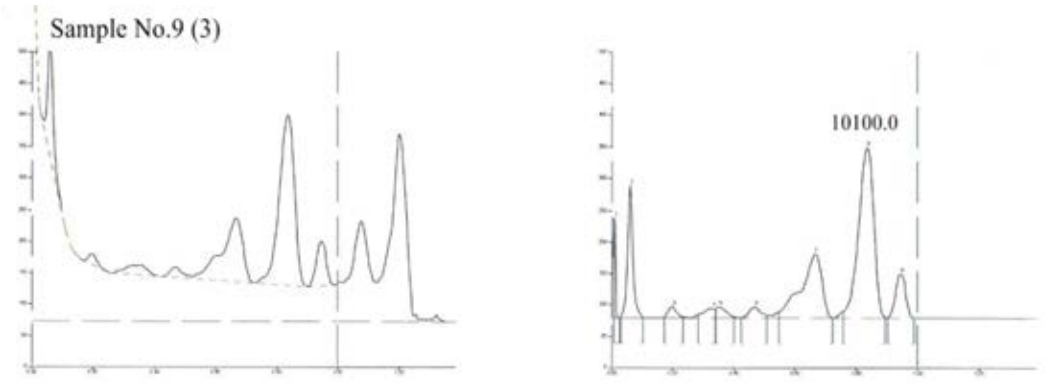


Figure 81. (Cont.) TLC densitometric chromatogram of accuracy method (Plate 1-accuracy and sample extracts No.9)

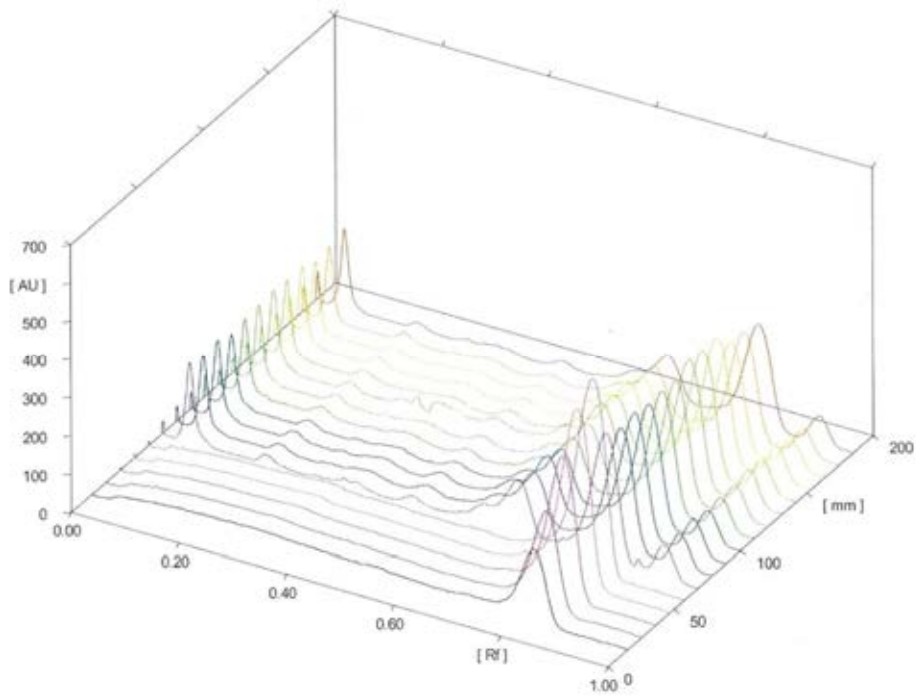


Figure 82. 3D TLC densitometric chromatogram of precision method (Plate 1- precision and sample extracts No.9)

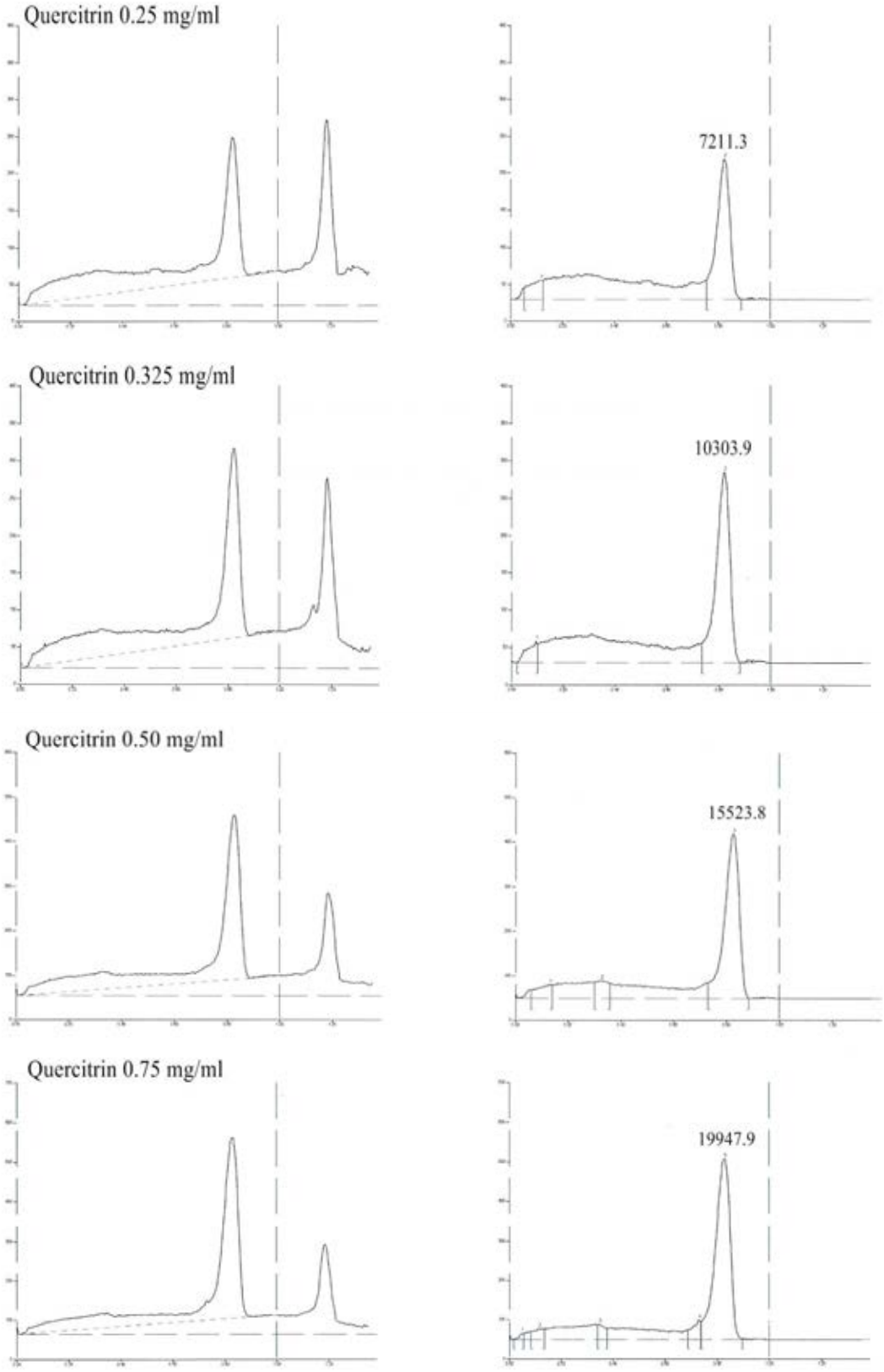


Figure 83. TLC densitometric chromatogram of precision method (Plate 1- precision and sample extracts No.9)

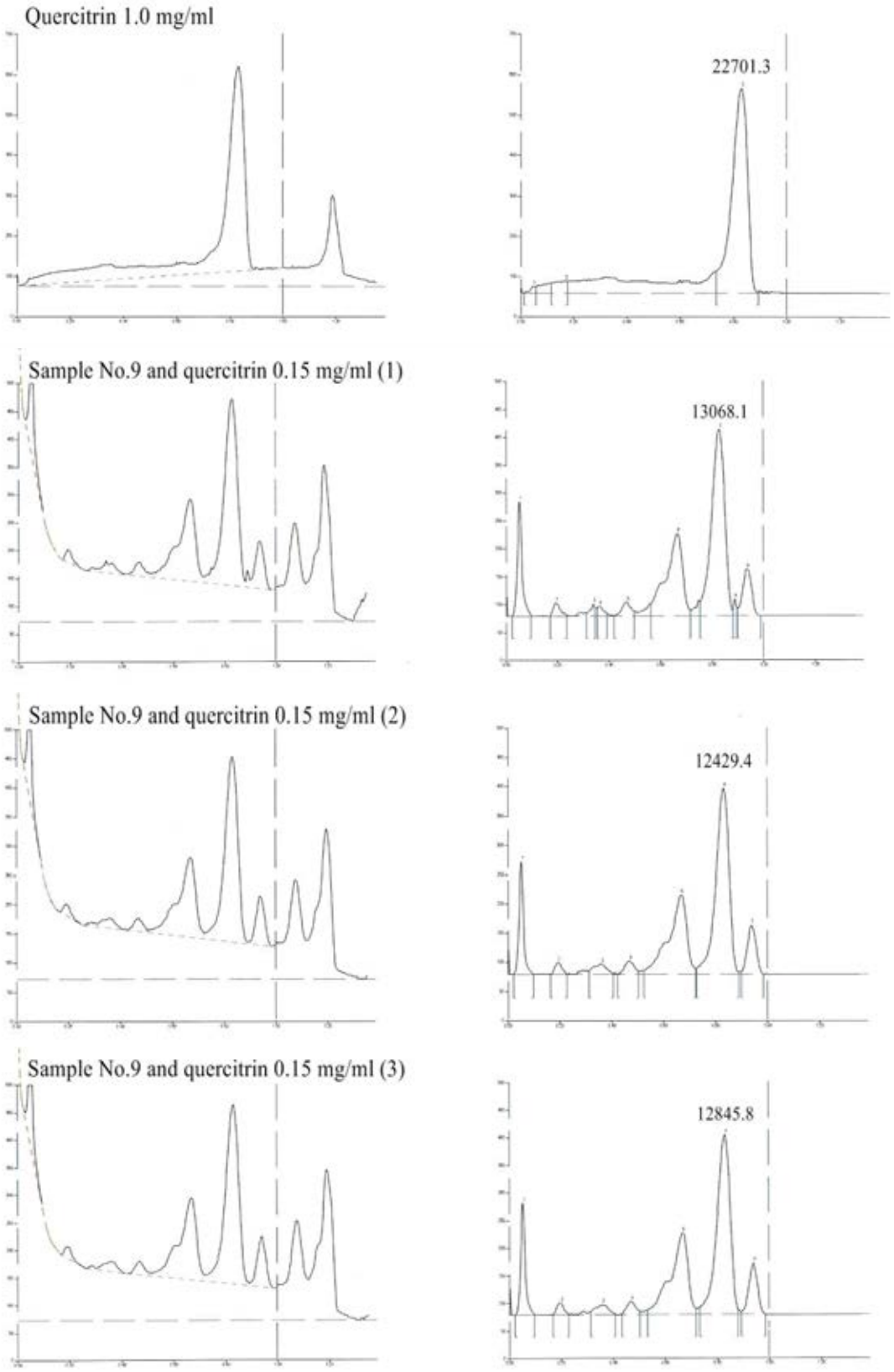


Figure 84. (Cont.) TLC densitometric chromatogram of precision method (Plate 1-precision and sample extracts No.9)

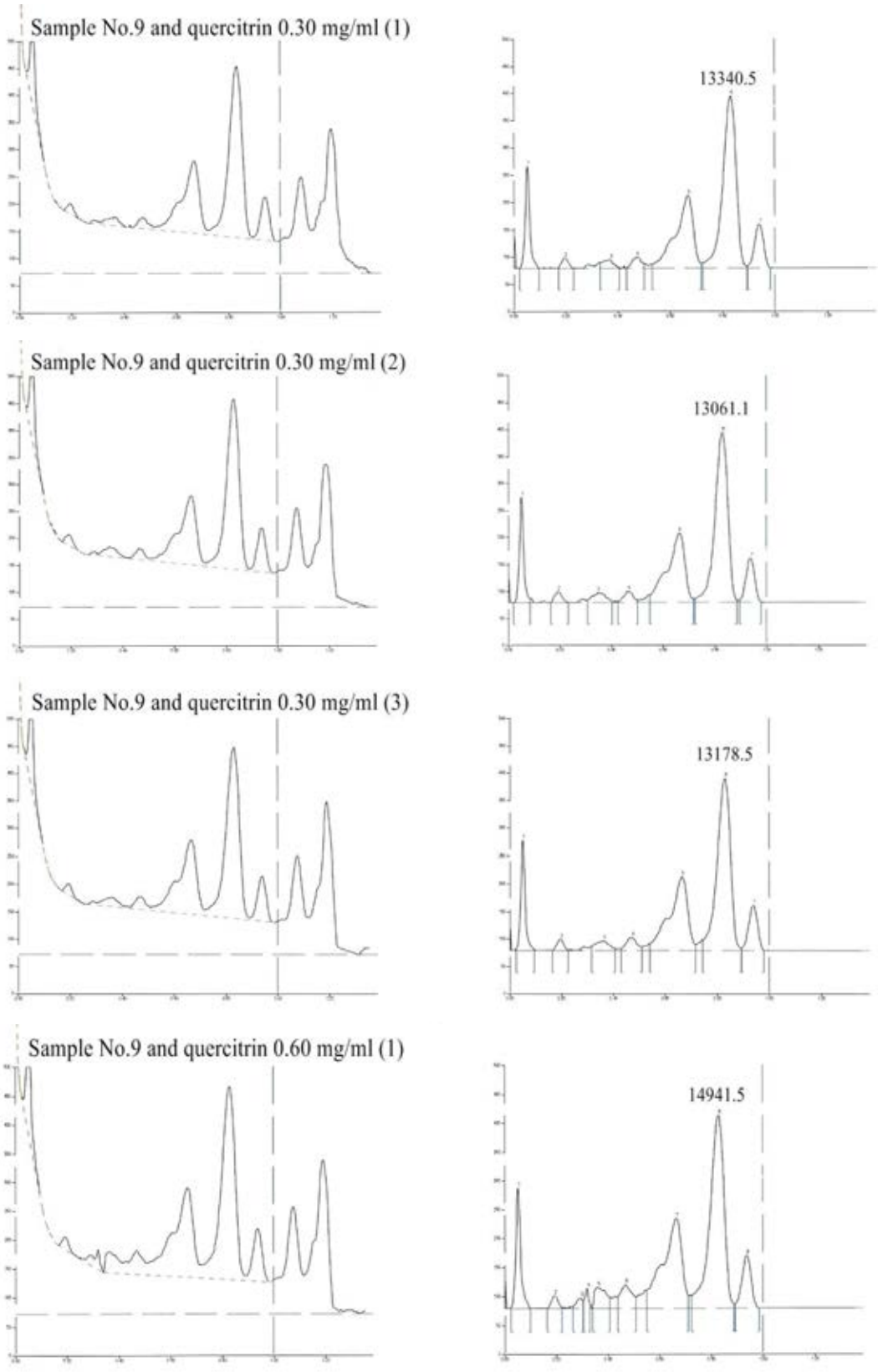


Figure 85. (Cont.) TLC densitometric chromatogram of precision method (Plate 1-precision and sample extracts No.9)

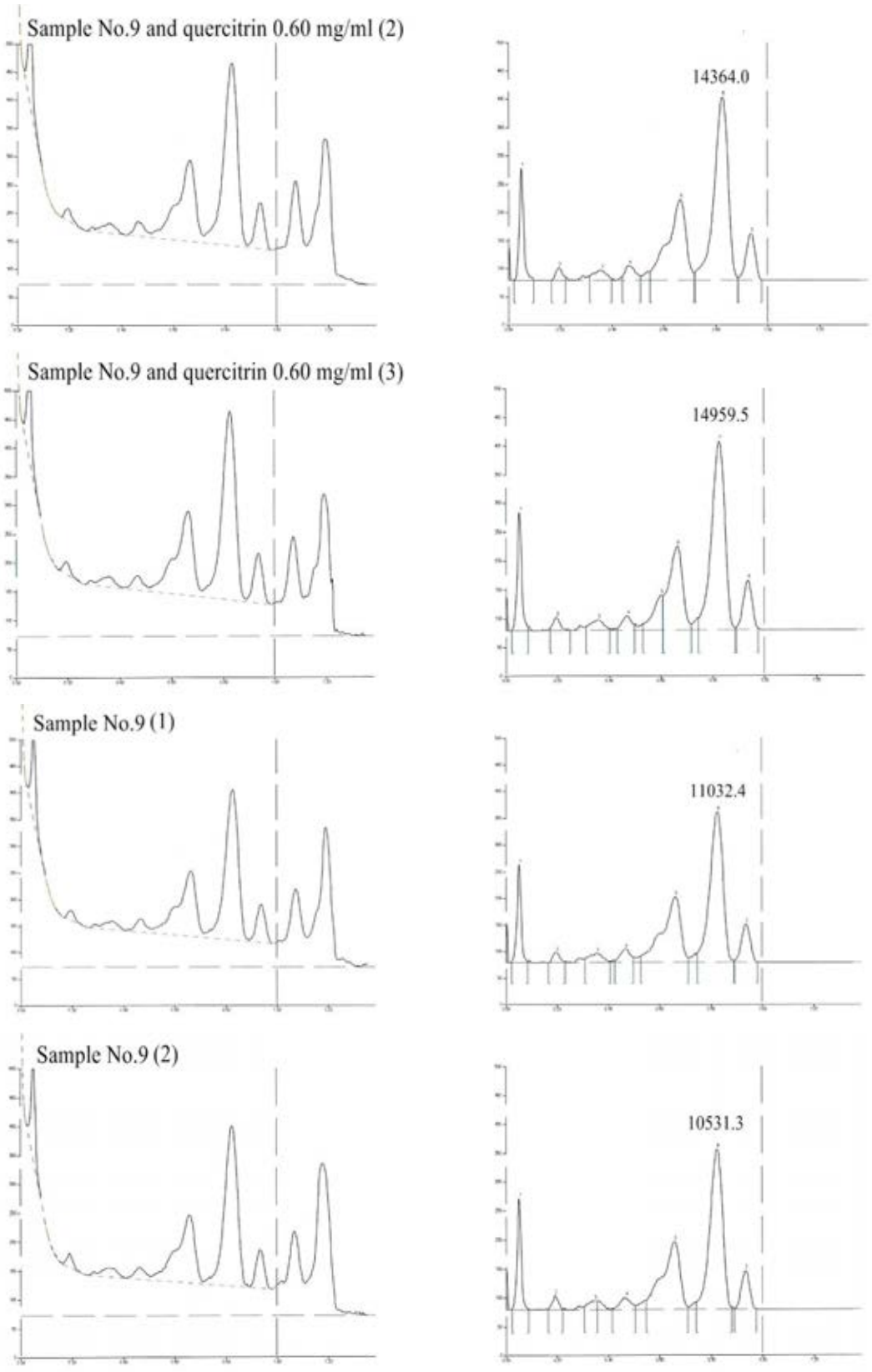


Figure 86. (Cont.) TLC densitometric chromatogram of precision method (Plate 1-precision and sample extracts No.9)

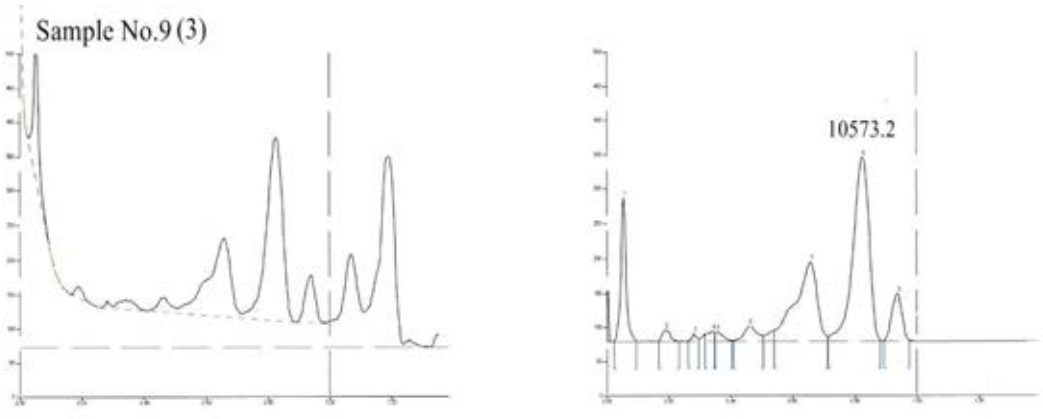


Figure 87. (Cont.) TLC densitometric chromatogram of precision method (Plate 1-precision and sample extracts No.9)

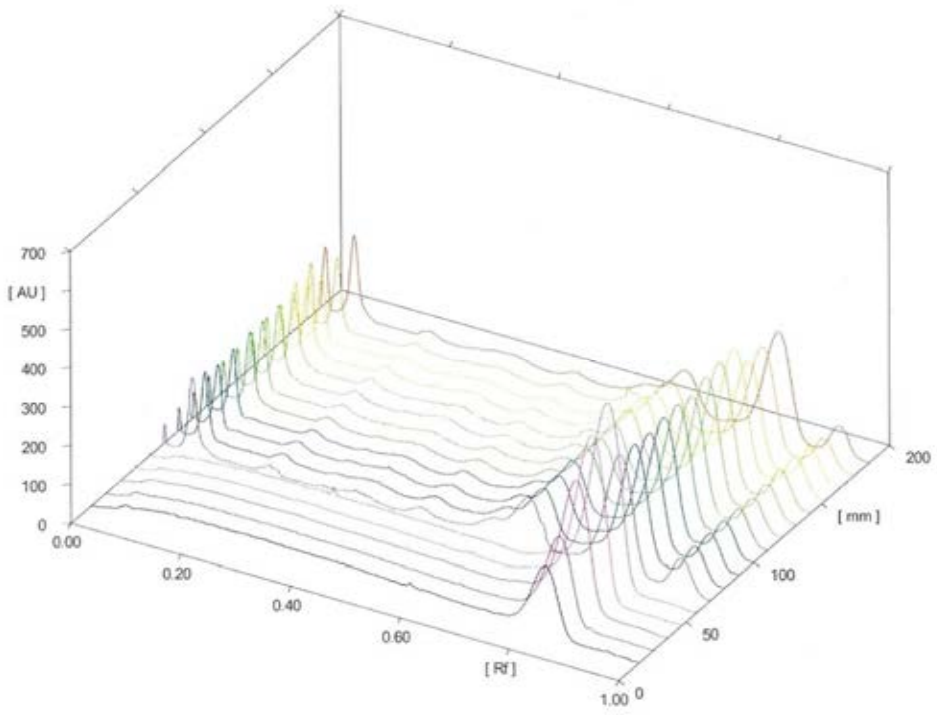


Figure 88. 3D TLC densitometric chromatogram of precision method (Plate 2-precision and sample extracts No.9)

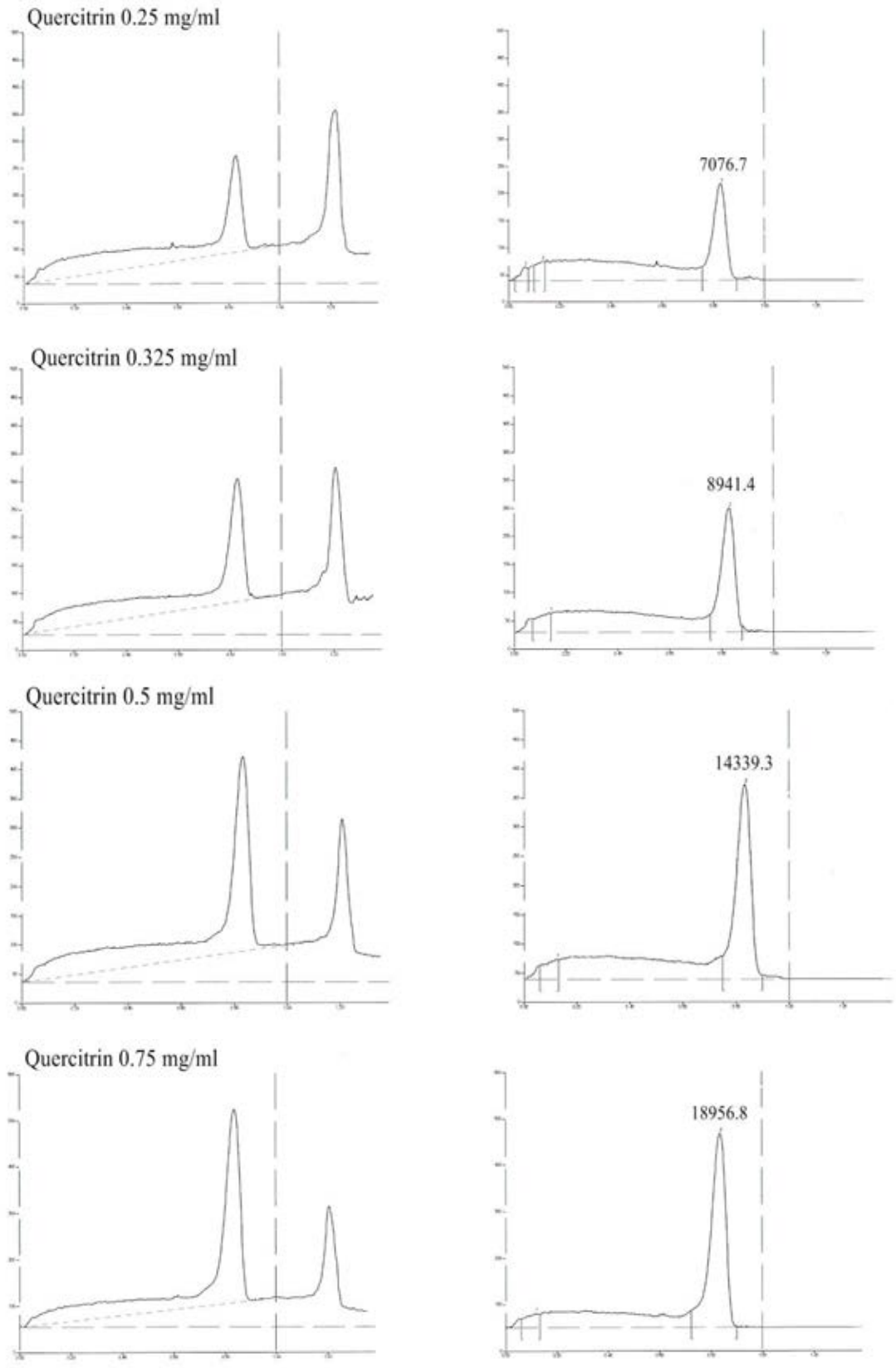


Figure 89. TLC densitometric chromatogram of precision method (Plate 2- precision and sample extracts No.9)

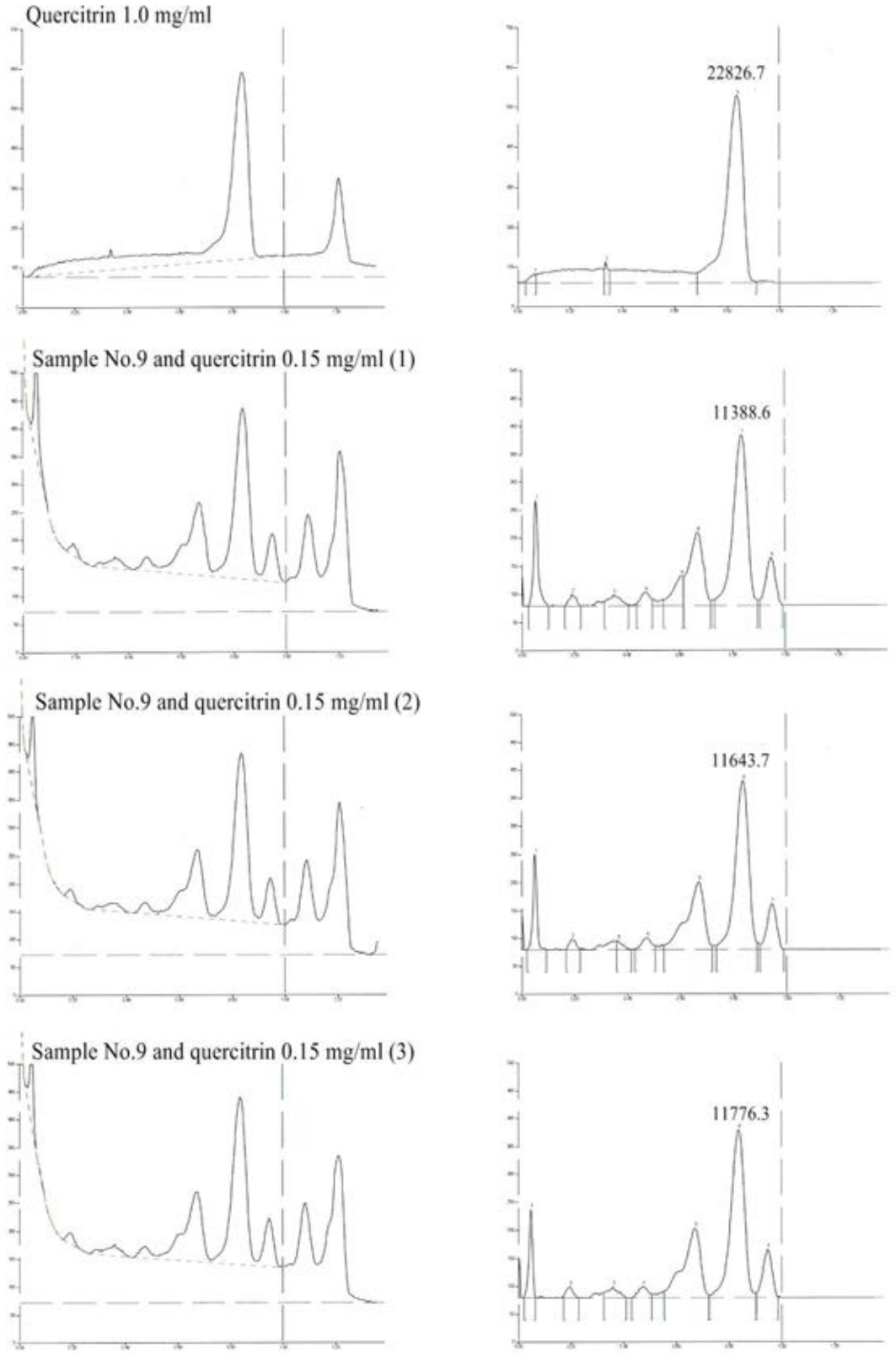


Figure 90. (Cont.) TLC densitometric chromatogram of precision method (Plate 2-precision and sample extracts No.9)

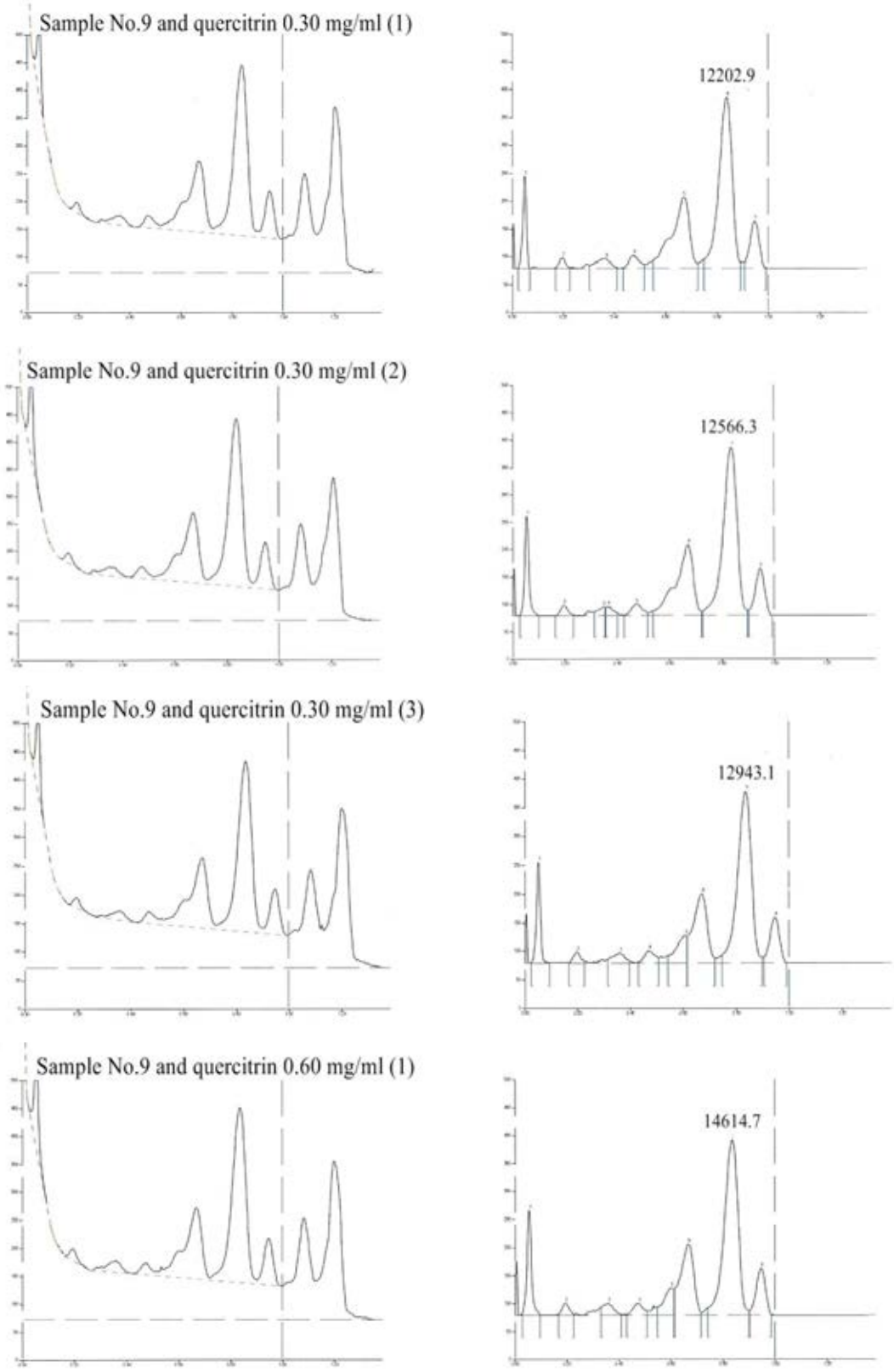


Figure 91. (Cont.) TLC densitometric chromatogram of precision method (Plate 2-precision and sample extracts No.9)

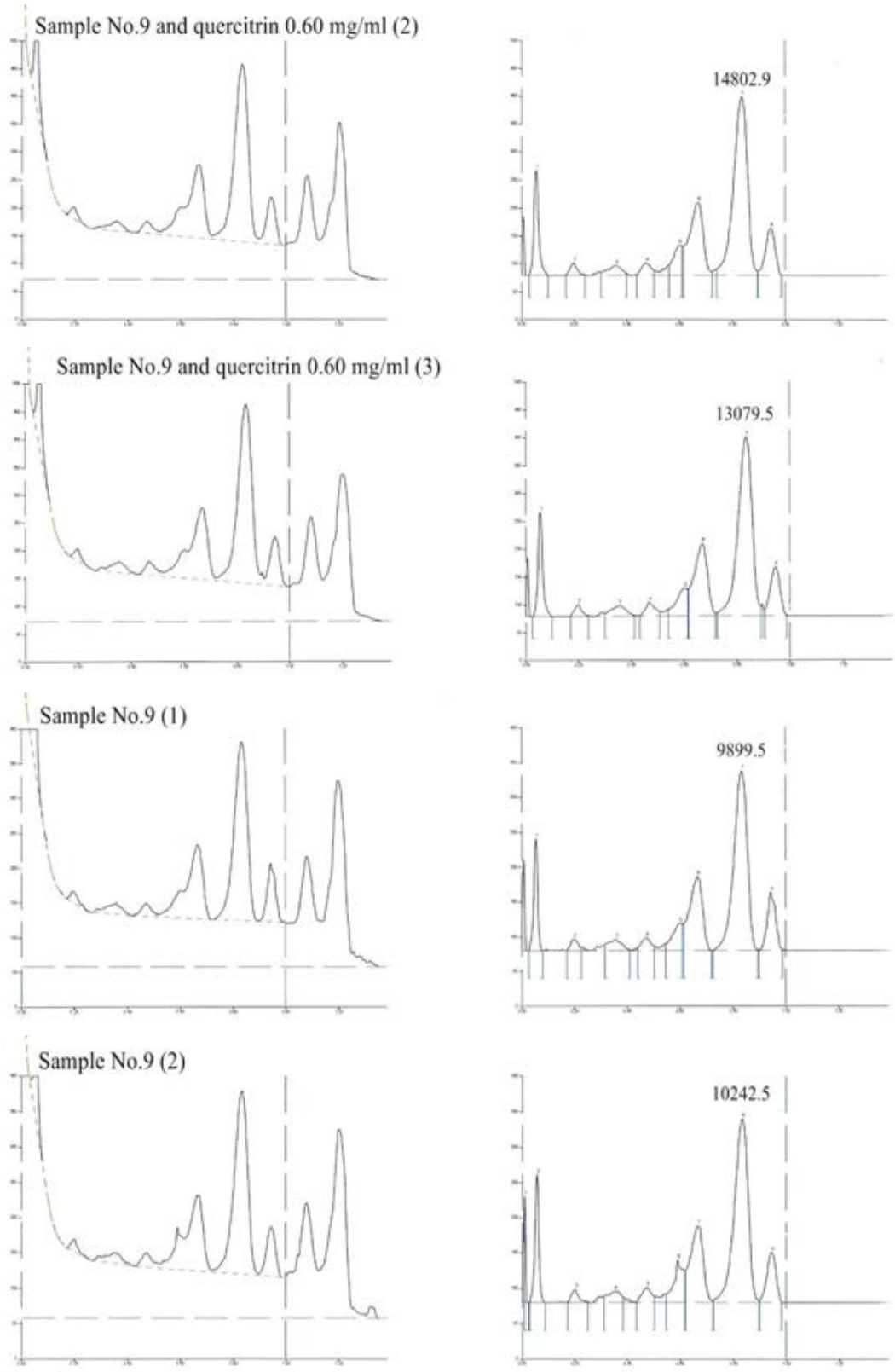


Figure 92. (Cont.) TLC densitometric chromatogram of precision method (Plate 2-precision and sample extracts No.9)

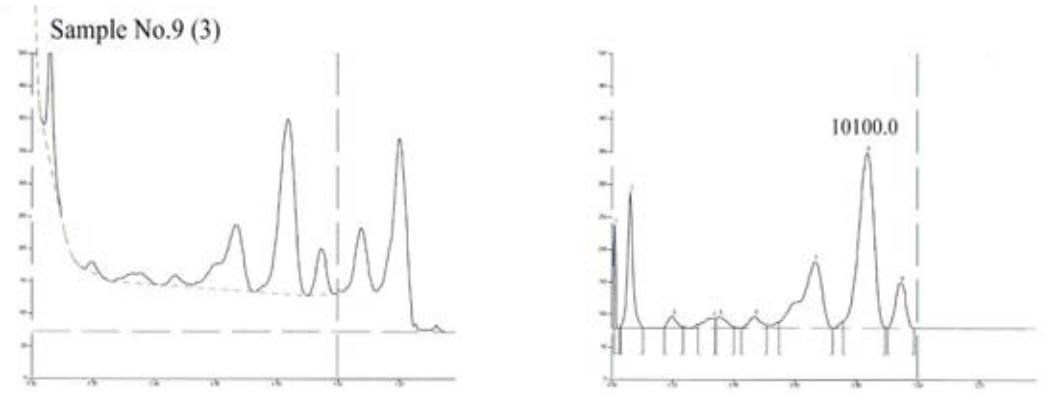


Figure 93. (Cont.) TLC densitometric chromatogram of precision method (Plate 2-precision and sample extracts No.9)

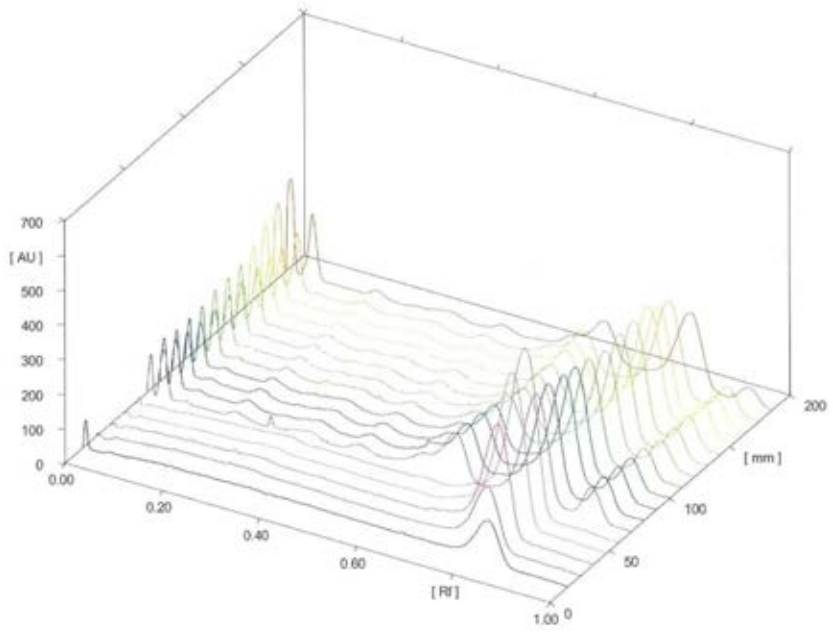


Figure 94. 3D TLC densitometric chromatogram of precision method (Plate 3- precision and sample extracts No.9)

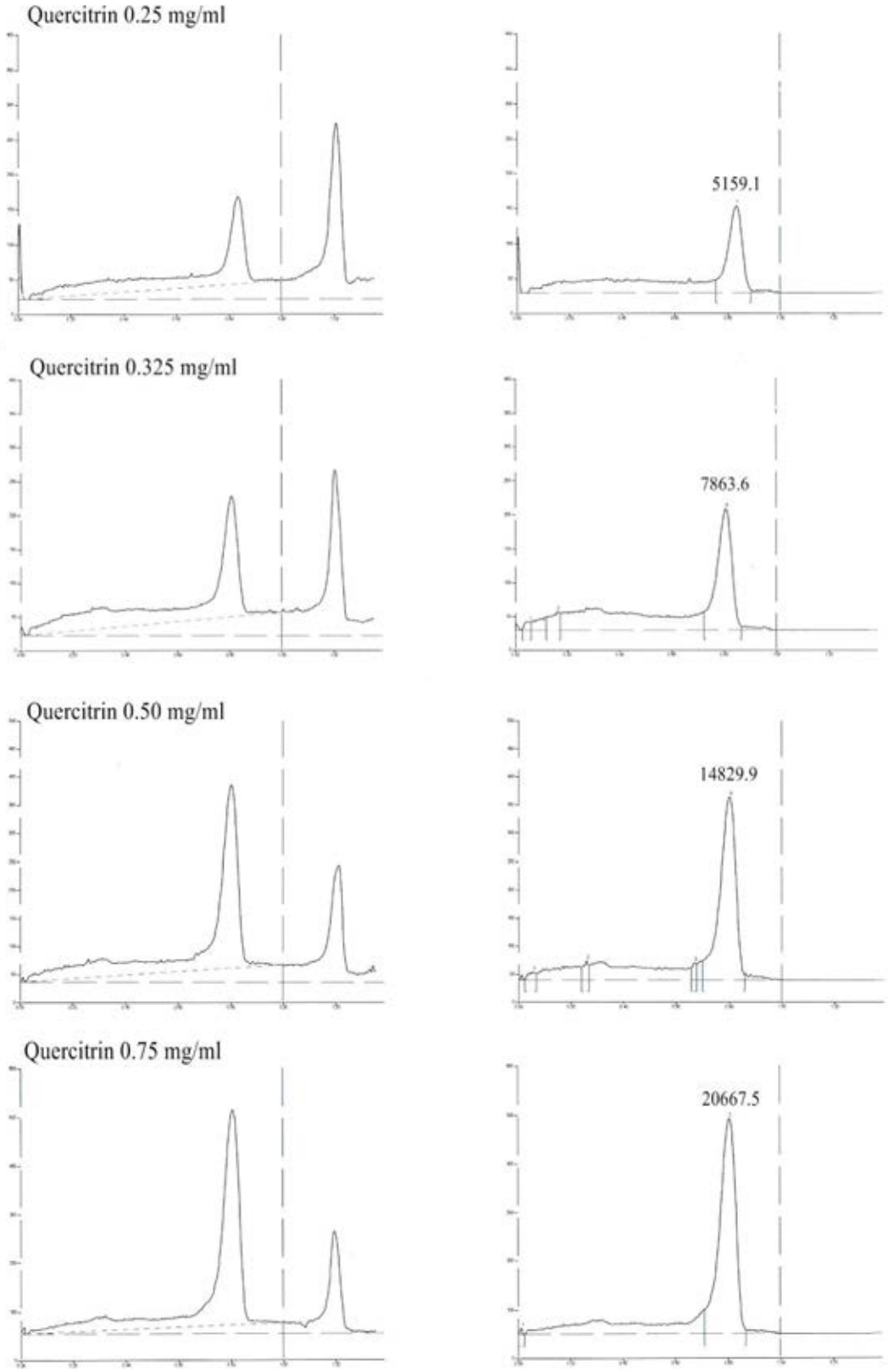


Figure 95. TLC densitometric chromatogram of precision method (Plate 3-precision and sample extracts No.9)

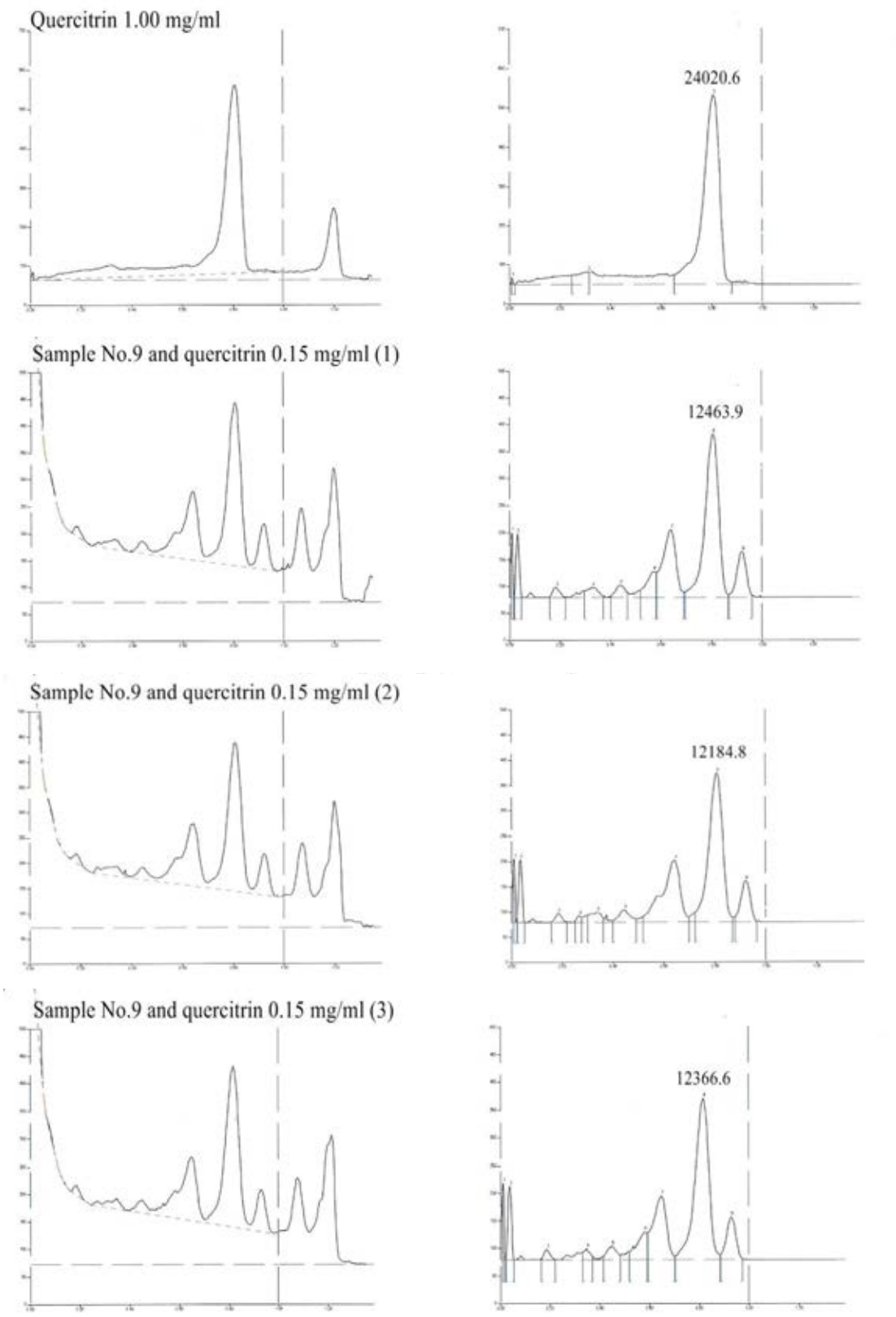


Figure 96. (Cont.) TLC densitometric chromatogram of precision method (Plate 3-precision and sample extracts No.9)

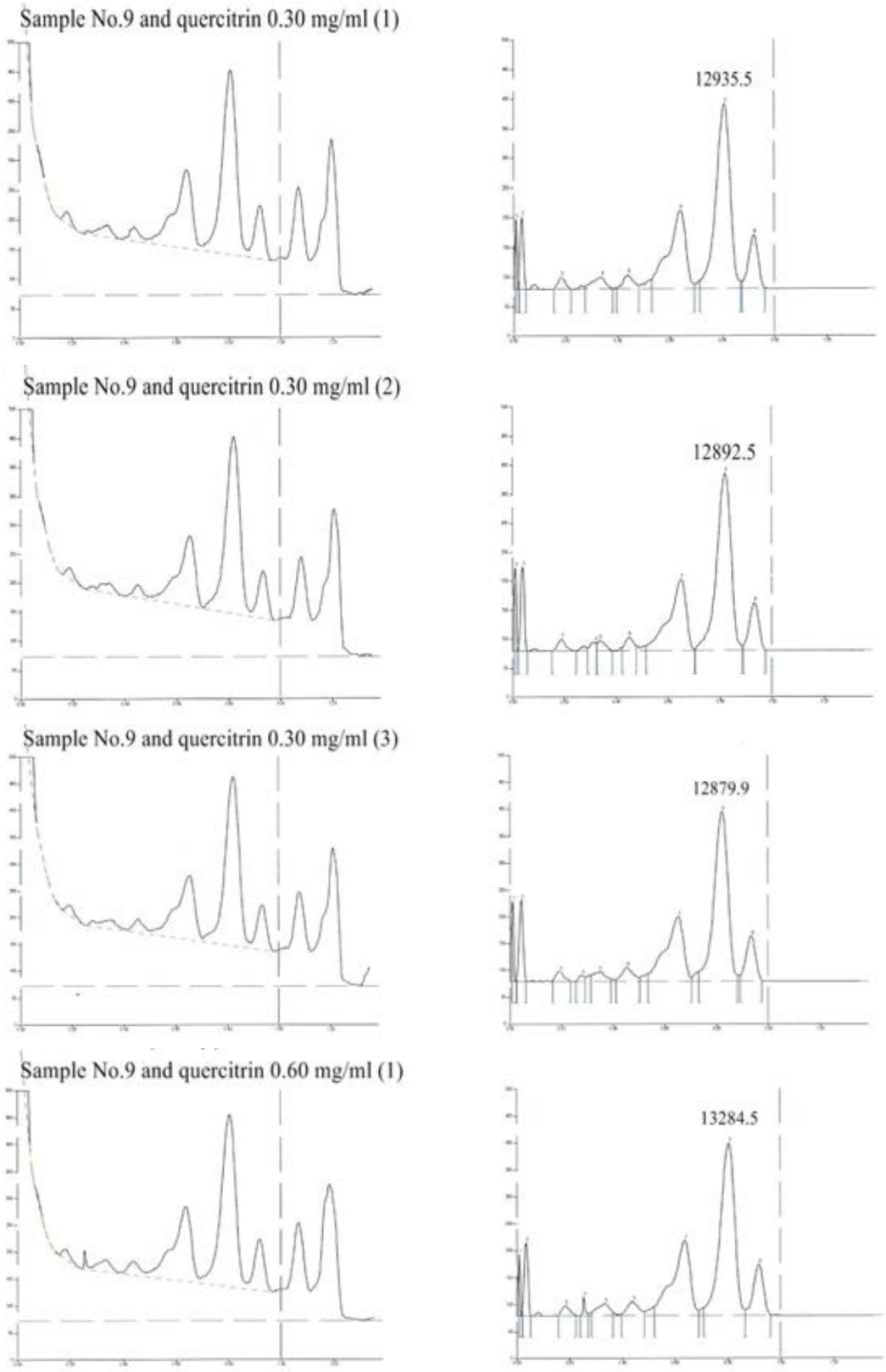


Figure 97. (Cont.) TLC densitometric chromatogram of precision method (Plate 3-precision and sample extracts No.9)

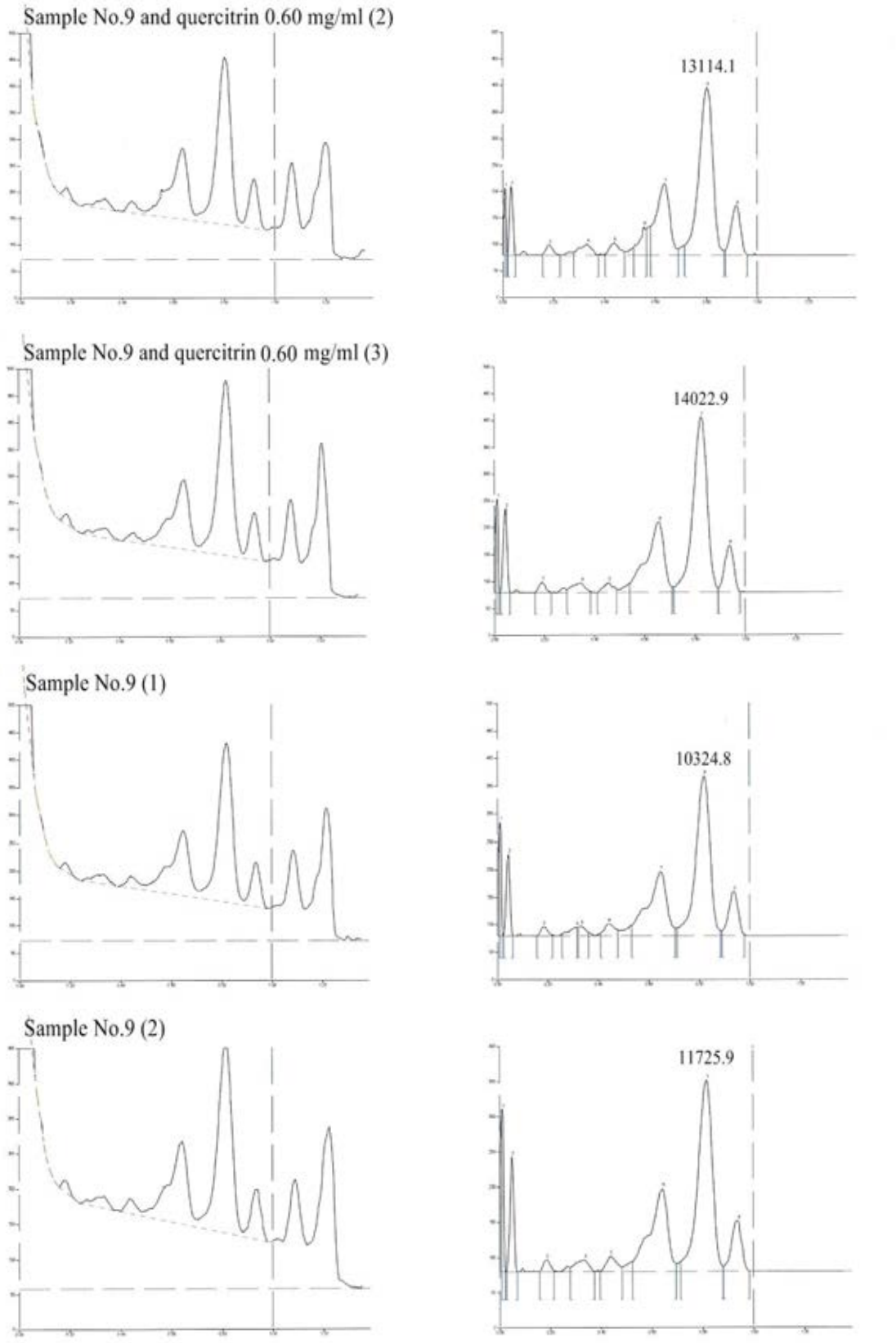


Figure 98. (Cont.) TLC densitometric chromatogram of precision method (Plate 2-precision and sample extracts No.9)

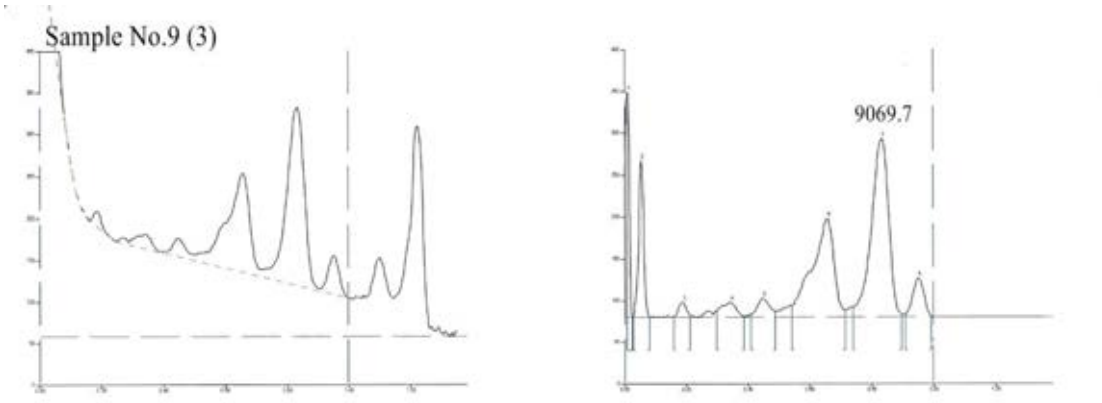
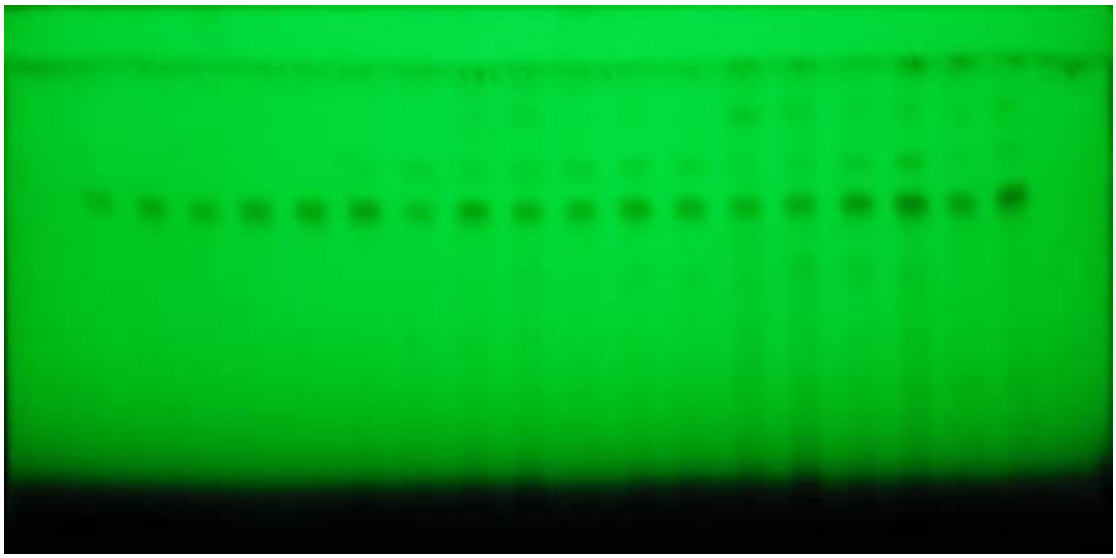


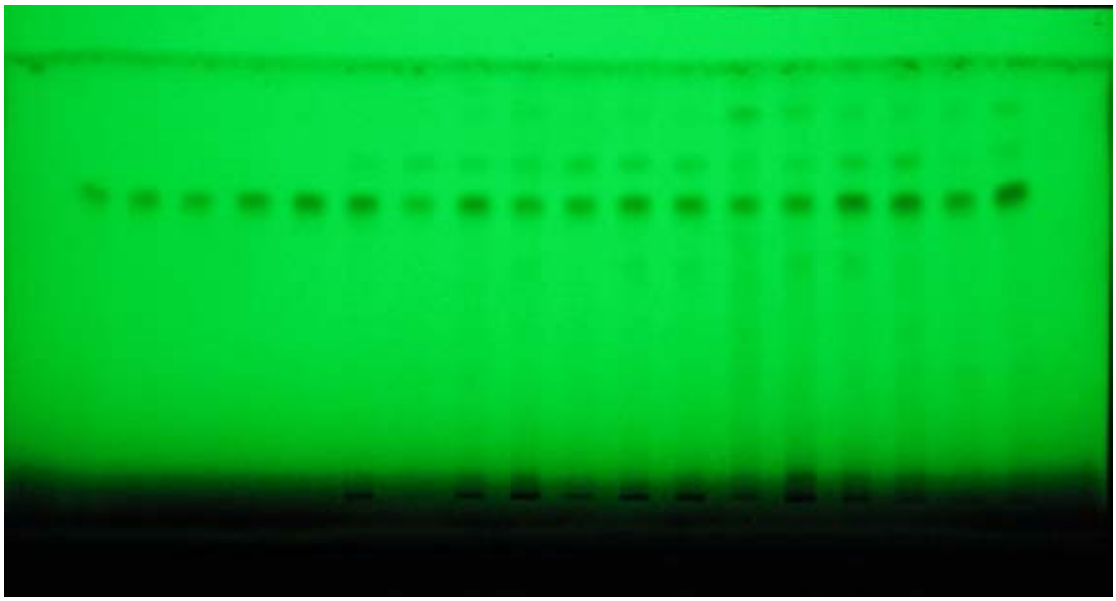
Figure 99. (Cont.) TLC densitometric chromatogram of precision method (Plate 3-precision and sample extracts No.9)



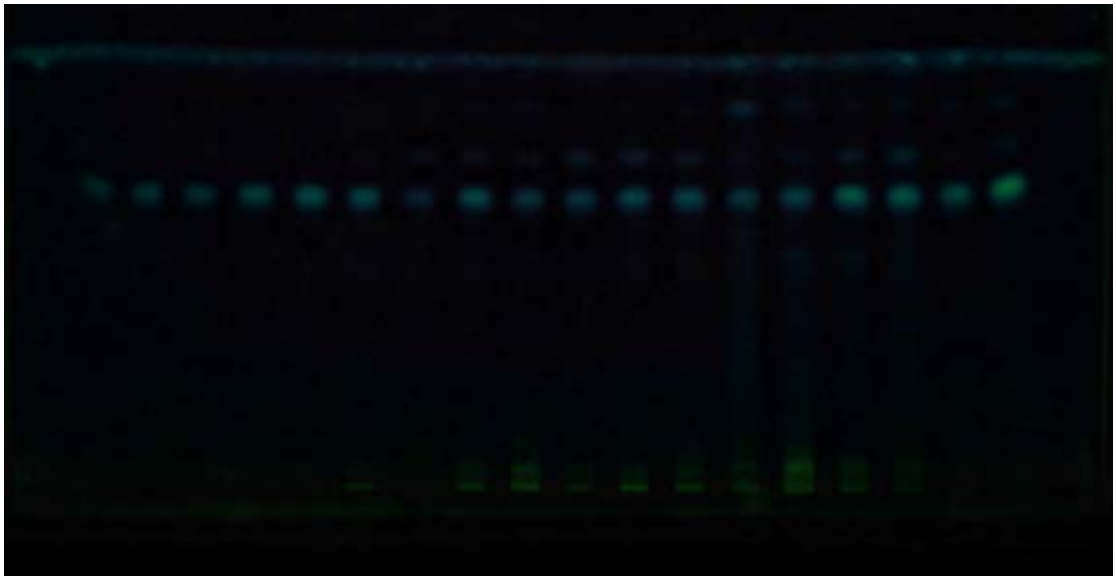
(A)

(B)

Figure 100. TLC plate 1 of quercitrin standard (0.25-1.0 mg/ml) and all sample extracts (13 locations); TLC plate visual with 254 nm (A), TLC plate subtrack background by imageJ software (B)

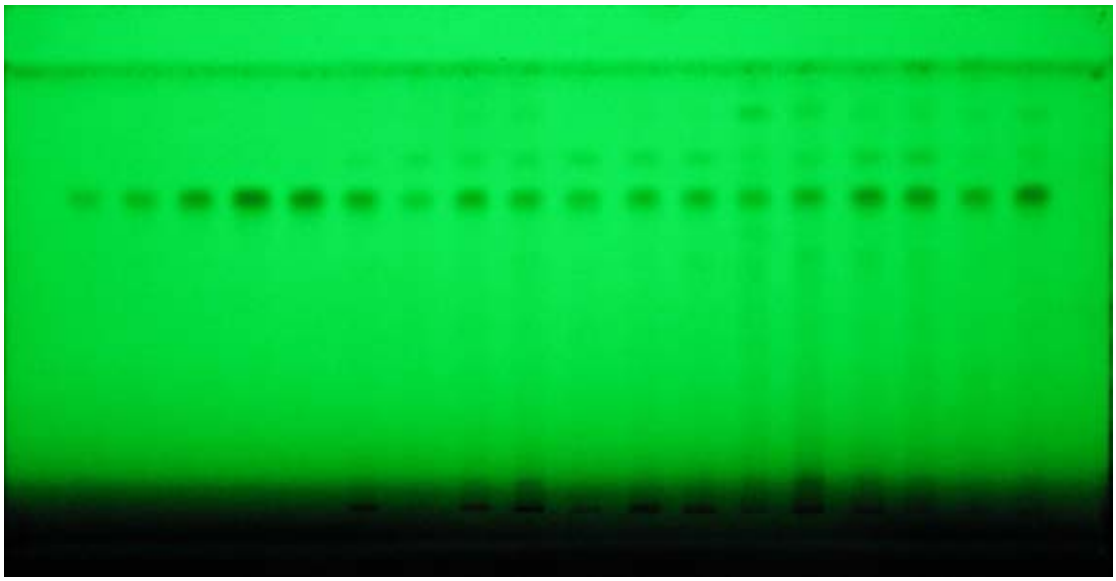


(A)

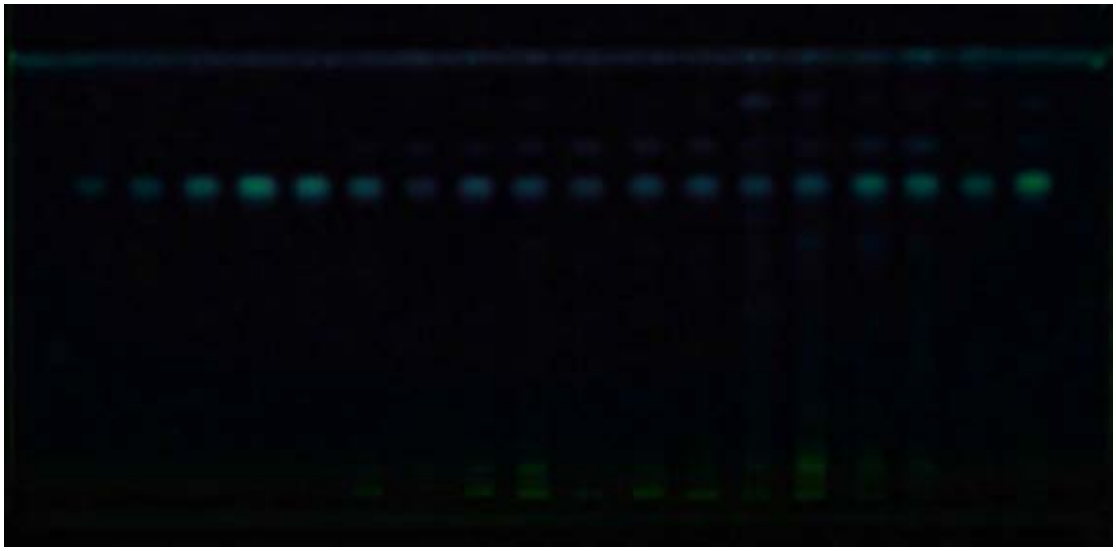


(B)

Figure 101. TLC plate 2 of quercitrin standard (0.25-1.0 mg/ml) and all sample extracts (13 locations); TLC plate visual with 254 nm (A), TLC plate subtrack background by imageJ software (B)

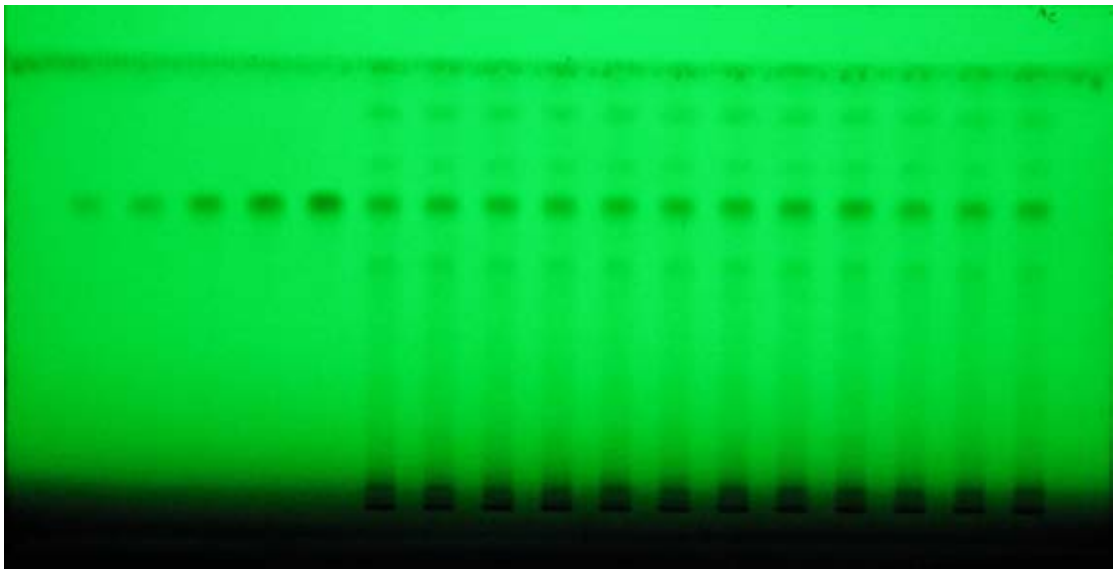


(A)

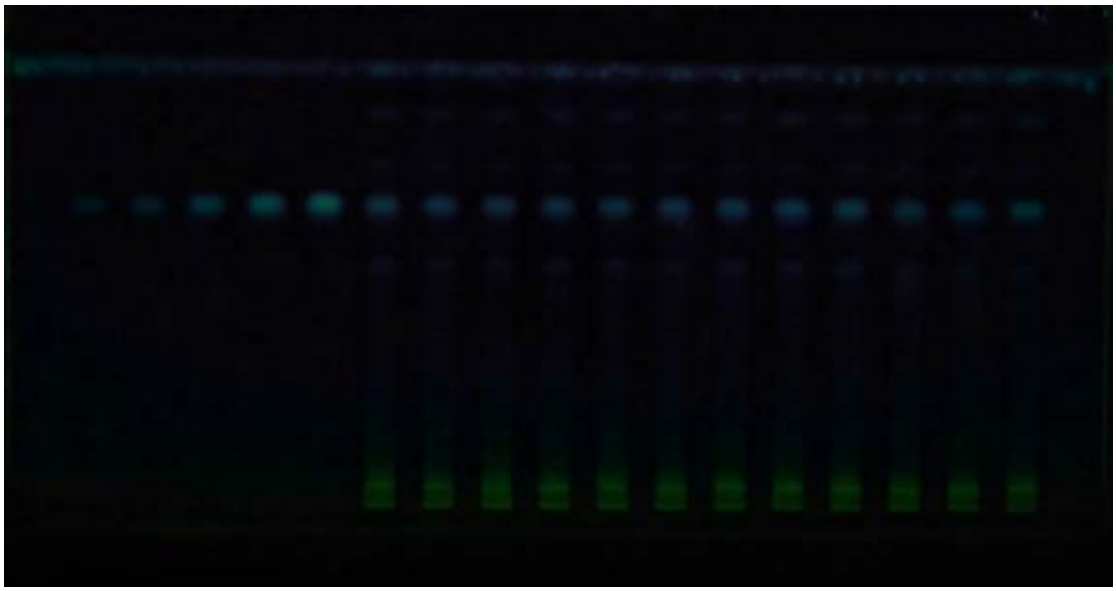


(B)

Figure 102. TLC plate 3 of quercitrin standard (0.25-1.0 mg/ml) and all sample extracts (13 locations); TLC plate visual with 254 nm (A), TLC plate subtrack background by imageJ software (B)

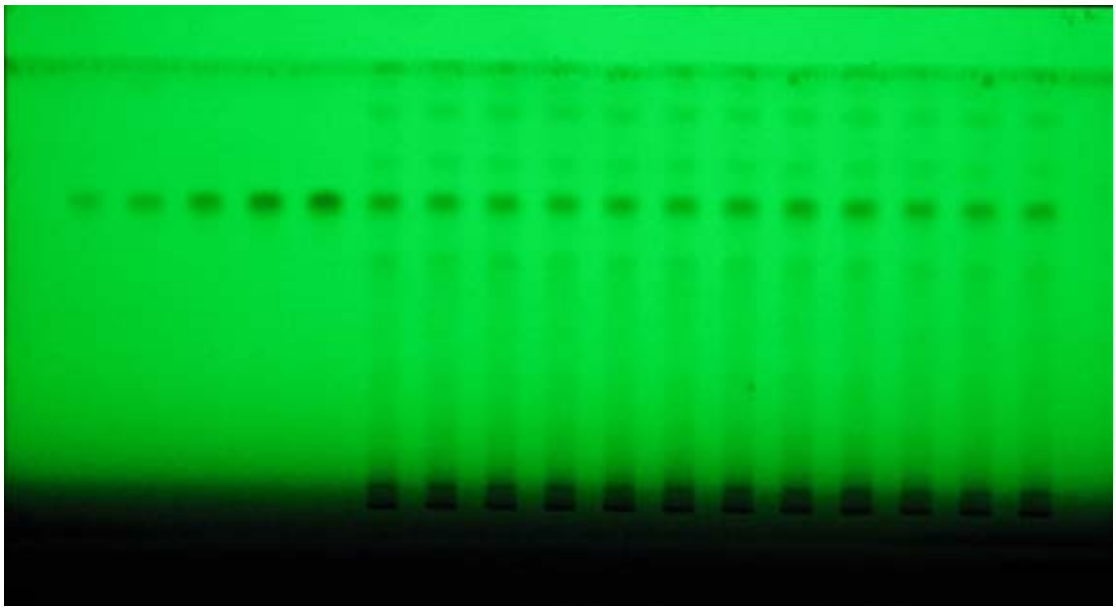


(A)

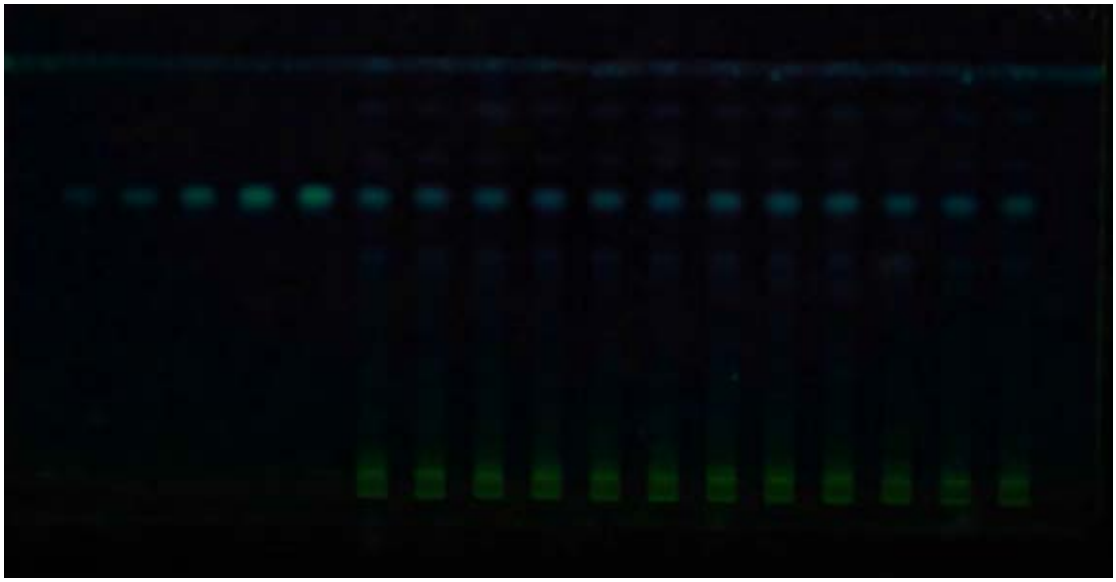


(B)

Figure 103. TLC plate 1 of accuracy method; TLC plate visual with 254 nm (A), TLC plate subtrack background by imageJ software (B)

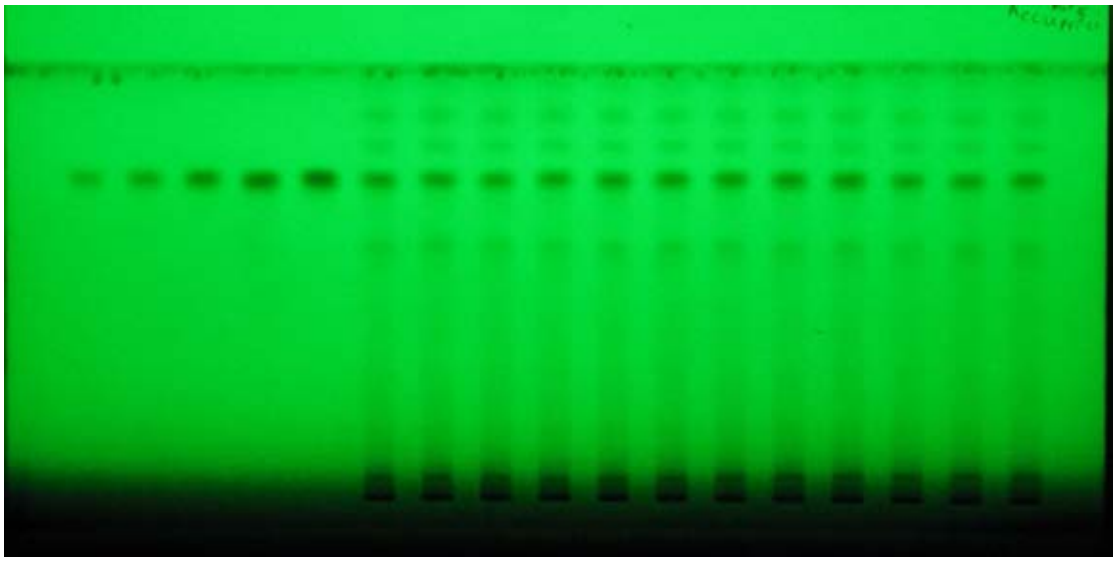


(A)

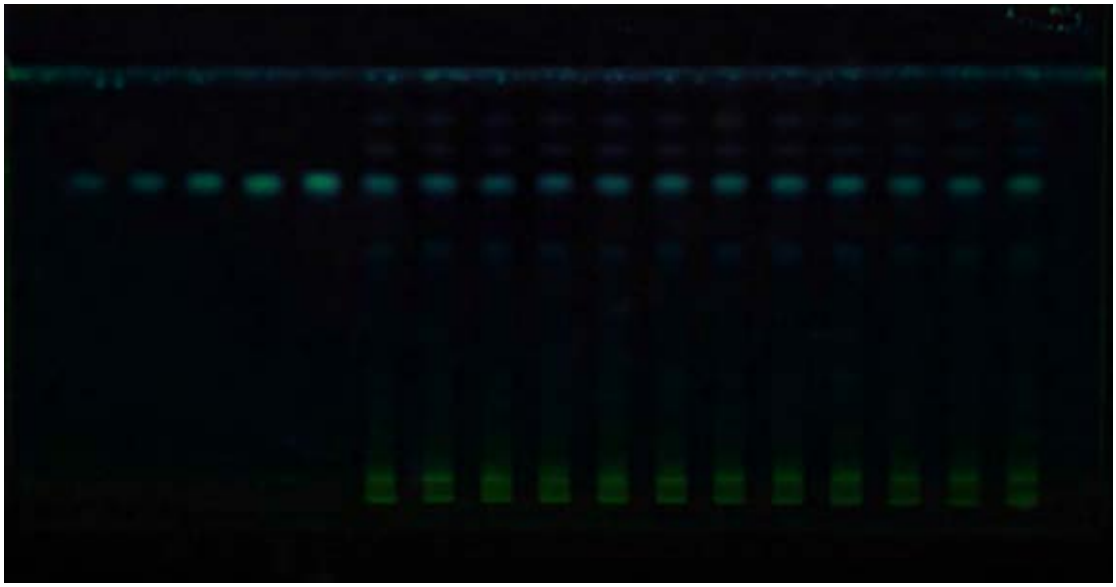


(B)

Figure 104. TLC plate 1 of precision method; TLC plate visual with 254 nm (A), TLC plate subtrack background by imageJ software (B)

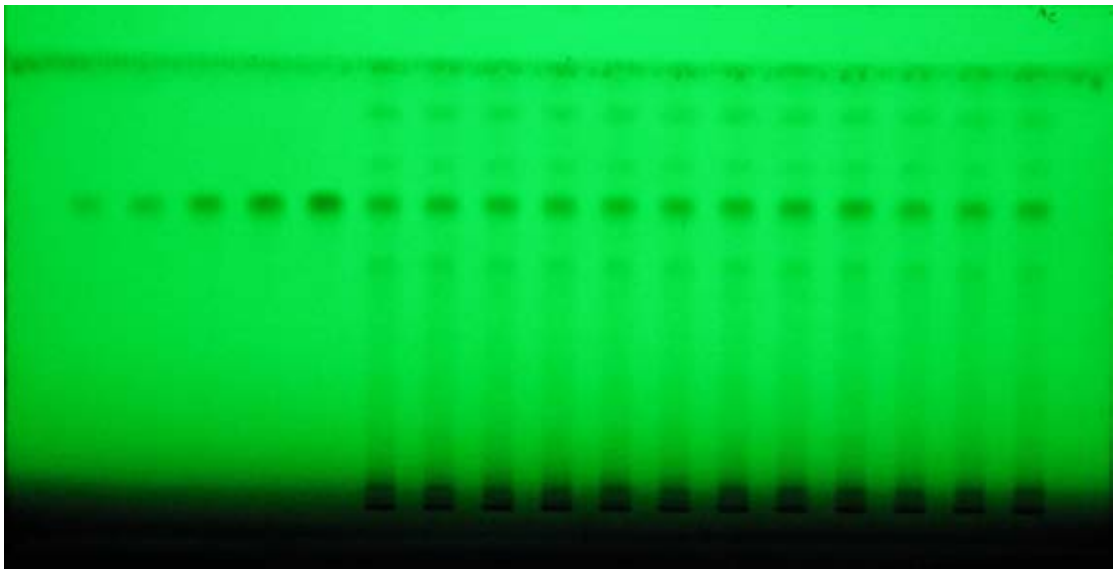


(A)

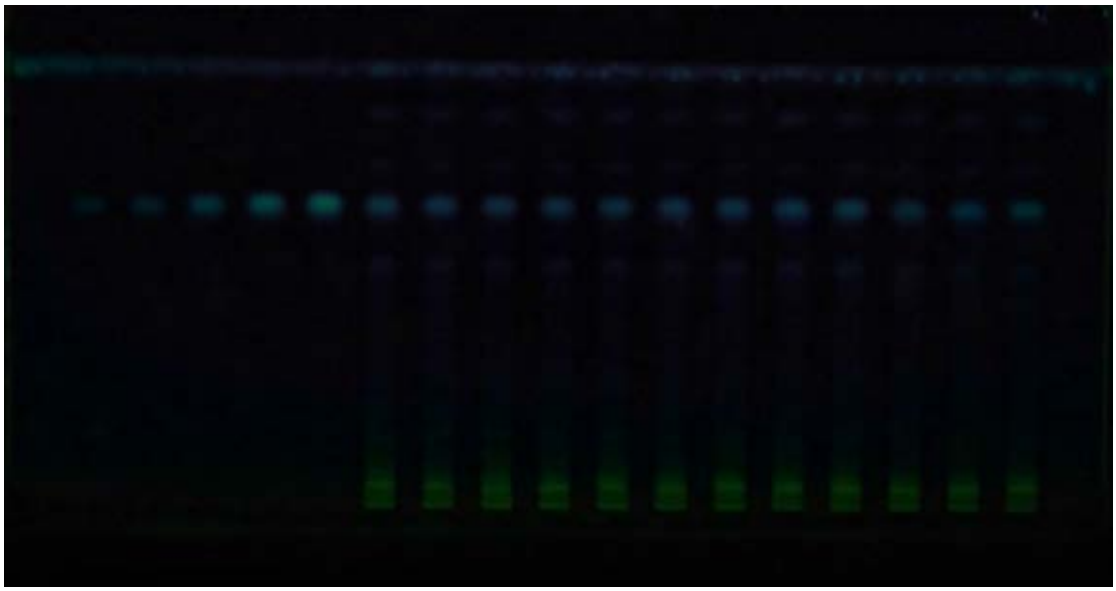


(B)

Figure 105. TLC plate 2 of precision method; TLC plate visual with 254 nm (A), TLC plate subtrack background by imageJ software (B)



(A)



(B)

Figure 106. TLC plate 3 of precision method; TLC plate visual with 254 nm (A), TLC plate subtrack background by imageJ software (B)

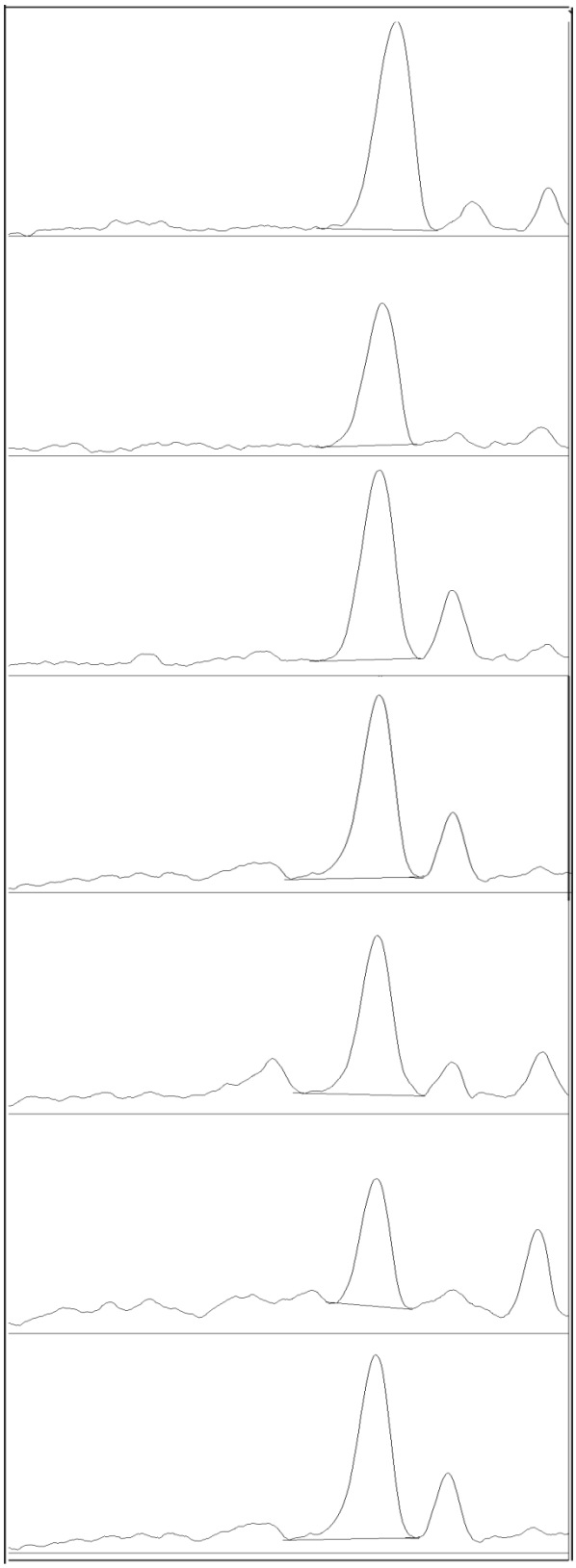


Figure 107. TLC image analysis chromatogram with imageJ software (13 sample extracts)

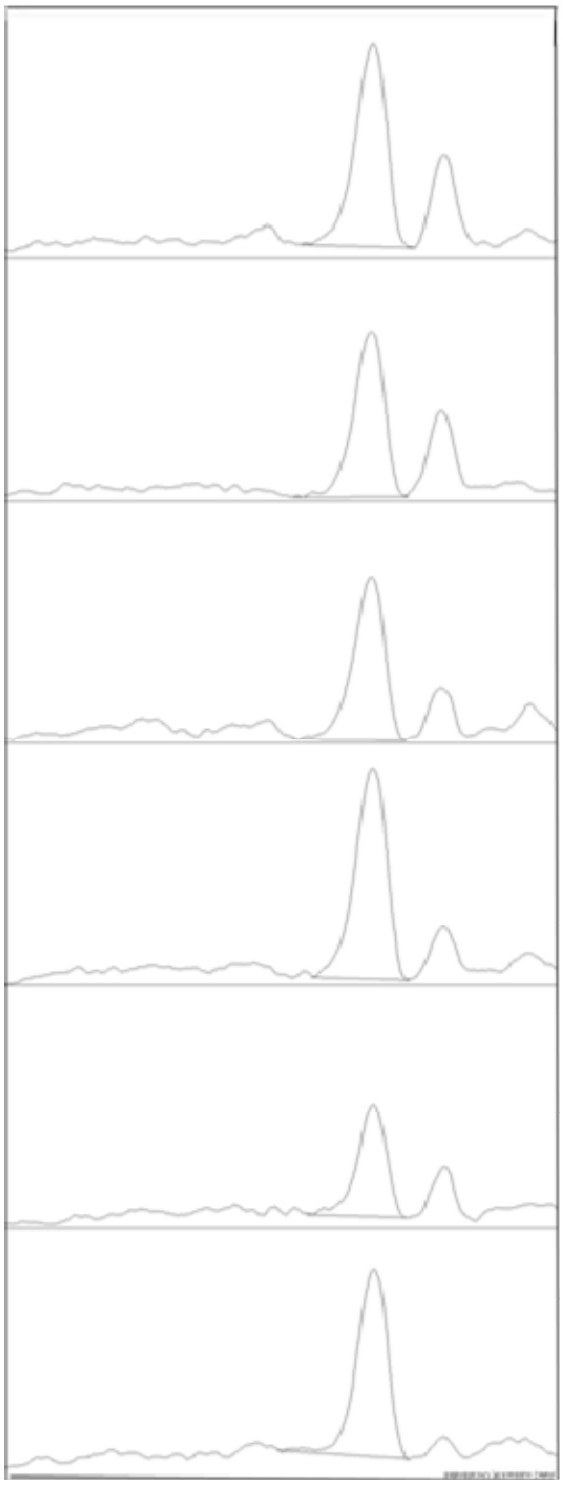


Figure 108. (Cont.) TLC image analysis chromatogram with imageJ software (13 sample extracts)

VITAE

Miss Supattra Prom-in was born on April 23, 1988 in Songkhla, Thailand. She got Bachelor's degree of Science, traditional Thai medicine faculty, Prince of Songkla University, Thailand in 2009.

Publication

Prom-in, S., Palanuvej, C., and Ruangrungsri, N. Qualitative and quantitative analyses of *Dendrophthoe pentandra* leaves with the special reference to quercitrin marker. Proceedings of the 39th Congress on Science and Technology of Thailand. Bangkok, 2013.