PHARMACOGNOSTIC SPECIFICATION AND ROSMARINIC ACID CONTENT OF *MENTHA CORDIFOLIA* LEAVES AND STEMS IN THAILAND

Miss Ploychat Chamnanthongpiwan

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ข้อกำหนดทางเภสัชเวทและปริมาณกรดโรสมารินิกในใบและลำต้นของ
สะระแหน่ในประเทศไทย

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น.ส.พลอยฉัตร ชำนาญท่องไพวัลห์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยา
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พลอยฉัตร ชำนาญท่องไพวัลห์ : ข้อกำหนดทางเภสัชเวทและปริมาณกรดโรสมารินิกในใบและลำต้นของสะระแหน่ในประเทศไทย. (PHARMACOGNOSTIC SPECIFICATION AND ROSMARINIC ACID CONTENT OF *MENTHA CORDIFOLIA* LEAVES AND STEMS IN THAILAND) อ.ที่ปรึกษาหลัก : อัญชลี ประสารสุขลาภ, อ.ที่ปรึกษาร่วม : ชนิดา พลานุเวช

สะระแหน่ (*Mentha cordifolia* Opiz ex Fresen.) เป็นสมุนไพรคู่ครัวไทย ที่มีกลิ่นหอม และมีฤทธิในการรักษาโรค เช่น ช่วยลดไข้ ลดอาการกระสับกระส่าย ขับเหงื่อ และช่วยย่อยอาหาร การศึกษานี้มีวัตถุประสงค์ในการระบุเกณฑ์มาตรฐานคุณภาพและเป็นเกณฑ์อ้างอิงปริมาณกรดโรสมารินิกของใบและลำต้นพืชสมุนไพรสะระแหน่แห้ง โดยจัดทำข้อกำหนดทางเภสัชเวทประกอบด้วยลักษณะทางจุลทรรศน์ของภาคตัดขวางของใบและลำต้น รวมทั้งค่าคงที่ของใบ วิเคราะห์ข้อกำหนดทางกายภาพและเคมีของเครื่องยา ได้แก่ ปริมาณเถ้าที่ไม่ละลายในกรด ปริมาณเถ้ารวม น้ำหนักที่หายไปเมื่อทำให้แห้ง ปริมาณความชื้น ปริมาณสิ่งสกัด และปริมาณน้ำมันระเหย วิเคราะห์องค์ประกอบทางเคมีของน้ำมันระเหยโดยวิธี GC/MS จัดทำลายพิมพ์ทางเคมีโดยวิธี TLC วิเคราะห์ปริมาณกรดโรสมารินิกโดยเตรียมสิ่งสกัดในเอธานอลด้วยเครื่องสกัดซ็อกห์เล็ต แยกและหาปริมาณโดยวิธี RP-HPLC ใช้คอลัมน์ intentsil® ODS-3 เป็นวัฏภาคคงที่ และใช้สารเมทานอล: กรดฟอสโฟริก 0.2% (45% : 55%)เป็นวัฏภาคเคลื่อนที่ ผลการศึกษาแสดงลักษณะกายวิภาคของใบและลำต้นพืชสะระแหน่ พบปากใบชนิดไดอะไซติก ทั้ง 2 ด้านของใบ มีค่าดัชนีของปากใบด้านบน 2.85 ± 0.64 ดัชนีของปากใบด้านล่าง 19.08 ± 2.65 พื้นที่เซลล์ผิวด้านบนและด้านล่าง 2128.64 ± 182.80 และ 1293.52 ± 262.45 ตารางไมโครเมตร ตามลำดับ ค่าดัชนีของขนด้านบน 0.45 ± 0.44 ค่าดัชนีของขนด้านล่าง 0.46 ± 0.33 มีต่อมน้ำมันมันกระจายอยู่ทั่วผิวใบด้านบนและด้านล่าง ค่าดัชนีของต่อมน้ำมันด้านบน 3.09 ± 0.89 ดัชนีของต่อมไขมันด้านล่าง 5.97 ± 1.75 ค่าดัชนีของเซลล์รั้ว 4.63 ± 0.35 ข้อกำหนดทางกายภาพและเคมีของใบสะระแหน่พบว่าปริมาณเถ้าที่ไม่ละลายในกรด ปริมาณเถ้ารวม น้ำหนักที่หายไปเมื่อทำให้แห้ง และปริมาณความชื้น ไม่มากกว่าร้อยละ 1.98, 9.41, 7.06 และ 10.50 โดยน้ำหนักตามลำดับ ปริมาณสิ่งสกัดด้วยเอธานอล ปริมาณสิ่งสกัดด้วยน้ำ และปริมาณน้ำมันระเหย ไม่น้อยกว่าร้อยละ 5.04, 15.23 และ 0.36 โดยน้ำหนักตามลำดับ ผลการศึกษาองค์ประกอบทางเคมีของน้ำมันระเหยพบว่าร้อยละ 73.22 คือ piperitenone oxide ข้อกำหนดทางกายภาพและเคมีของลำต้นสะระแหน่พบว่าปริมาณเถ้าที่ไม่ละลายในกรด ปริมาณเถ้ารวม น้ำหนักที่หายไปเมื่อทำให้แห้ง และปริมาณความชื้น ไม่มากกว่าร้อยละ 1.63, 8.41, 7.04 และ 10.01 โดยน้ำหนักตามลำดับ ปริมาณสิ่งสกัดด้วยเอธานอล และปริมาณสิ่งสกัดด้วยน้ำ ไม่น้อยกว่า 5.20 และ 16.63 โดยน้ำหนักตามลำดับ ผลการวิเคราะห์กรดโรสมารินิกโดยวิธี RP-HPLC พบว่า ปริมาณกรดโร

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Mentha cordifolia Opiz ex Fresen. is one of the popular Thai kitchen herbs due to its unique aroma and benefits to human health such as helping to relieve colds, fever, motion sickness and poor digestion problems. This study aimed to specify the quality parameters of *M. cordifolia* dried leaves and stems with special reference to rosmarinic acid (RA) contents. The pharmacognostic specifications of leaf and stem crude drugs were investigated including microscopic cross-sectioning of leaf and stem as well as leaf constant numbers; physico-chemical contents i.e. acid-insoluble ash, total ash, loss on drying, moisture, extractive matters and volatile oil. The chemical constituents of the volatile oil were analyzed by GC/MS. TLC fingerprints of these crude drugs were demonstrated. The ethanolic extracts were prepared exhaustively using Soxhlet apparatus. RA quantitative analysis was made by reverse phased high performance liquid chromatography (RP-HPLC) using intentsil® ODS-3 column as stationary phase and methanol: 0.2% phosphoric acid (45% : 55%) as mobile phase. The results demonstrated the anatomical characters of leaf and stem cross-sections. The stomata (diacytic type) were found in upper and lower sides of leaf. The microscopic leaf constant numbers were found to be as follows: upper stomatal index 2.85 ± 0.64, upper trichome index 045 ± 0.44, upper oil gland index 3.09 ± 0.89, upper epidermal cell area 2128.64 ± 182.80 μm^2, palisade ratio 4.63 ± 0.35; lower stomatal index 19.95 ± 2.65, lower trichome index 0.46 ± 0.33, lower oil gland index 5.97 ± 1.75 and lower epidermal cell area 1793.52 ±262.45 μm^2. The physico-chemical specification demonstrated the contents of acid-insoluble ash, total ash, loss on drying and moisture in leaf should not be more than 1.98, 9.41, 7.06 and 10.50 % by weight respectively; the ethanol-soluble extractive matter, water-soluble extractive matter and volatile oil in leaf should not be less than 5.04, 15.23 and 0.36 % by weight respectively. The main chemical compound in the volatile oil was piperitenone oxide (73.22%). The specification contents of acid-insoluble ash, total ash, loss on drying and moisture in stem should not be more than 1.63, 8.41, 7.04, 10.01 % by weight respectively; the ethanol-soluble extractive matter and water-soluble extractive matter in stem

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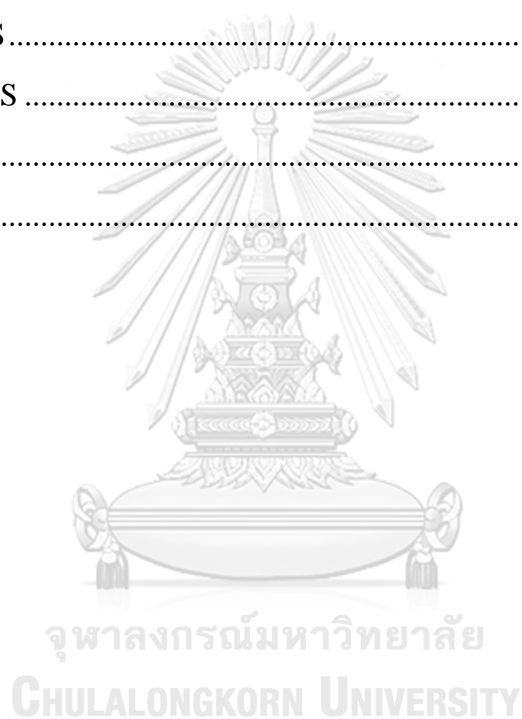

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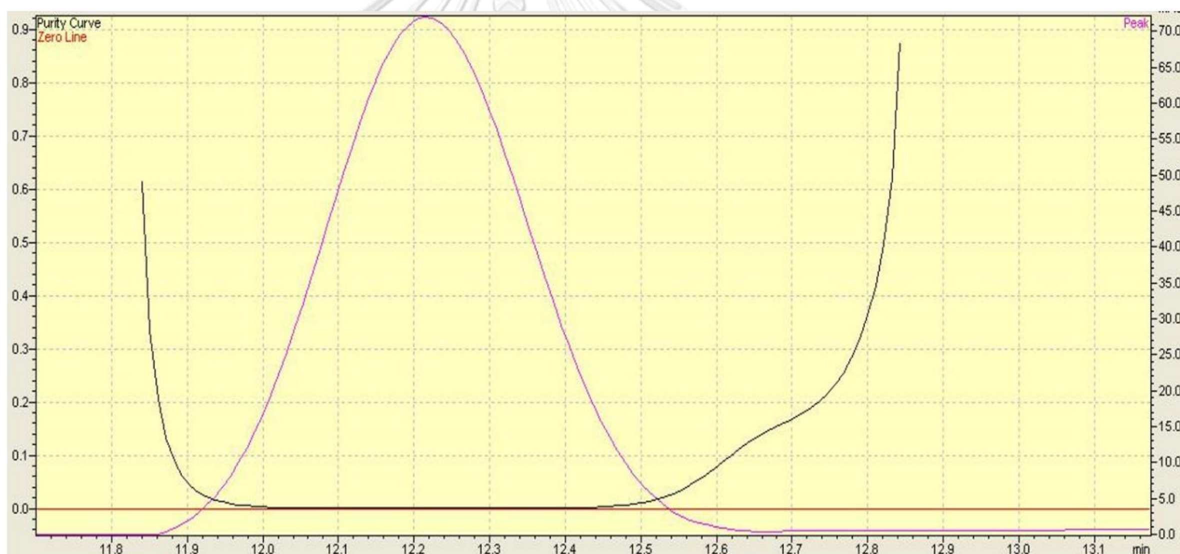

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CHAPTER I

INTRODUCTION

1.1 Background and rationale

Mentha cordifolia Opiz ex Fresen. (สะระแหน่) is one of the popular Thai kitchen herbs in Mentha species that emerged due to hybridization of *Mentha suaveolens* Ehrh. and *Mentha spicata* L.(Lawrence B. M., 2007) It was in the famous herbal preparation such as Ya Lueat Ngam (ยาเลือดงาม) (GPO, 2013), which was treated as a blood tonic in menstrual disorders and leucorrhea use. (Chotchoungchatchai et al., 2012) A major component of *M. cordifolia* is rosmarinic acid (RA); a phenolic compound and ester of caffeic acid, which is naturally occurring in several plants of the Lamiaceae family.

RA is the second most common ester of caffeic acid in the plant kingdom, especially in Lamiaceae and Boraginaceae. It is biosynthesized *via* shikimic acid pathway from phenylalanine and tyrosine. (Ellis and Towers, 1970) Many *in vitro* and *in vivo* pharmacological activities of RA have been studied and reported such as antioxidative, anti-inflammatory, antiproliferative, antimutagenic, cytoprotective and immunomodulation properties. Clinical studies with RA showed atopical dermatitis-mitigating and seasonal allergic rhinoconjunctivitis effects. (Amoah et al., 2015)

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A system of standardization is very important to ensure the quality of medicinal plant materials in the market. World Health Organization (WHO) is continuously emphasizing in quality control of medicinal plant products especially using modern techniques for chemical marker analysis. (WHO, 2011)

High performance liquid chromatography (HPLC) is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture. (Boligon and Athayde, 2014.) The characterization of HPLC is to force a mobile phase solution through a column; stationary phase, the differences of their

partition and adsorption behaviors between the mobile phase and the stationary phase allow separation of complex mixture by the use of high pressure. The aim of the present study was to evaluate pharmacognostic specification of *M. cordifolia* in Thailand and to determine the rosmarinic acid contents by high performance liquid chromatography (HPLC) for used as a tool for quality control of this Thai herbal crude drug.

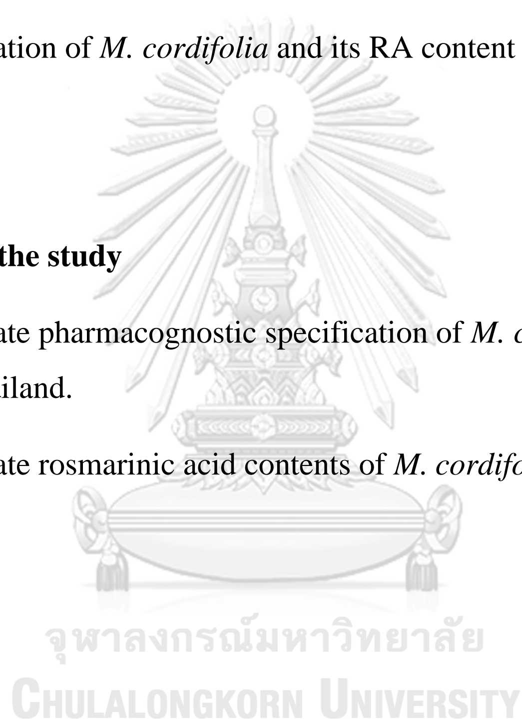
1.2 Research gap

Standardization of *M. cordifolia* and its RA content have not been reported in Thailand.

1.3 Objectives of the study

1.3.1 To evaluate pharmacognostic specification of *M. cordifolia* leaf and stem crude drugs in Thailand.

1.3.2 To evaluate rosmarinic acid contents of *M. cordifolia* leaf and stem crude drugs in Thailand.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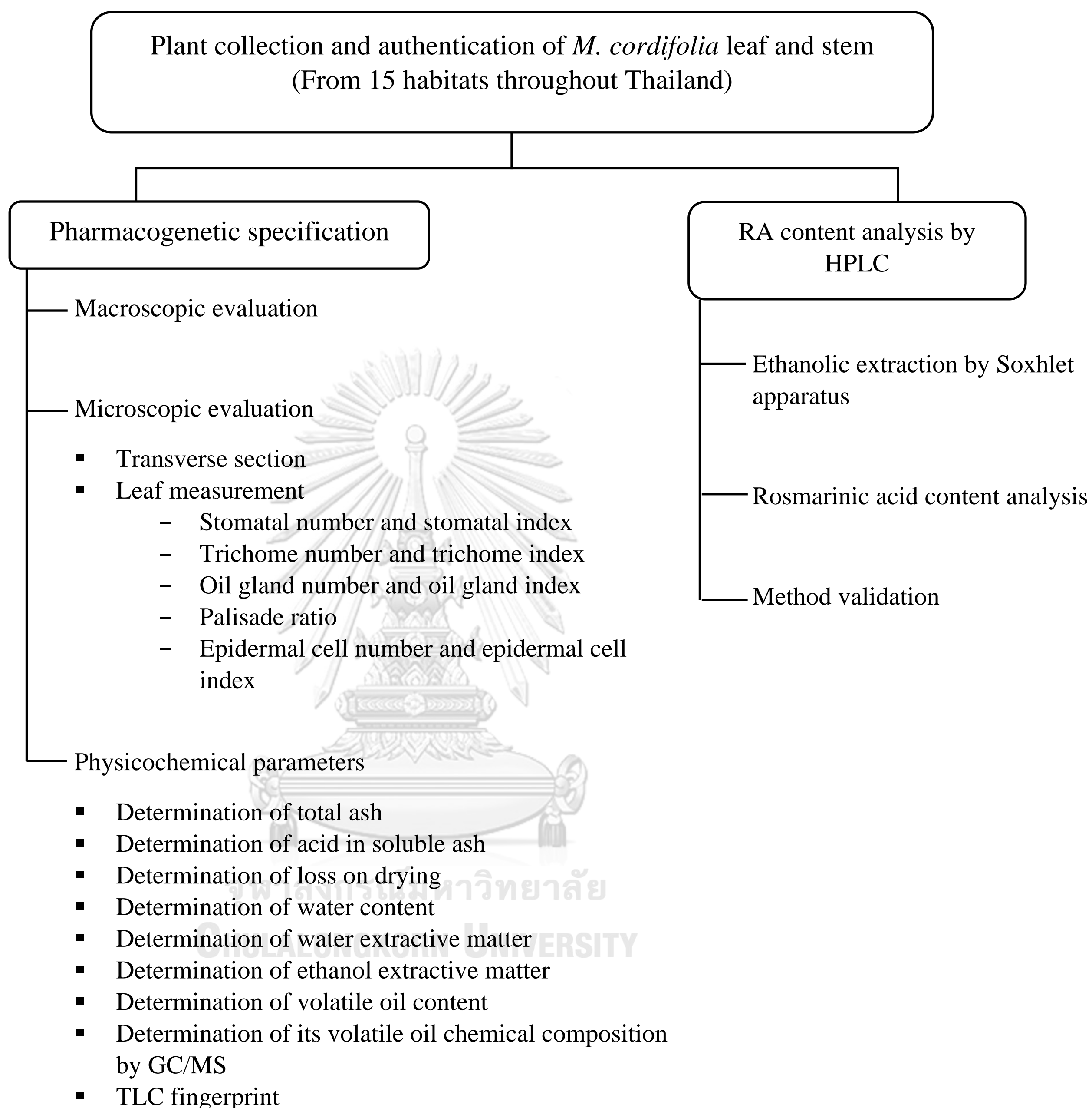
1.5 Conceptual framework



- Determination of total ash
- Determination of acid in soluble ash
- Determination of loss on drying
- Determination of water content
- Determination of water extractive matter
- Determination of ethanol extractive matter
- Determination of volatile oil content
- Determination of its volatile oil chemical composition by GC/MS
- TLC fingerprint

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CHAPTER II

LITERATURE REVIEWS

Part I : Overview of *Mentha cordifolia* Opiz. ex Frezen.

2.1 *Mentha cordifolia* Opiz ex Fresen.

2.1.1 Classification of *M. cordifolia* (GBIF, 2019)

Kingdom: Plantae

Phylum: Trecheophyta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus: Mentha

Species: *Mentha cordifolia* Opiz ex Fresen.

2.1.2 Synonyms: *Mentha* × *villosa* Huds.

2.1.3 Vernacular names

Bowles mint, Apple mint, Mojito mint, Mash mint, Kitchen mint and Saranae

2.1.4 Plants description (Soares, 2010)

M. cordifolia is an herbaceous, low growing plant with erect branches, opposite and short petiolate oval leaves, with strong and characteristic aroma.

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2.1.5 Distribution of *M. cordifolia* (Bezerra et al., 2019)

M. cordifolia is a plant from subtropical climate susceptible to intense winter.

2.1.6 Traditional use

Its leaves are also being used empirically in traditional medicine in Cameroon to treat insomnia and muscle relaxant. (Bum et al., 2011) In Thailand *M. cordifolia* is not simply a famous kitchen herb due to its tantalizing aroma, in fact due to its unique aroma and benefits to human health such as helping to relieve colds, flu, fever, motion sickness and poor digestion problems. (Başer et al., 2012) It also was in the famous herbal preparation such as Ya Lueat Ngam (ยาเลือดงาม) (GPO, 2013), which was treated as a blood tonic in menstrual disorders and leucorrhea use. (Chotchoungchatchai et al, 2012)

2.1.7 Pharmacological activities of *M. cordifolia*

Bum E.N. et al., 2011 found the extracts of *M. cordifolia* increased in a dose-dependent manner the sleeping time induced by sodium thiopental or diazepam. The decoctions of plants multiplied by a factor of 2 the sleeping time of their control group: *M.cordifolia* (from 10 ± 2 to 24 ± 3 min at a dose of 140 mg/kg) protected mice against strychnine- induced convulsions, protected against convulsions induced by pentylenetetrazol and protected mice against convulsions induced by picrotoxine These sedative properties could be related to the presence of some components in the extracts activating the benzodiazepine, barbiturate and/or GABA receptors in the GABAA receptor complex. The antagonism of pentylenetetrazol- and picrotoxine -induced seizures suggests the interaction of these plants with the GABA-ergic neurotransmission. The antagonism of strychnine -induced convulsions suggests the presence of anticonvulsant effect through glycine- strychnine -sensitive receptors.

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Amaral et al., 2015 studied the cytotoxic and antitumor effects of the essential oil from *M. cordifolia* (EOMC). The results show that non-polar hexane extract is analgesic, anti-intestinal parasitic and central nervous system depressant. The essential oil from leaves has reported that possesses significant cytotoxic and antitumor activity with low systemic toxicity. It is possible that these actions of the essential oil are related to the synergistic action of its minor constituents. According to many studies have demonstrated that several constituents found in EOMC, including sabinene, β-pinene, myrcene, limonene and germacrene D show cytotoxic and anticancer activity justifying the in-vitro effect shown by EOMC and its possible action in vivo.

Fialovaa et al., 2015 reported confirmation of high antioxidant activity of this phenolic-rich water extract in *vitro* by DPPH and ABTS tests. They also tested *ex vivo* in the ischemia-reperfusion injured rat superior mesenteric artery. The result showed that antioxidant activity of the water extract of *M. cordifolia* leaves possible to prevent oxidative stress tissue injury.

Lahlou et al., 2002 investigated the effected of EOMC by intravenous injection in chronic treatment with deoxycorticosterone-acetate (DOCA)-salt on cardiovascular conscious rats. The results showed that both of DOCA-salt-hypertensive and uninephrectomized control and bolus injections of EOMC (1 to 20 mg/kg body weight), conscious rats, decreased mean aortic pressure, heart rate and decreases blood pressure in conscious DOCA-salt-hypertensive rats dose-dependently. These actions could be related to an increase in EOMC-induced vascular smooth muscle relaxation, rather than to enhanced sympathetic nervous system activity in this hypertensive model.

2.1.8 Phytochemical

The main phenolic compounds in mint are phenolic acids and flavonoids (Fialová et al., 2015). *M. cordifolia*.is one of the mint species. The chemical constituent of *M. cordifolia* has been reported in some previous studies which were shown in the Table 1.

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Table 1 Compounds in *M. cordifolia* plants.

Compound	Plant part	References
3β-O-acetylolean-12-en-28-oate	Aerial parts	Monte et al., 2001
Methyl 3β- O-acetylurs-12-en-28-oate	Aerial parts	Monte et al., 2001
Methyl 2α,3α-di-O-acetylurs-12-en-28-oate	Aerial parts	Monte et al., 2001
Methyl 2cz,3r acetylolean-12-en-28-oate	Aerial parts	Monte et al., 2001
Methyl 3β,24-di-O-acetylurs-12-en-28-oate	Aerial parts	Monte et al., 2001
2α,3α-di-O-acetylolean-12-en-28-oate;	Aerial parts	Monte et al., 2001
Methyl 2α,3β,24-tri-O-acetylurs-12-en-28-oate	Aerial parts	Monte et al., 2001
Methyl 2α,3β,24-tri-Oacetylolean-12-en-28-oate	Aerial parts	Monte et al., 2001
2α,3β,24-tri-O-acetylolean-12-chloro-28-13-olide	Aerial parts	Monte et al., 2001
6,7-bis-(2,2-dimethoxyethene)- 2,11-dimethoxy-2Z,4E,8E,10Z-dodecatetraendioic acid.	Leaf	Villaseñor and Sanchez, 2009
β-Sitosterol	Leaf	Villaseñor et al., 2002
β-sitosteryl-β-D-glucoside	Leaf	Villaseñor et al., 2002

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Compound	Plant part	References
Menthalactone	Leaf	Villaseñor and Sanchez, 2009
Eriodictyol-7-O-rutinoside	Leaf	Fialova et al., 2015
Luteolin-7-O-glucuronide	Leaf	Fialova et al., 2015
Hesperetin-7-O-rutinoside	Leaf	Fialova et al., 2015
Quinic acid	Leaf	Fialova et al., 2015
Chlorogenic acid	Leaf	Fialova et al., 2015
p-coumaroyl-hexoside	Leaf	Fialova et al., 2015
Coumarolyquinic acid	Leaf	Fialova et al., 2015
Apigenin-glucuronide	Leaf	Fialova et al., 2015
Kaempferol-3-O-glucuride	Leaf	Fialova et al., 2015
Rosmarinic acid	Leaf, underground part	Tekeľová et al.,2016; Fialova et al., 2015; Fialova et al., 2020
Lithospermic acid	Leaf, underground part	Fialova et al., 2015; Fialova S. et al., 2020
Salvianolic acid A	Leaf, underground part	Fialova et al., 2015; Fialova et al., 2020
Lutenolin-7-O-rutinoside	Leaf, underground part	Fialova et al., 2015; Fialova et al., 2020
Luteolin	Leaf, rhizome	Tekeľová et al.,2016;; Fialova et al., 2013
Photocatechuic aldehyde	Underground part	Fialova et al., 2020

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Compound	Plant part	References
Caffeic acid	Underground part	Fialova et al, 2015; Fialova et al, 2020
Eriodictyol-7-O-rutinoside	Underground part	Fialova et al., 2020
2-(3,4-dihydroxyphenyl) ethyl ester of Salvianolic acid D	Underground part	Fialova et al., 2020
Hesperetin-7-O-rutinoside	Underground part	Fialova et al., 2020
Salvianolic acid B	Underground part	Fialova et al., 2020
Caffeic acid tetramer	Underground part	Fialova et al., 2020
Ursolic acid	-	Villaseñor and Sanchez, 2009
Sitosterol	-	Villaseñor et al., 2002
Stigmasterol	-	Villaseñor et al., 2002

In *M. cordifolia* essential oil also has reported the chemical identification. Previous study identified and compared the chemical in essential oil obtained from plants cultivated in north eastern Brazil, Greece and USA which shown in the Table 2 below. The oil was found to be rich in piperitenone oxide (55.4 %) and γ-muurolene (13.1%). (Matos et al, 1999)

Table 2 Percentage composition of oil of *M. cordifolia* from Northeastern Brazil, Greece and USA. (Matos et al, 1999)

Compound	RI	Northeastern Brazil	Greece		USA
			1	2	
α - pinene	926	0.7	0.32	0.51	0.4 – 0.8

Compound	RI	Northeastern Brazil	Greece		USA
			1	2	
Camphene	-	-	0.01	0.01	-
β - pinene	957	1.1	1.38	1.85	0.3 – 0.8
Sabinene	955	0.8	0.12	0.21	0.1 – 0.6
Myrcene	976	3.2	0.50	0.35	0.1 – 1.2
3 – octanol	-	-	-	-	0.1 – 0.3
ρ - cymene	-	-	0.05	0.02	0.0 – 0.2
α - terpinene	-	-	-	-	0.0 – 0.3
Limonene	1009	2.5	1.18	2.02	1.0 – 6.8
1, 8 - cineole	1005	2.1	5.93	7.31	0.2 – 4.3
(Z) – β - ocimene	1020	3.5	-	-	0.0 – 0.6
(E) – β - ocimene	1030	0.4	-	-	0.0 – 0.2
γ - terpinene	-	-	0.21	0.15	0.0 – 0.2
Cis – sabinene hydrate	-	-	-	-	0.0 – 1.5
Terpinolene	-	-	0.01	0.01	0.0 – 0.2
Linalool	1078	0.4	<0.01	<0.01	0.7 – 2.5
Trans – sabinene hydrate	-	-	-	-	0.0 – 1.5
Octyl acetate	1108	0.7	-	-	-
Borneol	1138	0.3	-	-	-
3 – octyl acetate	-	-	0.21	0.38	0.0 – 0.3
1 – octen – 3 - ol	-	-	-	-	0.0 – 9.1

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Compound	RI	Northeastern Brazil	Greece		USA
			1	2	
Menthone	-	-	0.10	1.02	0.0 – 2.1
Isomenthone	-	-	-	-	0.1 – 0.3
Terpinene – 4 - ol	-	-	0.19	0.28	0.0 – 1.8
β - copaene	-	-	-	-	Tr – 0.4
Cis - dihydrocavone	-	-	-	-	5.4 – 14.1
Trans - dihydrocarvone	-	-	-	-	0.0 – 1.4
α - terpineol	-	-	-	-	0.2 – 1.8
Trans - carveol	-	-	-	-	0.4 – 0.8
Cis – carveol.	-	-	-	-	0.6 – 1.4
Pulegone	-	-	0.18	0.30	0.0 – 0.4
Dihydrocarvyl acetate	-	-	-	-	1.7 – 18.0
Neodihydrocarveol	-	-	-	-	Tr – 0.6
Piperitenone oxide	1342	55.4	0.15	61.18	-
β - bourbonene	1382	0.3	0.15	0.24	0.7 – 2.6
β - elemene	1390	0.5	-	-	-
Caryophyllene	1414	3.4	2.95	4.51	0.6 – 1.2
Cis – muurola – 4(14), 5 - diene	1440	1.3	-	-	-
α - humulene	1445	0.6	-	-	-
Trans - muurola – 4(14), 5 - diene	1454	1.4	-	-	-

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Compound	RI	Northeastern Brazil	Greece		USA
			1	2	
(E) – β - farnesene	1458	1.4	-	-	-
γ - muurolene	1474	13.1	-	-	-
Bicyclogermacrene	1486	1.1	-	-	-
Germacrene A	1493	0.6	-	-	-
Cis - calamenene	1505	0.3	-	-	-
δ - cadinene	1513	0.3	1.53	0.95	-
1, 10 – di -epi -cubenol	1590	0.5	-	-	-
T - muurolol	1621	0.44	-	-	-
Total	80.4		-	-	-

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Part II Description, biosynthesis and biological activities of rosmarinic acid

2.2 Rosmarinic acid (Pubchem, 2004)

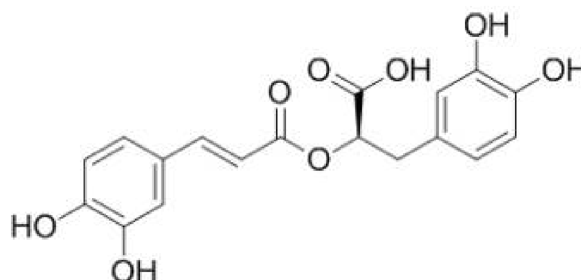

Table 3 Structure of RA molecule

2.2.1 Description

IUPAC Name:	(2R)-3-(3,4-Dihydroxyphenyl)-2-{[(2E)-3-(3,4-dihydroxyphenyl)-2-propenoyl]oxy}propanoic acid
Molecular formula:	$C_{18}H_{16}O_8$
Molecular weight:	360.318 g/mol
Melting point:	171-175 °C

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RA is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, one of the polyphenolic substances contained in culinary herbs. It was first isolated and purified from the plant, Rosmary (*Rosmarinus officinalis*) in the family Lamiaceae. These herbs are commonly grown in the garden as kitchen herbs, and while used to add flavor in cooking, are also known to have several potent physiological effects.

The precursors from primary metabolism for the biosynthesis of RA have been identified by feeding radioactive amino acids to plants of Mentha. Phenylalanine was mainly incorporated into the caffeic acid part of the molecule, whereas tyrosine and DOPA gave rise to the 3,4-dihydroxyphenyllactic acid moiety (Ellis and Towers, 1970). The sample of Biosynthetic pathway for RA as found in suspension cultures of *Coleus blumei* was shown in Figure 2.

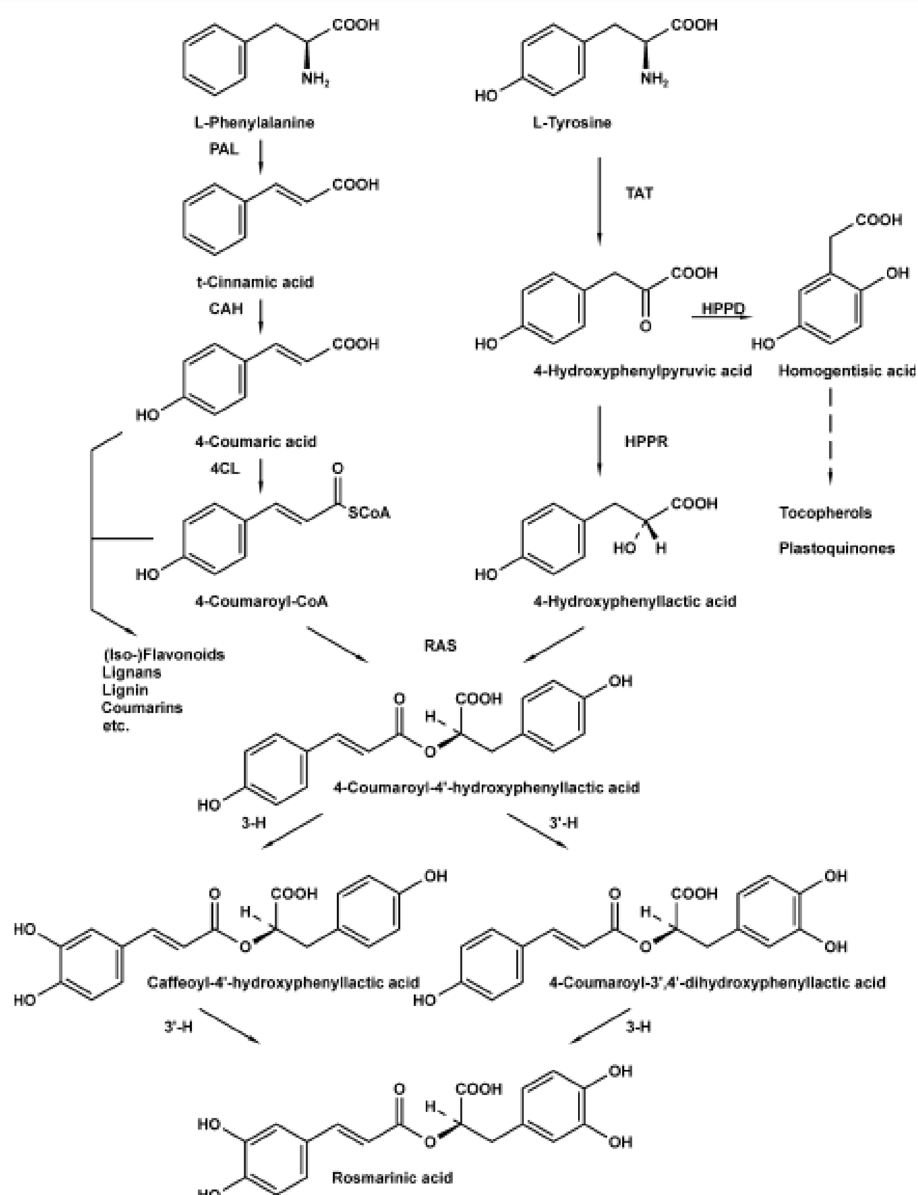

Table 4 Biosynthetic pathway for RA as found in suspension cultures of *Coleus blumei*. PAL=phenylalanine ammonia-lyase, CAH= cinnamic acid 4-hydroxylase, 4CL=hydroxycinnamate:coenzyme A ligase, TAT=tyrosine aminotransferase, HPPR=hydroxyphenylpyruva (Petersen and Simmonds, 2002.)

2.2.2 Biological and pharmacological activities of RA

The biosynthesis of RA, a phenolic ester has been widely investigated. This compound has shown many remarkable biological and pharmacological activities, which have led to its pharmaceutical and analytical development

RA has a range of biological activities, making it an interesting material for the pharmaceutical, food, and cosmetics industries. In the Table 3 was shown its biological and pharmacological activities.

Table 5 Some biological and pharmacological activities of RA

Biological and pharmacological activities	Potential usage	References
Antioxidant and anti-inflammatory effects	Prevented the diabetes-induced aortic disorder	Sotnikova et al., 2013
Inhibit activation of the nuclear factor- kappa B pathway by inhibiting IkappaB kinase activity	Inactivate inflammatory response in sepsis	Jiang et al., 2009
Inhibited the enhanced protein expression of IL-4 and IL-5, and eotaxin in the lungs of sensitized mice	Intervention for allergic asthma	Sanbongi et al., 2004
Inhibition of the inflammatory response and scavenging of ROS	An effective intervention for patients with seasonal allergic rhinoconjunctivitis	Osakabe et al., 2004; Osakabe et al., 2008
Reducing numbers of leukocytes/eosinophils in bronchoalveolar lavage (BAL)	Therapeutic potential in this murine model of respiratory allergy	Costa et al., 2012
Inhibition of RA on ADR-induced apoptosis in H9c2 cardiac muscle cells at a mechanistic level	Inhibitory effect on cardiotoxicity in tumor patients	Kim et al., 2005
Inhibit the viability of pterygium epithelial cells (PECs) through regulation of	A therapeutic medication for pterygium	Chen et al., 2017

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Biological and pharmacological activities	Potential usage	References
extrinsic and intrinsic apoptosis pathways		
RA-mediated neuroprotection in SH-SY5Y cells was involved in the attenuation of apoptotic cell death and modulation of antioxidative molecule heme oxygenase-1 (HO-1)	The prevention of neurodegenerative diseases	Lee et al., 2008
Antioxidant activity and membrane stabilization		Perez-Fons et al., 2010
Reduction of the frequency of micronuclei and the extent of DNA damage induced by doxorubicin	Prevention against chemically induced chromosome breakage and primary DNA damage	Panya et al., 2010
Increase of the physical and oxidative stability of liposomes		Furtado et al., 2009
Suppression of UVB-induced alterations to human keratinocytes	Skin protection against UVB light	Vostalova et al., 2010
Reduction of IFN-ɣ and IL-4 production by activated T cells	Skin protection against atopic dermatitis	Jang et al., 2011
Inhibiting NF-kB activation, and inducing PPARg	RA is a promising neuroprotective compound of	Fallarini et al., 2009

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Biological and pharmacological activities	Potential usage	References
expression	potential use at the nutritional/pharmaceutical interface	
Cognitive-enhancing effect		Park et al., 2010
RA reduced both Aβ deposition and rabbit amyloid -positive oligomers		Hamaguchi et al., 2009
RA imparted a prominent effect on motor performance, body weight loss, morphology of motor neurons, and clinical scoring as well as the survival of ALS model mice		Shimojo et al., 2010
RA down-regulates the LPS-induced production of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory Protein-1α via the MAPK Pathway in bone-marrow derived dendritic cells	The development of therapeutic drugs for the treatment of dendritic cells-related acute and chronic diseases	Kim et al., 2008
Inhibition of TNF-a-induced ROS generation and NF-kB activation and activation of TNF-a-induced apoptosis	Promising for cancer prevention and treatment of variety of human cancer that are resistant to chemotherapy	Moon et al., 2010
Inhibition of bone metastasis from breast carcinomas		Xu et al., 2010
Antifibrotic activity	Drug candidate for	Li et al., 2010

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Biological and pharmacological activities	Potential usage	References
	ameliorating liver fibrosis	
Dramatic apoptotic activity on potentially pathogenic CD4 CD45RO effector T cells	Treatment of rheumatoid arthritis	Hur et al., 2006
Inhibiting the ROS–NLRP3 inflammasome–CRP axial	Protective role of RA in nicotine-induced atherosclerosis	Yao et al., 2018

2.2.3 Quantitative analysis of RA in *M. cordifolia* by HPLC

Various chromatographic methods are used for RA content analysis such as High-Performance Liquid Chromatographic (HPLC), Capillary Zone Electrophoresis. This study used HPLC.

High performances liquid chromatography (HPLC)

HPLC is an extremely versatile technique where analytes are separated by passage through a column packed with micrometer-sized particles. It is the most accurate analytical methods widely used for the quantitative. In the modern pharmaceutical industry, HPLC is the major and integral analytical tool applied in all stages of drug discovery, development, and production. This technique depends on difference rate of a mixture moves through a porous medium i.e. stationary phase under the influence of some solvent i.e. mobile phase under high pressure. (S.L. et al., 2015) The results in the separation of components will show that the molecules in the sample will have distinct affinities for the stationary phase. The component of sample which shows strong interactions with the stationary phase will move slowly through the column than the component shows weaker interactions. (Kupiec, 2004)

Reversed-phase HPLC (RP-HPLC) is the most commonly used mode of HPLC. Generally, RP-HPLC has a nonpolar stationary phase, e.g., C8 silica (Table 4), and a moderately polar aqueous mobile.

Table 6 Commonly used stationary phases and their associated modes in HPLC Stationary phases Modes

Stationary phases	Modes
C6 silica	Reversed-phase
C8 silica	Reversed-phase
C18 silica	Reversed-phase
Silica	Normal phase
Diol	Normal and reversed-phase
Cyano (CN)	Normal and reversed-phase
Benzene sulphonic acid	Strong cation exchange
Polystyrene	Size exclusion

The mobile phase used for eluent in RP-HPLC is usually composed of a mixture of water and miscible organic solvents, usually acetonitrile, MeOH, or tetrahydrofuran (Table 5). Sometimes, buffers, acids, or bases are also added to suppress compound ionization or to control the degree of ionization of free unreacted silanol groups to reduce peak tailing and improve chromatography. (Latif and Sarker, 2012)

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Table 7Commonly used mobile phases in RP-HPLC

Mobile phases	**Polarity index (Snyder)**	**UV-cutoff (nm)**
Acetonitrile	6.2	190
Isopropanol	4.3	210
Methanol	6.6	205
Tetrahydrofuran	4.2	212 - 230
Water	9.0	180

In RP-HPLC there is strong attraction between the polar solvent and polar molecules in the mixture being passed through the column, but there is not much attraction between the hydrocarbon chains attached to the stationary phase and the polar molecules in the solution. Therefore, polar molecules in the mixture spend most of their time moving with the solvent. Nonpolar compounds in the mixture tend to form attractions with the hydrocarbon groups because of van der Waals dispersion forces. They are less soluble in the solvent because of the need to break hydrogen bonds as they squeeze in between the water or methanol molecules. They spend less time in solution in the solvent, and this slows them down on their way through the column, which means longer retention time. In RP-HPLC the polar molecules travel through the column more quickly. RP-HPLC allows purification of most classes of compounds, including compounds present in various herbal products, and is often the most preferable choice when analyzing and attempting to separate and identify compounds from a complex mixture (Snyder et al, 1997)

Part III : Quality control methods for herbal materials

Current Regulations for Standardization of Crude Drugs Internationally several pharmacopoeias have provided monographs stating parameter and standard of many herbs and some product made from these herbs. Several pharmacopoeias such as Pharmacopoeia Committee, Chinese Herbal Pharmacopoeia, United States Herbal Pharmacopoeia, British Herbal Pharmacopoeia, British Herbal Compendium, Japanese Standards for Herbal Medicine, The Ayurvedic Pharmacopoeia of India (API) and so on contain information including plant name (both Russian and Latin), plant part, recommended collection time, macroscopic evaluation for whole and pulverized plant material, microscopic observation, quantitative data (loss of material on drying, concentration of chemical constituents or biological activity, ash content, acid in soluble ash, broken parts, organic and mineral contamination). HPLC traces and TLC chromatograms of adulterants, qualitative assay (chemical reactions or chromatography) fraction sieve analysis (for pulverised material), packaging, storage conditions, shelf life, and pharmacological group. (Shikov et al., 2014; Mehta et al., 2011)

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Standardization is the gathering of complete data on medicinal plants including the qualitative and quantitative parts of analysis. Qualitative analysis covers the identification of the compound, performed by measuring the level of a chemical in a crude drug extract. The standardization will help in authentication of the plants and ensures reproducible quality of herbal medicines which will lead to safety and efficacy.

Authentication is one of the most important step of identification methods for Identification of right variety and search of adulterants.

2.3.1 Macroscopic evaluation

The macroscopic characteristics are useful for determining the identity and purity of the herbal drug including shape, size, color, texture, fracture aspects and characteristics of the cut surface. These techniques may be used to discriminate between the desired plant species or plant part and morphologically similar, yet distinguishable species that could occur as potential adulterants (Abu-Hamdah et al., 2008)

2.3.2 Microscopic evaluation

The microscopic characteristics helps to identify the herbal drug and may be crucial in the identification of adulterants. This analysis is necessary for powdered or fragmented drugs. Using microscope detecting various cellular tissues, trichrome, stomata, starch granules, calcium oxalate crystals are some of important parameters which play important role in identification of certain crude drug. The measurement contains many parameters such as stomatal number, stomatal index, epidermal cell number, epidermal cell area and palisade ratio. (Indian Pharmacopoeia, 2010)

Crude drug can also be identified microscopically by cutting the thin transverse section or longitudinal section. Some of the chemicals which are used in obtaining clear sections are phloroglucinol, chloral hydrate, etc. The microscopic characters are usually described by words or coupled with pictures drawn by hands or photos of both transverse sections and powder characteristics. The microscopic

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analysis should be supplemented with data from chemical and physico-chemical analysis (Thomas et al., 2008).

Stomata

There are four types of stomata, distinguished by the forms and arrangement of the surrounding cells. (Patil et al., 2013)

(a) Anomocytic (Ranunculaceous) irregular cell

(b) Anisoytic (Cruciferous) unequal cell

(c) Diacytic (Caryophyllaceous) cross cell

(d) Paracytic (Rubiaceous) parallel cell

Palisade ratio

Palisade ratio is another criteria for identification and evaluation of herbal drugs. The palisade cell present beneath upper epidermal cell. It can be found in fine powders. This value remains constant within a range for a given plant species and is of diagnostic value in differentiating the species. It is very useful diagnostic feature for characterization and identification of different plant species. The palisade ratio is the average number of the palisade cells under one epidermal cell. The palisade ratio values were calcurated follow divided the number of palisade cells counted in four epidermal cells by four. The procedure was repeated and the mean palisade ratio values were found out (Mukherjee P.K., 2002).

Palisade ratio is a reliable taxonomic character, which is constant for a taxon and it will not vary with environment (Simon, 2018).

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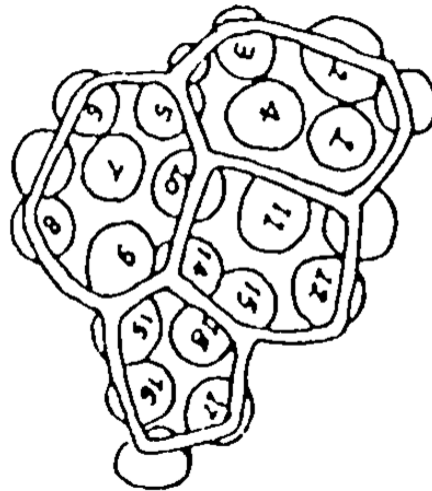

Table 8 Palisade cell under four epidermal cells

Trichomes

Trichomes are divided and subdivided as follows (Patil et al., 2013)

Covering Trichomes

(a) Unicellular trichomes

(b) Uniseriate multicellular unbranched trichomes

(c) Biseriate multicellular unbranched trichomes

(d) Multiseriate multicellular unbranched trichomes

(e) Multicellular branched trichomes

Glandular Trichomes

(a) Unicellular glandular trichomes

(b) Multicellular glandular trichomes

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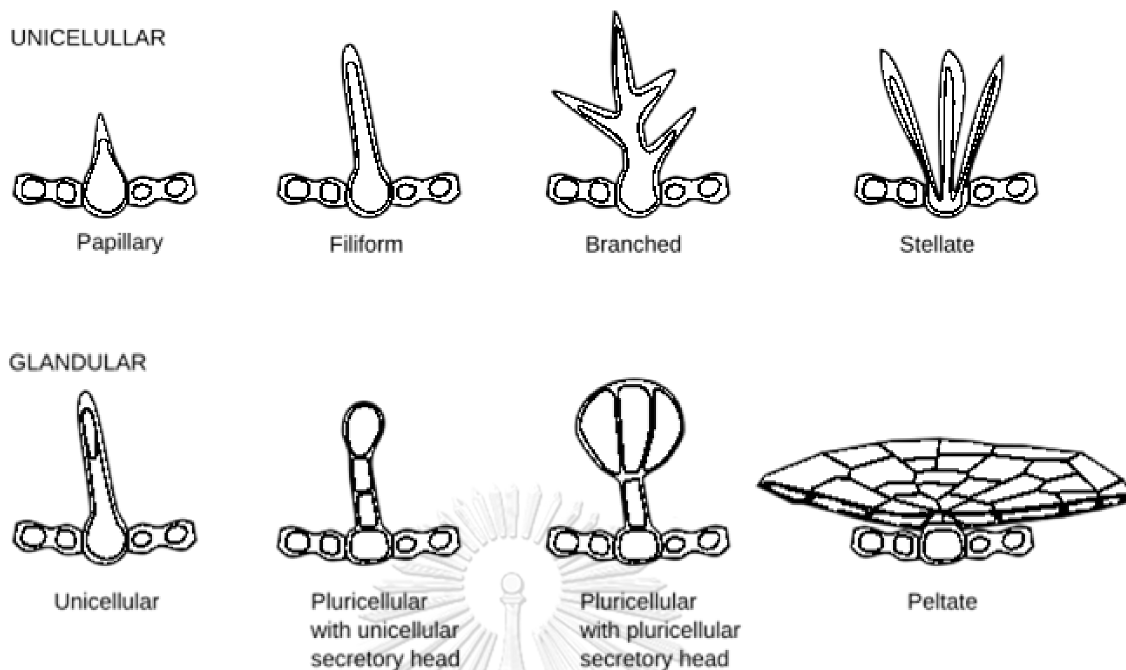

Table 9 Type of trichome

2.3.3 Physiochemical evaluation

This specification is criteria to judge the identity and purity of crude drug

2.3.3.1 Determination of total ash and acid insoluble ash (Leung, 2006; Kim, 2012)

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The total ash is the amount of inorganic elements in plant. It is the residue remaining after complete combustion of the organic matters. Total ash content reveals how many minerals are physiologically contained in the medicinal plant material.

The acid insoluble ash content is another index to demonstrate the quality of herbal material. Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and re-incinerating that acid insoluble matters. Acid-insoluble ash consists of indigestible mineral components, such as silicon in cell wall. Contamination or adulteration from non-physiological silicon material such as sand and soil affect this ash contents. Therefore, the total ash and acid-insoluble ash contents serve as another supplementary piece of evidence to illustrate the quality of the plant materials. .

2.3.4 Determination of loss on drying (Kim, 2012)

This gravimetric method is wildly used to determine and control the moisture content of herbal drugs by heating the sample until no further weight loss The moisture in medicinal plants affect quality due to toxigenic fungi and damage from insects if the herbs are poorly dried and stored. This method compares the weight of an herbal drug before and after it is oven dried, However, the weight loss is not only from water but also from volatile matters as well.

2.3.5 Determination of water content (WHO, 2011)

The water content determination is commonly measured properties of plant materials. The presence of excessive amounts of water in plant drugs is responsible for the growth of bacteria, fungi, insects an as well as the hydrolysis of constituents. The pharmacopoeia monographs limit the water content, especially in drugs that have the facility to absorb it, or in which the excessive amounts of water cause deterioration. The azeotropic method may be applied, which consists of distilling the crude drug with toluene or xylene, which should be saturated with water before using, as if the solvent is anhydrous, and not yet saturated, water containing in plant materials may combine together with solvent, which can lead to inaccurate result. This method requires special equipment. The water and the solvent are distilled together and separated in the receiving tube on cooling.

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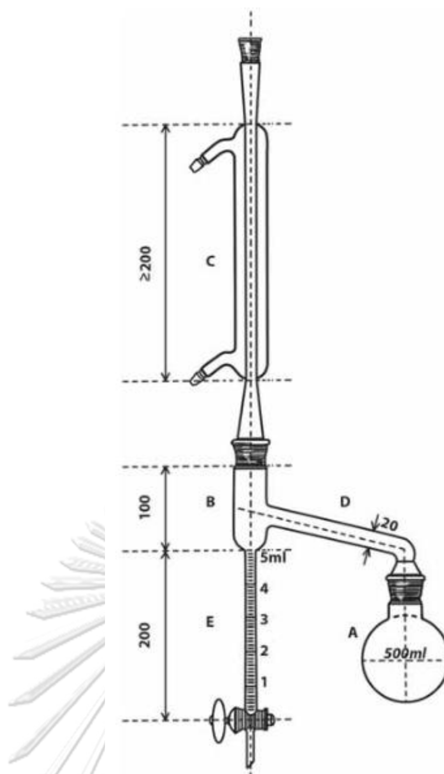

Table 10 Azeotropic apparatus of water content (dimentions in mm), (A) a glass flask, (B) a cylindrical tube, (C) a reflux condenser, (D) a receiving tube, a graduated receiving tube

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2.3.6 Determination of volatile oil (WHO, 2011)

The determination of volatile oils is characterized by their odor, appearance and capability to volatilize at room temperature. Volatile oils are composed of a mixture of chemical compounds especially aromatic compounds such as terpenes and terpenoids. Volatile oils are considered to be the fragrance of the medicinal herbs. This method is applied by hydro distillation using Clevenger equipment.

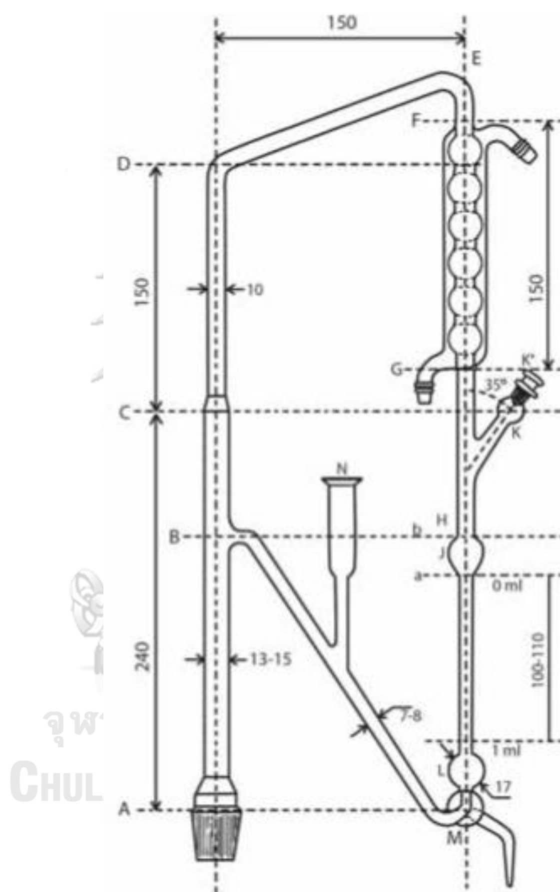

Table 11 Clevenger apparatus for determination of volatile oil content

2.3.7 Determination of solvent extractive matters (WHO, 2011)

The determination of extractable matters refers to the amount of active constituents in a plant material when extracted with a specific solvent such as water and ethanol. Ethanol is used for the slightly non-polar substances whereas water was used for the polar substances. The method is based on the solubility of active substances in a given solvent. This value provides an indication of the extent of non-polar, medium polar and polar components present in the plant material.

2.3.8 Thin layer chromatographic identification (TLC fingerprint) (Tistaert et al., 2010)

Chromatographic technique is valuable additional information to establish the identity of plant material. This method is effective and convenient to perform, and the equipment required is inexpensive. TLC fingerprint is the important key for herbal medicines made up of the complex mixture of chemical constituents such as. The basic parameter used to describe migration in TLC is the retention factor (Rf). The Rf is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

$$\mathbf{h}\mathrm{Rf} = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}} \times 100$$

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The Rf for a compound is a constant from one experiment to the next only if the chromatography conditions are also constant such as solvent system, adsorbent, thickness of the adsorbent, amount of material spotted and temperature. It is frequently used for evaluating medicinal plant materials and their preparations.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Material	Source
Cover glasses (24 × 50 mm), Menzel Glâser	Thermo Scientific, Brunswick, Germany
Filter paper No.4	Whatman, England
Filter paper No.40, Ashless	Whatman, England
Inersil® ODS-3 HPLC column (5 µm × 4.6 mm × 250 mm)	GL Sciences, Tokyo, Japan
Microscope slides (25.4 × 76.2 mm)	Sail Brand, China
Nylon membrane syringe filters (46 mm × 0.45 µm)	National Scientific, Tennessee, USA
PTFE membrane syringe filters (13 mm × 0.45 µm)	ANPEL Laboratory Technology (Shanghai), Shanghai, China
ReproSil®-Pur ODS-3 HPLC guard column (5 µm × 4.0 mm × 10 mm)	Dr. Maisch GmbH, Ammerbuch, Germany
Syringe	Nipro, Phra Nakhon Si Ayutthaya, Thailand
TLC aluminum sheet, silica gel 60 GF_{254}	Merck, Darmstadt, Germany

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3.2 Chemicals and reagents

Ethanol (Analytical grade)	RCI Labscan Limited, Bangkok, Thailand
Ethyl acetate (Analytical grade)	RCI Labscan Limited, Bangkok, Thailand
Formic acid 98-100% (Analytical grade)	Merck, Darmstadt, Germany
Methanol, HPLC grade	RCI Labscan Limited, Bangkok, Thailand
Rosmarinic acid (CAS No. 20283-92-5, purity 96%)	Sigma-Aldrich., St. Louis, Missouri, USA
Toluene (Analytical grade)	RCI Labscan Limited, Bangkok, Thailand
Ultra-pure water	NW20VF, Heal Force, China

3.3 Equipment and instruments

Ashing Furnace (AAF 11/18)	Carbolite, Hope Valley, England
CAMAG TLC Chamber	CAMAG, Muttenz, Switzerland
CAMAG TLC Scanner 4	CAMAG, Muttenz, Switzerland
CAMAG Visualizer	CAMAG, Muttenz, Switzerland
Centifuge (Model: SIGMA 1-14)	Sartorius, GÖttingen, Germany
Digital camera (Canon Power Shot A650 IS)	Canon Marketing Co. Ltd., Thailand

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3.3 Equipment and instruments (cont.)

Digital Orbital Shaker (Model: SHO-2D)	Daihan Scientific, Gangwon-do, Korea
Gas chromatography / mass spectrometry	Thermo Finnian model Trace GC Ultra equipped with Finnigan DSQ MS detector, USA
Hot air oven	WTC Binder, Tuttlingen, Germany
Laminar hood (Model: Class II BSC)	ESCO, Singapore
Microscope (Axio Imager. A2)	Carl Zeiss, Jena, Germany
Rotary evapolator (Model: B-210)	Buchi, Flawil, Switzerland
Ultraviolet fluorescence analysis cabinet	Spectronics Corporation, USA
Ultra-pure water purification (Model: NW20VF)	Heal Force, China
Ultrosonic bath (Model : CC-80)	Analytical Lab Science, Bangkok, Thailand
Ultraviolet viewing cabinet	Spectronics Corpolation, New York, USA
Water bath (Model: SC/48 R)	Brinkmann, USA
winCAT software (version: 1.4.6.2002)	CAMAG, Switzerland

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3.4 Methods

3.4.1 Plant sample

3.4.1.1 Plant sample collection

Fresh *M. cordifolia* samples were collected from 15 different locations of Thailand. The sample was authenticated by specialist, Associate Professor Dr.Nijsiri Ruangrungsi, The herbarium specimens was prepared and deposited at College of Public Health Sciences, Chulalongkorn University.

3.4.1.2 Crude drug preparation

The plant samples were cleaned, the leaves and stems were separated and dried in a hot air oven at 45 °C.

3.4.2 Pharmacognostic specification

Pharmacognostic parameters such as microscopically morphological characters, microscopic leaf measurement, physicochemical properties including ethanol soluble extractive matter, water soluble extractive matter, moisture, loss on drying, total ash, acid in soluble ash and volatile oil as well as and TLC fingerprint was performed following World Health Organization (WHO) guideline with some modifications.

3.4.2.1 Morphological character

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Whole plant of *M. cordifolia* was demonstrated botanically by hand drawing. *M. cordifolia* samples were identified with shape, color, texture and other by visual inspection.

Morphological characters of leaf and stem crude drugs were demonstrated.

3.4.2.2 Microscopic evaluation

Transverse section

Fresh and mature midrib and stem of *M. cordifolia* were transversely sectioned. The cross section was done by hand with a razor as thin as possible and investigated with the aid of microscope. All plant photos were taken by digital camera and illustrated by hand drawing with dimensions of a specific ratio to actual size.

Quantitation of leaf constant numbers

Fresh and mature laminae of *M. cordifolia* from 3 different sources were investigated for microscopic leaf constant number i.e. stomatal number, stomatal index, trichome number, trichome index, oil gland number, oil gland index, epidermal cell area and palisade ratio. Chlorophyll was clearly removed by soaking in Haiter (containing 6% sodium hypochloride) : water (1 : 1 v/v) for 24-48 hours. The lamina section was heated in chloral hydrate : water (4 : 1 w/v) on water bath for 48 hours, then transferred to slide and observed the cells under microscope. The image was recorded using an AxioVision software. The 40X power was used for the magnification. Both sides of the lamina were examined for 30 fields per location. The average of 90 fields from 3 locations were demonstrated.

Stomatal number and stomatal index

The stomatal number is an average number of stomata per square millimeters (mm^2) of epidermis of the leaf.

The stomatal index is the ratio of the number of stomata to the total number of ordinary epidermal cells in the same area.

$$\text{Stomatal index} = \frac{S}{E + O + T + S} \times 100$$

Trichome number and trichome index

Trichome number is an average number of trichomes or cicatrices per 1 mm^2 of epidermis. Trichome index is a percentage proportion of trichome number to all epidermal cell number in one square millimeter.

$$\text{Trichome index} = \frac{T}{E + S + O + T} \times 100$$

Oil gland number and oil gland index

Oil gland number is an average number of oil gland cells per square millimeter of epidermis. Oil gland index is a percentage proportion of oil gland number to all ordinary epidermal cell number in one square millimeter.

$$\text{Oil gland index} = \frac{O}{E + T + S + O} \times 100$$

O = Number of oil gland in a given area of the leaf

E = Number of epidermal cells in the same area of leaf

T = Number of trichome and cicatrix in the same area of leaf

S = Number of stomata in the same area of leaf

Palisade ratio

Palisade ratio is the average number of palisade cells under one epidermal cell of the leaf. The palisade cells under four continuous epidermal cells were counted the divided by four to obtain the palisade ratio.

Epidermal cell number and epidermal cell area

Epidermal cell number per square millimeters of epidermis was counted both sides of leaf. The epidermal cell area was calculated by dividing one square millimeter by the epidermal cell numbers.

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3.4.3 Physiochemical evaluation

The leaf and stem crude drugs from 15 locations were evaluated. All the tests of physicochemical parameters were done in triplicate.

3.4.3.1 Determination of loss on drying

Three grams of dried powder of *M. cordifolia* leaves and stems were put in pre-weighed crucible and carried into an oven at 105°C until constant weight. The loss of weight was calculated in percentage of dried sample.

3.4.3.2 Determination of total ash and acid insoluble ash

The aforementioned crucible was incinerated to ash at 500°C in a furnace until it become white color indicating the absence of carbon, cooled in a desiccator and weighed. The content of total ash was calculated in percentage of dried sample.

Twenty-five milliliters of hydrochloric acid (70g/l) was added to the crucible containing the total ash and boiled gently for 5 minutes and filtered through ashless filter paper Whatman #40. The insoluble matters were transferred to the original crucible, dried on a hot-plate an incinerated to ash again. After cooling down in desiccator, the content of acid insoluble as was weighted and calculated in percentage of dried sample.

3.4.3.3 Determination of water and ethanol extractive matters

Five grams of dried powdered samples were macerated with 70 ml of water or 95% ethanol in closed flask for 24 hours (the first 6 hours by shaking bath and 18 hours allow standing). After filtration through filter paper Whatman No.4, the marc was washed by water or ethanol and final volume was adjusted to 100 ml. Twenty milliliters of the filtrate were transferred to a pre-weighed beaker, evaporated to dryness on hot plate and dried in oven at 105℃ for 6 hours. After cooling the beaker in a desiccator, the contents of water and ethanol soluble extractable matters were calculated in percentage.

3.4.3.4 Determination of moisture content

The water content was determined using azeotropic method. Thirty grams of dried powdered samples were added with 300 ml of water-saturated toluene in a round bottom flask. The flask was boiled until the water and toluene were distilled over. The condenser tube was allowed to cool and the toluene and water in

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receiving tube was separated completely, the volume of water distilled over were recorded and report in percentage.

3.4.3.5 Determination of volatile oil content

Ground samples (50 g) were added with 550 ml water in a round bottom flask and distilled in Clevenger apparatus. The oil was received and the volume of oil was measured and reported in percentage.

3.4.3.6 Thin layer chromatographic fingerprint

The ethanolic extract from maceration (3.4.3.3 Determination of ethanolic extractive matter) were dried and re-dissolved in ethanol to 10 mg/ml. Three milliliters were applied to the TLC plate coat with silica gel G60 F254. The TLC plate was developed in the chamber with the suitable solvent system of toluene: ethyl acetate: formic acid (5:4:1.2 v/v/v). After development, the TLC plate was removed after that the produced spots were observed, under ultraviolet light at 254 and 365 nm. Then the plate was spray with ρ-anisaldehyde reagent and heated at 105 °C for 10 minutes on TLC-plate heater.

3.4.3.7 Gas Chromatography / Mass Spectrometry (GC/MS)

The essential oil from determination of volatile oil content was analyzed by a Finnigan Trace GC Ultra with DSQ Quadrupole detector. Zebron ZC-5 MS fuse silica column (30 m × 0.25 µm, 0.25 µm film thicknesses) was used as stationary phase. The oven temperature started from 60 °C up to 240 °C with the rate of 3°C/min. The carrier gas was helium with the flow rate of 1 ml/min. One microliter of *M. cordifolia* essential oil solution (1:100 in hexane) with injected by Finnigan Autoinjector A3000 with split ratio of 100:1. MS was performed by EI positive mode at 70 eV ionization. The chemical constituents of oil were identified by matching mass spectra and retention indices with Adams R.P. essential oil database and NIST05.

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3.4.4 Quantitative analysis of rosmarinic acid

3.4.4.1 Preparation of the ethanoic extract of *M. cordifolia*

The dried powders (5 g) of *M. cordifolia* leaves and stems were exhaustively extracted with 300 ml of 95% ethanol in a Soxhlet apparatus. The prepared extract was filtered and evaporated till dryness.

3.4.4.2 Preparation of rosmarinic acid solution

One milligram of standard rosmarinic acid was dissolved in 1 ml of methanol and filtered through a 0.45 µm PTFE membrane syringe filter. This stock solution was diluted serially for calibration curves. (0.1, 0.2, 0.3, 0.4, 0.5 mg/ml)

3.4.4.3 Preparation of sample solutions

One milligram of each sample extracts was dissolved in 1 ml of methanol and filtered through a 0.45 µm PTFE membrane syringe filter.

3.4.4.4 HPLC analysis

HPLC system and data analysis were processed with Shimadzu LC solution software. Shimadzu HPLC LC-20A system (Shimadzu, Japan) consists of a system controller (CMB-20A), a column (CTO-20A), an auto-sampler (SIL-20A), an on-line degassing unit (DGU-20A3), two solvent delivery units (LC-20A) and photo-diode array detector (SPD-M20A). The mobile phase was filtered through 0.45 nylon membrane filters and degassed using an ultrasonic bath 10 minutes before analysis. The column temperature was maintained at 30 ^{o}c and injection volume was 5 µl. Chromatographic separation was conducted using the reversed-phased C18 and coupled with C18 guard column. The sample were analyzed using 0.2% phosphoric acid in water (solvent A) and methanol (solvent B) as mobile phase. The program was set in isocratic mode at 55% solvent A for 20 minutes at flow rate of 1 ml/min. The wavelength was set at 330 nm for monitory chromatographic profile. The measurement was done in triplicate.

3.4.5 Method validation

3.4.5.1 Calibration curve

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Calibration curve was created by plotting peak area compared five different levels of standard RA. Linear regression and coefficient of determination were analyzed by Microsoft Excel program.

3.4.5.2 Accuracy

The accuracy of an analytical procedure was analyzed by adding three different concentrations of standard RA (low, medium and high levels) into sample. All tests were done in triplicate. The accuracy was calculated as percentage recovery of RA.

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

A = the amount of rosmarinic acid found after spiking standard solution

B = the amount of rosmarinic acid found before spiking standard solution

C = the amount of standard rosmarinic acid actually added

3.4.5.3 Precision

The precisions of the HPLC method were evaluated by repeatability and intermediate precision. The samples with three different concentrations of rosmarinic acid were analyzed on the same day and three different days respectively. All sample tests were done in triplicate. The precision was evaluated as the relative standard deviation (RSD).

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

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

LOD and LOQ were evaluated based on calibration curve using the formula:

$$\text{Limit of detection (LOD)} = \frac{3.3 \times \text{SD res}}{\text{S}}$$

$$\text{Limit of detection (LOQ)} = \frac{10 \times \text{SD res}}{\text{S}}$$

S = Slope of regression line

SD res = Standard deviation of the regression line

3.4.5.5 Specificity

The specificity was evaluated by complete separation of RA peak from other peaks in the sample extract. The peak purity index of the analyte was processed with Shimadzu LC Solution software.

3.4.5.7 Robustness

Temperature in the system was varied for evaluation of the robustness and expressed as %RSD.

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3.4.6 Data analysis

The parameters due to physicochemical evaluation were express as grand mean ± pooled standard deviation.

CHAPTER IV

RESULTS

4.1 Macroscopic characteristics

Arial part of Sa-ra-nae or Marsh mint, Kitchen mint was illustrated in Figure 7.

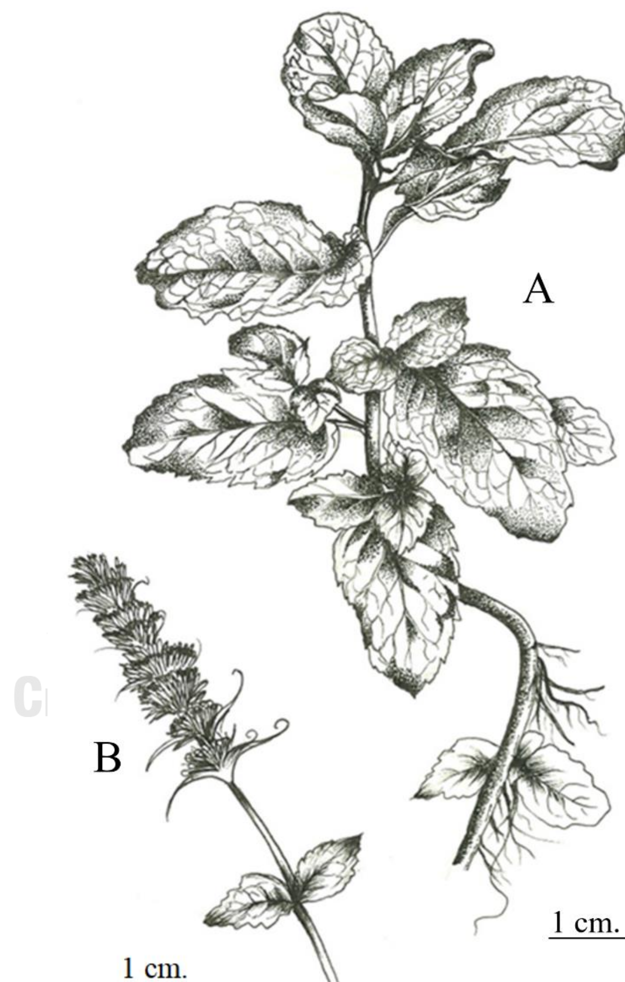

Table 12 *M. cordifolia* Opiz ex Fresen. (A) and inflorescence (B)

The dried leaf crude drug was brownish-green while the dried stem crude drug was light grayish-brown.

Table 13 *M. cordifolia* leaf crude drug

Table 14 *M. cordifolia* stem crude drug

4.3 Microscopic characteristics

Anatomical characters of *M. cordifolia* leaf and stem

The midrib characteristics were illustrated in Figure 10 presenting vascular bundle, glandular trichome, non-glandular trichomes and oil glands.

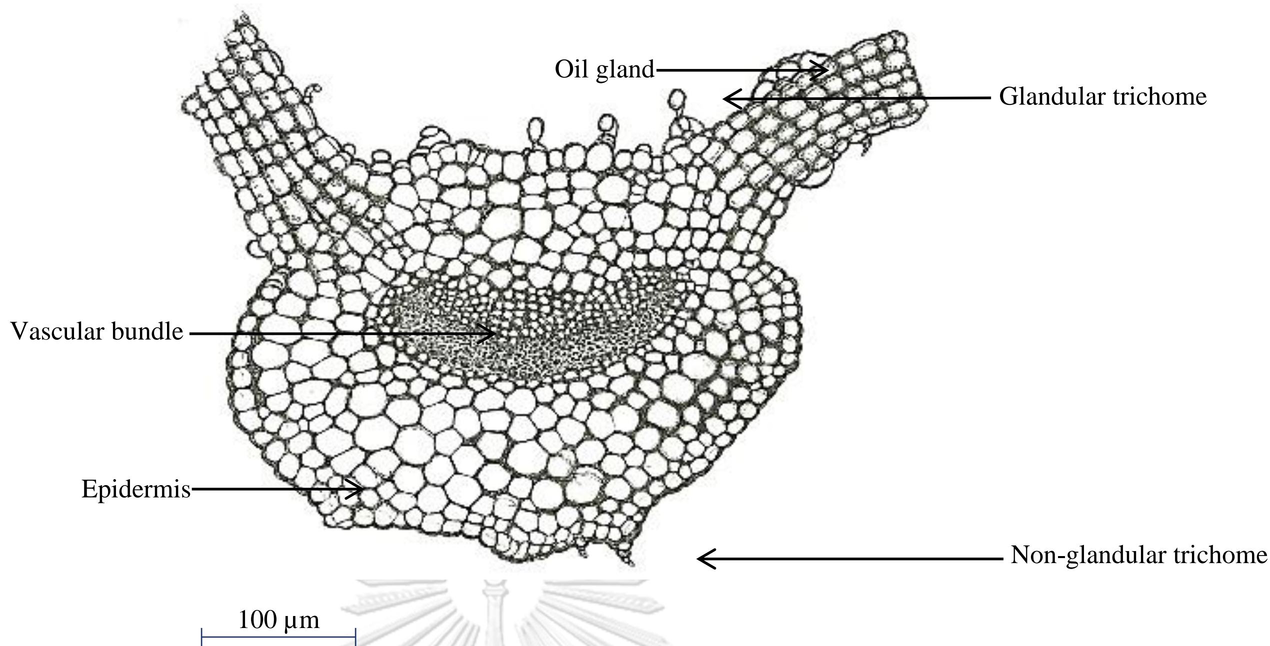

Table 15 Midrib cross section of *M. cordifolia*

The anatomy of stem was quadrangular shape with four main vascular bundles as demonstrated in Figure 11.

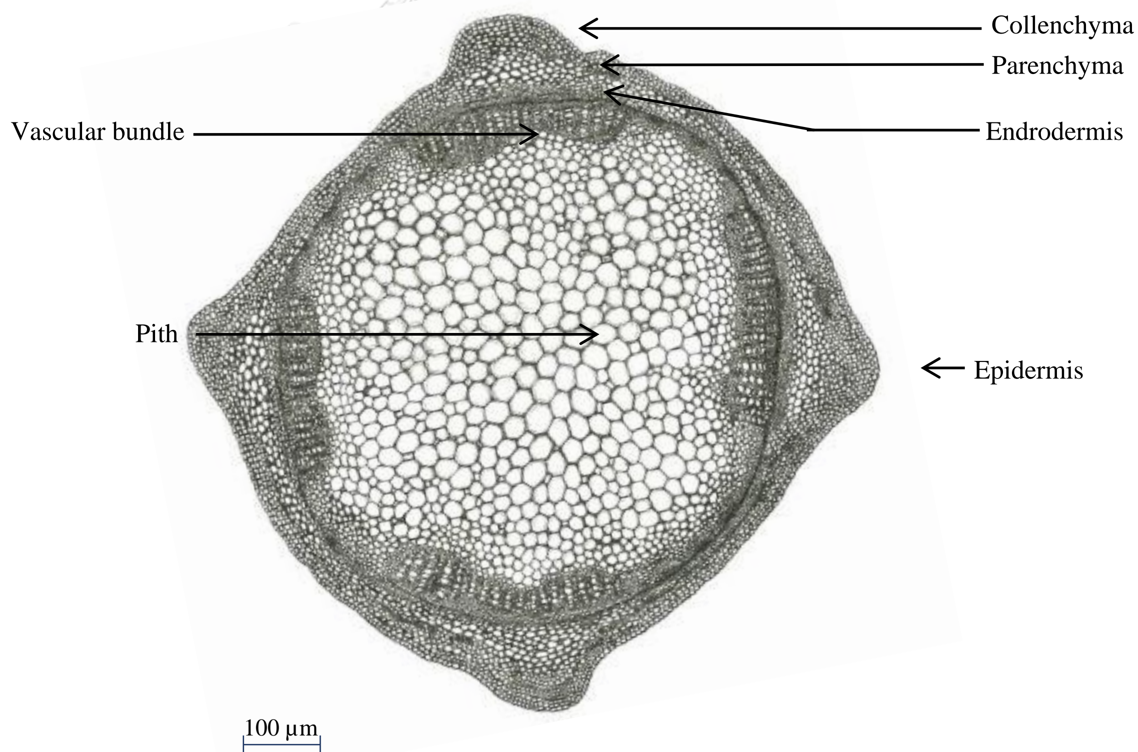

Table 16 Stem cross section of *M. cordifolia*

Quantitative and qualitative microscopic characters of leaf

Stomatal cell

The diacytic stomatal cells were found both upper and lower sides of the leaf surface. The photographs of stomatal cells under 40X microscope were shown in Figure 12, 13. The stomatal number and stomatal index values were shown in Table 6.

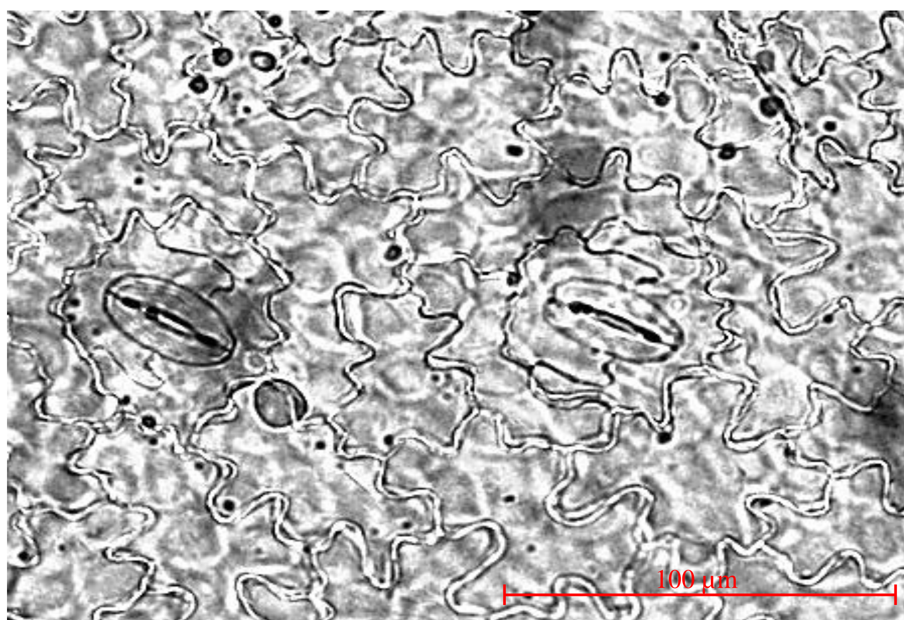

Table 17 Stomatal cells of upper leaf of *M. cordifolia*

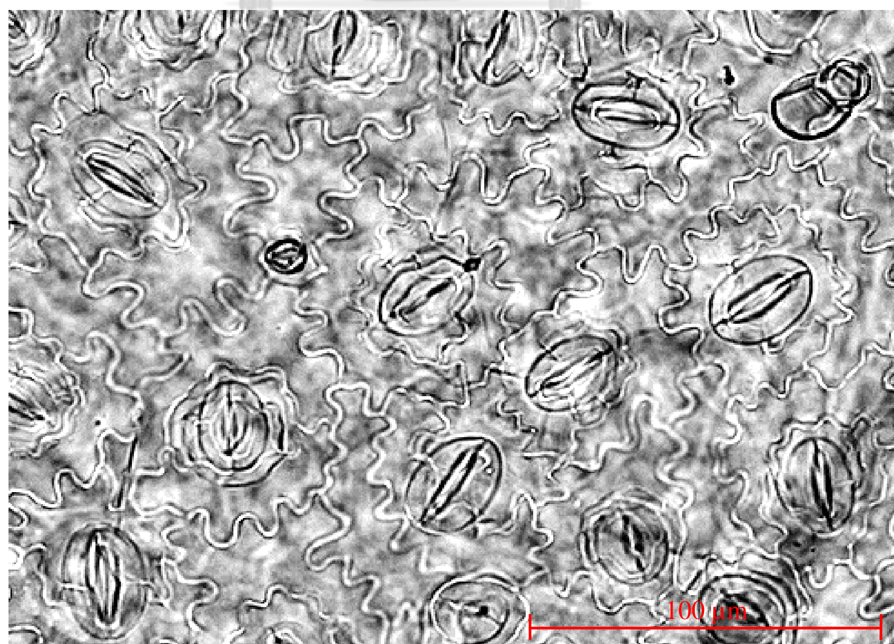

Table 18 Stomatal cells of lower leaf of *M. cordifolia*

Palisade cells

Palisade cells formed as layer below the upper epidermis. The photograph of palisade cells was presented in Figure 14. The palisade ratio was shown in Table 7.

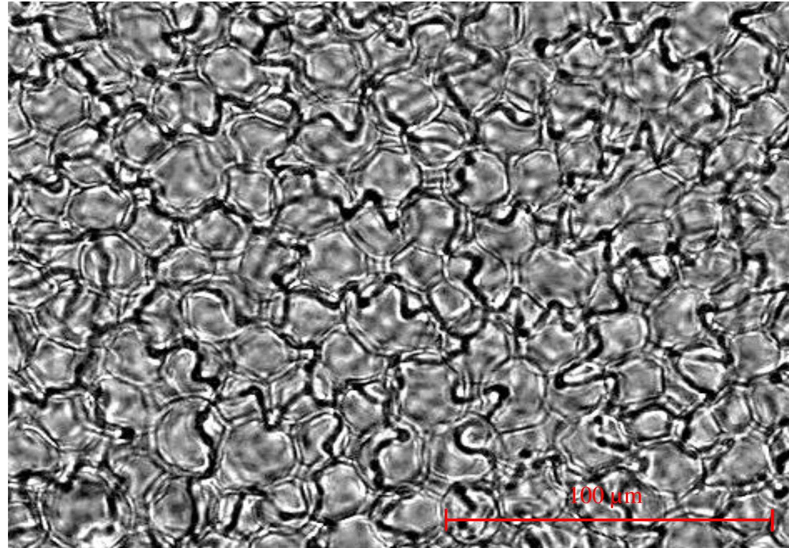

Table 19 Palisade cells of *M. cordifolia*

Epidermal cell

The epidermal cells which form wavy cell wall were presented in Figure 15. The epidermal cell area was shown in Table 7.

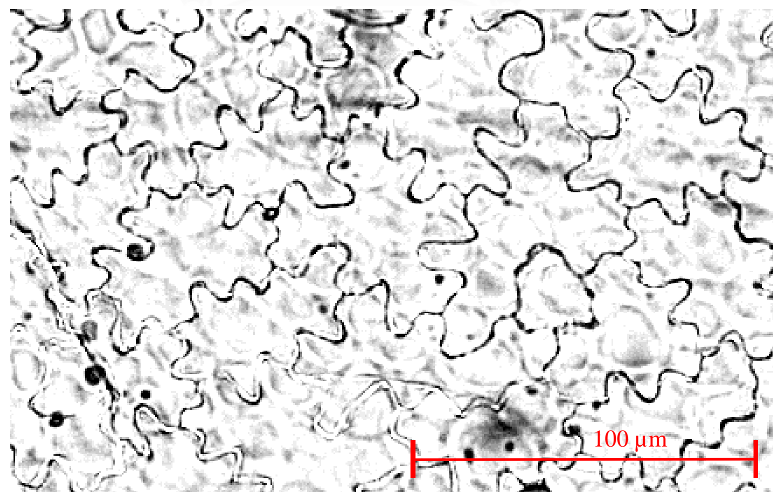

Table 20 Epidermal cells of *M. cordifolia*

Trichome

The uniseriate non-glandular and uniseriate glandular trichomes were found both upper and lower sides of the leaf blade (Figure 16). The midrib also showed non-glandular and uniseriate glandular trichomes (Figure 17). Trichome number and trichome index were shown in Table 8.

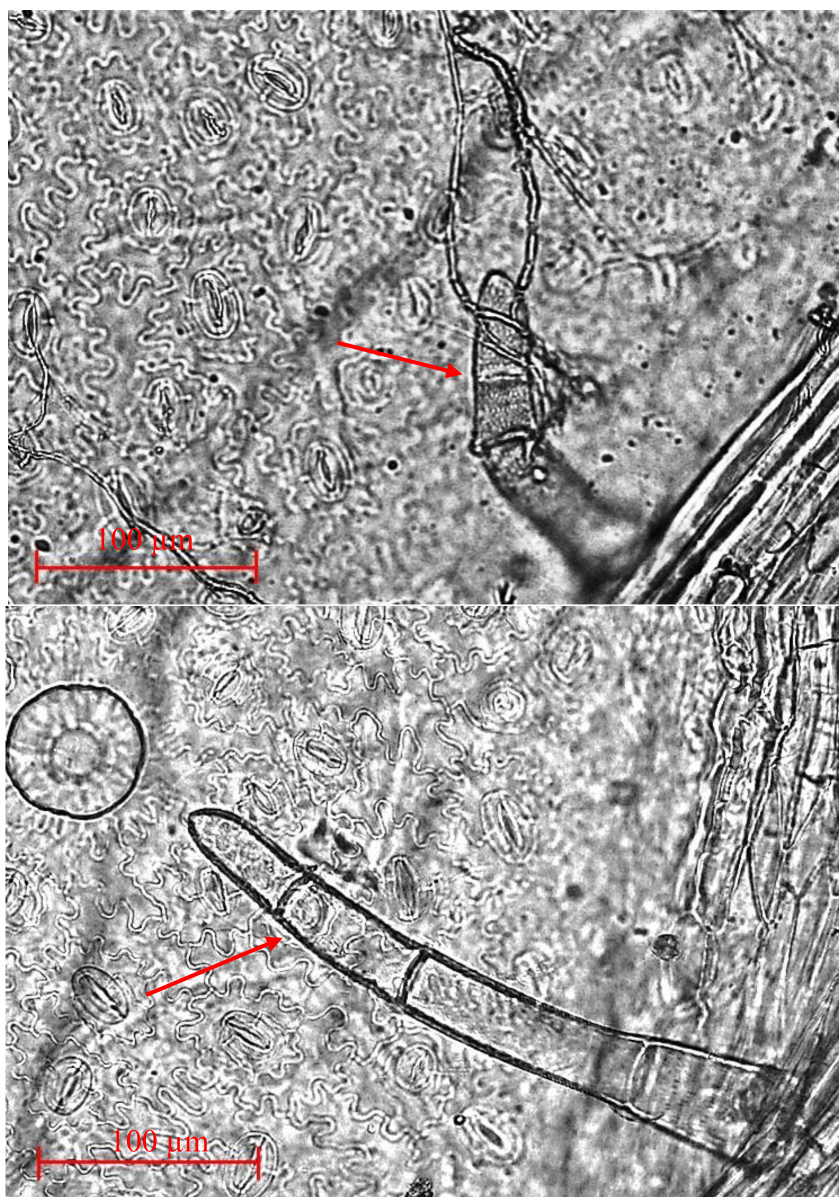

Table 21 The uniseriate non-glandular trichomes and cicatrices on the lower leaf of *M. cordifolia*

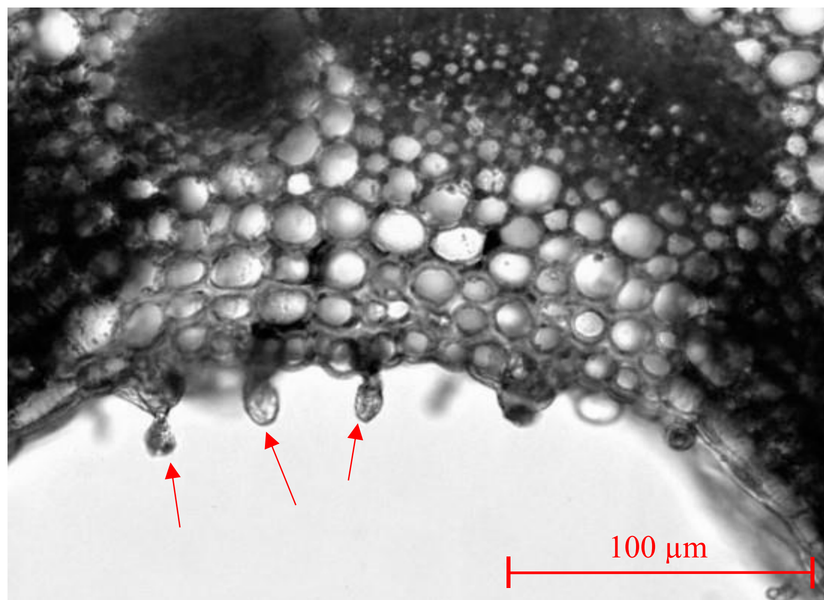

Table 22 The uniseriate glandular trichome on the midrib of *M. cordifolia*

Oil gland

The oil glands were found both of upper and lower sides of the leaf blades. The oil gland number and oil gland index were shown in Table 9. The photograph of oil gland was shown in Figure 18.

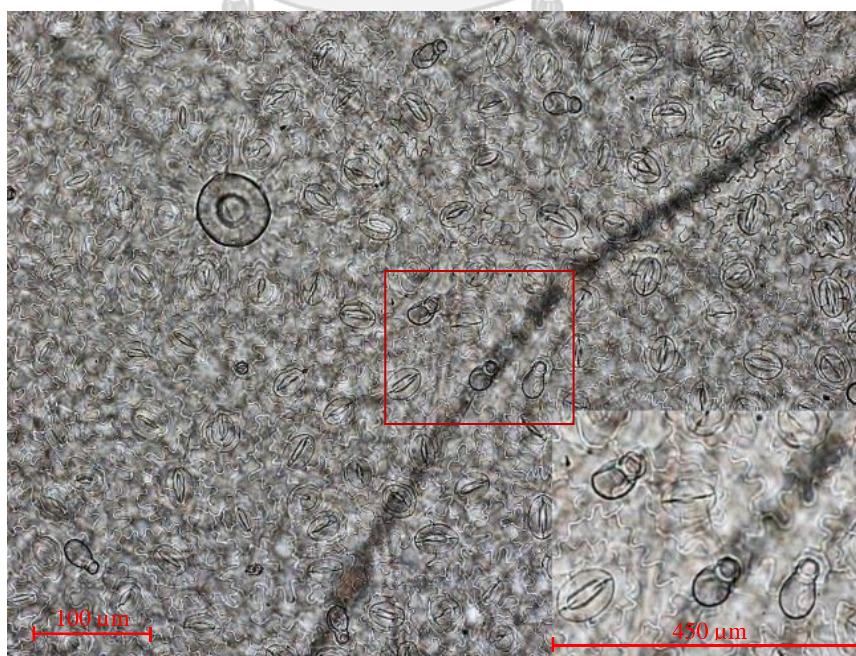

Table 23 Oil glands found on the *M. cordifolia* leaf

Leaf constant number

Microscopic leaf measurement was performed on the lamina of fresh and mature leaves collected from 3 different locations. Ninety fields (30 field from each location) were measured and mean, SD, min, max were shown in Table 6, 7, 8, 9.

Table 24 Microscopic leaf constant number of *M. cordifolia* based on stomatal number and stomatal index

	Stomatal number **Mean ± SD** **(Min – Max)**	**Epidermal cell number** **Mean ± SD** **(Min – Max)**	**Stomatal index** **Mean ± SD** **(Min – Max)**
Upper epidermis	14.58 ± 3.41 (8 -24)	480.71 ± 42.44 (408 -588)	2.85 ± 0.64 (1.39 - 4.24)
Lower epidermis	174.04 ± 54.44 (88 - 300)	665.02 ± 134.53 (424 - 924)	19.08 ± 2.65 (12.77 - 26.22)

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***Table* 25** Microscopic leaf constant number of *M. cordifolia* base on epidermal cell number, epidermal cell area and palisade ratio

	Epidermal cell number ($/mm^2$) **Mean ± SD** **(Min – Max)**	**Epidermal cell area (μm^2)** **Mean ± SD** **(Min – Max)**	**Palisade ratio** **Mean ± SD** **(Min – Max)**
Upper epidermis	480.71 ± 42.44 (408 -588)	2128.64 ± 182.80 (1724.68 – 2482.98)	4.63 ± 0.35 (3.75 – 5.25)
Lower epidermis	665.02 ± 134.53 (424 - 924)	1793.52 ± 262.45 (1370.25 – 2502.49)	-

Table 26 Microscopic leaf constant number of *M. cordifolia* base on trichome number, epidermal cell number and trichome index

	Trichome number **Mean ± SD** **(Min – Max)**	**Epidermal cell number** **Mean ± SD** **(Min – Max)**	**Trichome index** **Mean ± SD** **(Min – Max)**
Upper epidermis	2.31 ± 2.24 (0 – 8)	480.71 ± 42.44 (408 -588)	0.45 ± 0.44 (0 – 1.63)
Lower epidermis	4.00 ± 2.68 (0 - 12)	665.02 ± 134.53 (424 - 924)	0.46 ± 0.33 (0 – 1.95)

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Table 27 Microscopic leaf constant number of *M. cordifolia* base on oil gland number, lower epidermal cell number and oil gland index

	Oil grand number **Mean ± SD** **(Min – Max)**	**Epidermal cell number** **Mean ± SD** **(Min – Max)**	**Oil gland index** **Mean ± SD** **(Min – Max)**
Upper epidermis	15.69 ± 4.29 (8 – 24)	480.71 ± 42.44 (408 -588)	3.09 ± 0.89 (1.40 – 4.92)
Lower epidermis	50.80 ± 8.84 (28 - 80)	665.02 ± 134.53 (424 - 924)	5.97 ± 1.75 (2.56 – 9.89)

4.5 Physicochemical parameters

All the contents of physicochemical parameters of *M. cordifolia* leaf and stem were shown in Table 10, 22. The specification for quality control of *M. cordifolia* leaf crude drug indicated that the acid-insoluble ash, total ash, loss on drying and moisture contents should not be more than 1.98, 9.41, 7.06 and 10.50 % of dried weight. Conversely, ethanol-soluble extractive, water-soluble extractive matters and volatile content should not be less than 5.04, 15.23 and 0.36 of dried weight respectively.

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The specification for quality control of *M. cordifolia* stem crude drug indicated that acid-insoluble ash, total ash, loss on drying and moisture content should not be more than 1.63, 8.04, 7.04 and 10.01% of dried weight. In the other hand, ethanol-soluble extractive and water-soluble extractive matters should not be less than 5.20 and 16.60 % of dried weight respectively.

Table 28 Physico-chemical content of *M. cordifolia* leaf and stem (% by weight) from 15 sources throughout of Thailand.

Parameter	Leaf	Stem
	*Content (% of dry weight)	
Acid-insoluble ash	1.98 ± 0.07	1.63 ± 0.05
Total ash	9.41 ± 0.18	8.40 ± 0.18
Loss on drying	7.06 ± 0.14	7.04 ± 0.14
Ethanol-soluble extractive matter	5.04 ± 0.18	5.20 ± 0.20
Water-soluble extractive matter	15.23 ± 0.42	16.60 ± 0.48
Moisture content	10.50 ± 0.41	10.01 ± 0.38
Volatile oil content	0.36 ± 0.02	0

*The content was shown as grand mean ± pooled SD.

4.5 Thin layer chromatographic fingerprint

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The TLC fingerprint plate of *M. cordifolia* leaf and stem ethanolic extracts were observed under ultraviolet light at 254, 365 nm and detection with p-anisaldehyde reagent (Figure19, 20).

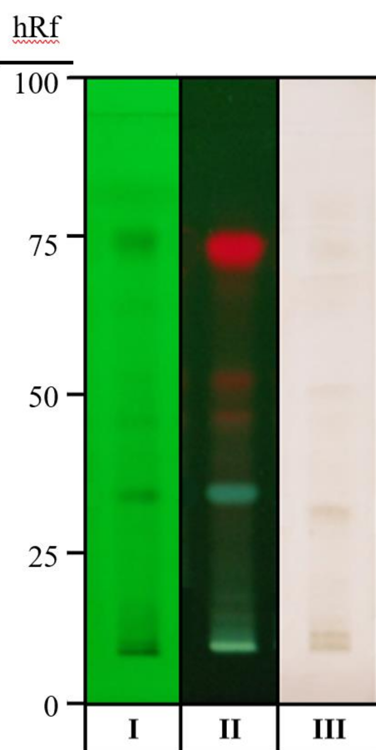

Table 29 TLC fingerprint of *M. cordifolia* leaf ethanolic extract

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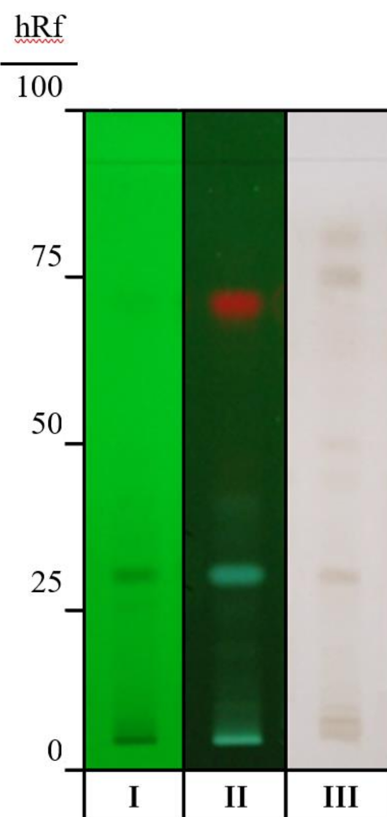

Table 30 TLC fingerprint of *M. cordifolia* stem ethanolic extract

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Stationary phase	Silica gel 60 GF_{254} TLC plate
Mobile phase	Toluene: ethyl acetate: formic acid (5:4:1.2 v/v)
Detection	I = detection under UV 254 nm
	II = detection under UV 365 nm
	III = detection with ρ-anisaldehyde reagent

4.6 Chemical constituents of *M. cordifolia* leaf volatile oil by GC/MS

The main component in *M. cordifolia* leaf volatile oil was piperitenone oxide (73.22%). The other compounds were shown in Table 11.

Table 31 Chemical constituent in *M. cordifolia* leaf volatile oil

Compound name	RT (min)	*Peak area %	KI
α-Pinene	6.20	0.74 ± 0.19	939
Sabinene	7.40	0.25 ± 0.20	975
β-Pinene	7.59	0.89 ± 0.23	979
Myrcene	7.88	0.75 ± 0.37	990
3-Octano	8.21	0.30 ± 0.24	991
Sylvestrene	9.37	2.16 ± 1.20	1030
1,8-Cineole	9.55	2.04 ± 0.61	1031
p-Cymenene	11.81	0.12 ± 0.47	1091
3-Octanol acetate	12.93	0.15 ± 0.19	1123
z-isopropyldenecyclohexanone	13.72	0.27 ± 0.24	-
Borneol	15.36	0.04 ± 0.12	1169
p-Cymen-9-ol	16.04	1.12 ± 0.54	1024
Coahuilensol, methyl ether	17.24	0.95 ± 0.40	1221
Carvone	18.53	1.61 ± 3.06	1249
Nonanal <dimethyl acetal>	19.19	0.03 ± 0.11	1279
dihydroedulan 1	20.51	0.14 ± 0.20	-
Piperitenone	22.65	0.53 ± 0.22	1343
Eugenol	23.12	0.04 ± 0.17	1359

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Compound name	RT (min)	*Peak area %	KI
Piperitenone oxide	23.58	73.22 ± 7.62	1368
Cinerolon	24.99	4.74 ± 1.70	-
(E)-Caryophyllene	25.79	0.87 ± 0.19	1419
(E)-β-Farnesene	27.18	0.02 ± 0.05	1456
cis-Muurola-4(14),5-diene	27.56	0.37 ± 0.13	1466
γ-cadinene	28.34	2.72 ± 0.56	1513
trans-Calamenene	29.94	0.39 ± 0.32	1522
chlorothymol	31.88	5.15 ± 4.63	1486
α-Cadinol	35.20	0.15 ± 0.20	1654

*The percentage of peak area calculated from 15 locations by GC/MS

Quatitative analysis of rosmarinic acid content in *M. cordifolia* leaf and stem ethanolic extracts by RP-HPLC

The dried powders of *M. cordifolia* leaves and stems from 15 different sources throughout Thailand were exhaustively extracted with 95% ethanol in a Soxhlet apparatus. The yield of leaf and stem ethanolic extract were 24.31 ± 3.77 (Table 12) and 20.92 ± 8.86 % by weight respectively (Table 13).

Table 32 The yield of *M. cordifolia* leaf ethanolic extracts from 15 different sources throughout Thailand

Source	Weight of sample (g)	Weight of extractive matter (g)	Yield (g/100g)
Ratchaburi	5.00	1.25	24.99

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Source	**Weight of sample (g)**	**Weight of extractive matter (g)**	**Yield (g/100g)**
Petchabun	5.00	1.34	26.78
Si sa ket	5.03	1.36	27.00
Lopburi	5.04	0.94	18.69
Nakhon Sawan	5.02	1.44	28.76
Kanchanaburi	5.01	1.48	29.54
Suphanburi	5.03	1.49	29.66
Prachinburi	5.02	1.41	28.07
Chachoengsao	5.01	0.96	19.08
Samutprakan	5.01	1.04	20.84
Nakornpathom	5.00	1.11	22.24
Bangkok	5.01	1.11	22.11
Saraburi	5.00	1.16	23.12
Nakhon Ratchasima	5.01	1.04	20.80
Petchaburi	5.00	1.15	22.89
		Average	24.31
		Min	18.69
		Max	29.66
		SD	3.77

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Table 33 The yield of *M. cordifolia* stem ethanolic extracts from 15 differnt sources throughout Thailand

Source	Weight of sample (g)	Weight of extractive matter (g)	Yield (g/100g)
Ratchaburi	5.06	1.97	38.93
Petchabun	5.00	2.18	43.60
Si sa ket	5.02	0.95	19.00
Lopburi	5.04	1.23	24.35
Nakhon Sawan	5.03	1.07	21.24
Kanchanaburi	5.00	1.02	20.31
Suphanburi	5.05	0.84	16.68
Prachinburi	5.04	1.00	19.92
Chachoengsao	5.00	0.82	16.45
Samutprakan	5.00	0.81	16.09
Nakornpathom	5.00	0.90	17.93
Bangkok	5.00	0.91	18.10
Saraburi	5.01	0.81	16.13
Nakhon Ratchasima	5.00	0.69	13.84
Petchaburi	5.00	0.56	11.27
		Average	20.92
		Min	11.27
		Max	43.60
		SD	8.86

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RA contents in leaf and stem of *M. cordifolia* were quantitated by HPLC, the results were shown in Table 13. The average RA content in leaf and stem among 15 sources were 1.92 ± 1.27 and 0.99 ± 0.41 g/100g by dry weight of crude drug respectively.

Table 34 RA content of *M. cordifolia* leaf and stem from 15 different sources throughout Thailand

		Leaf	**Stem**
No.	**Sources**	**Rosmarinic acid content (g/100g)**	**Rosmarinic acid content (g/100g)**
1	Ratchaburi	0.84	0.88
2	Petchabun	0.73	0.87
3	Si sa ket	1.43	0.38
4	Lopburi	0.96	0.83
5	Nakhon Sawan	2.26	1.31
6	Kanchanaburi	1.57	0.29
7	Suphanburi	3.56	1.82
8	Prachinburi	5.57	1.08
9	Chachoengsao	2.19	1.60
10	Samutprakan	0.83	0.65
11	Nakornpathom	1.51	1.07
12	Bangkok	1.21	1.22
13	Saraburi	2.47	0.83
14	Nakhon Ratchasima	1.57	1.07
15	Petchaburi	2.06	1.92

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No.	Sources	Leaf	Stem
		Rosmarinic acid content (g/100g)	**Rosmarinic acid content (g/100g)**
	Average	1.92	0.99
	SD	1.27	0.41

4.8. Method validation

4.8.1. Calibration curve

Calibration curve was linear in the range of 100-500 µg/ml (Figure 21). The chromatogram was shown in Figure 22.

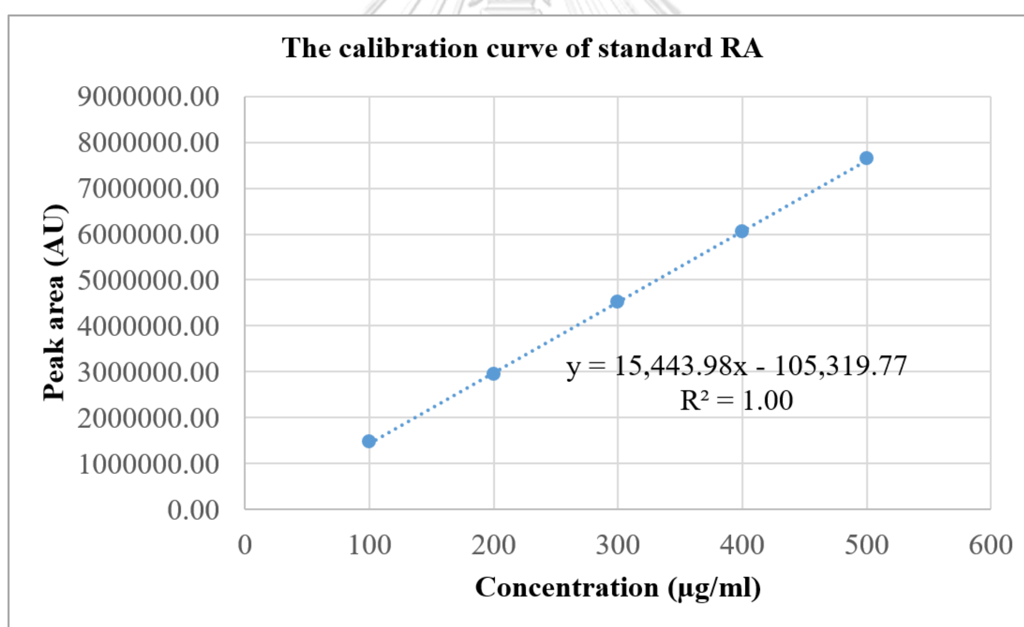

Table 35 The calibration curve of standard RA

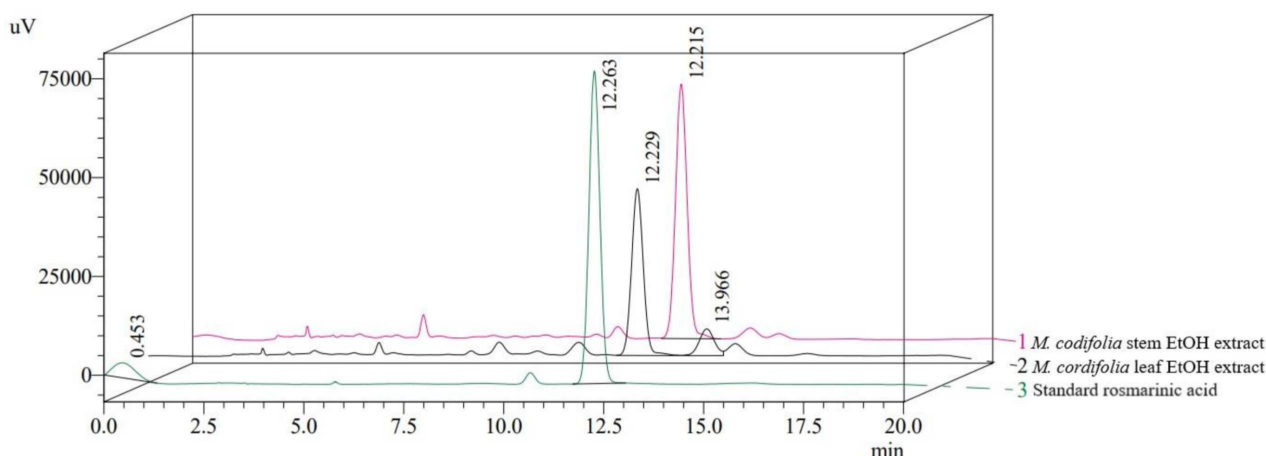

Table 36 The chromatogram of RA in *M. cordifolia* leaf and stem ethanolic extract

4.8.2 LOD and LOQ

LOD and LOQ were obtained from the calculation based on the slope of calibration curve and the standard deviation of regression line. The lowest concentration for analyte in a sample that could be detected was 0.006 mg/ml, whereas the lowest concentration for analyte in the sample that could be quantitatively defined was 0.019 mg/ml.

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4.8.3 Accuracy

The accuracy of an analytical procedure was analyzed by adding three different concentrations of standard RA (low, medium and high levels) into sample. All tests were done in triplicate. The accuracy of HPLC for RA quantitation of *M. cordifolia* leaf and stem ethanolic extract which calculated as percentage recovery of RA were shown on Table 15.

Table 37 Accuracy of HPLC for RA quantitation of *M. cordifolia* ethanolic extract

Rosmarinic acid added (µg/ml)	Leaf		Stem	
	Rosmarinic acid content found (µg/ml)	% Recovery	Rosmarinic acid content found (µg/ml)	% Recovery
-	61.26	100.00	90.48	100.00
50	112.73	101.32	139.27	99.15
200	289.19	110.70	304.32	104.77
400	468.91	101.66	491.07	100.12
Average	**-**	103.42	-	101.01

4.8.4 Precision

The repeatability and intermediate precision was evaluated with three different concentrations of RA was analyzed on the same day and three different days respectively. The values were shown as %RSD in Table 16.

Table 38 Repeatability and intermediate precision of RA in *M. cordifolia* leaf by HPLC

Rosmarinic acid added (µg/ml)	***M. cordifolia* leaf extract**		***M. cordifolia* stem extract**	
	Repeatability precision	**Intermediate precision**	**Repeatability precision**	**Intermediate precision**
-	2.93	0.37	2.24	0.15
50	1.91	0.42	1.14	0.26
200	0.66	0.97	0.55	0.50

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	M. cordifolia leaf extract		*M. cordifolia* stem extract	
Rosmarinic acid added (µg/ml)	**Repeatability precision**	**Intermediate precision**	**Repeatability precision**	**Intermediate precision**
400	0.68	0.59	0.51	0.75
Average	1.54	0.59	1.11	0.42

4.8.1.5 Specificity

The specificity was evaluated by peak purity test which processed with Shimadzu LC Solution software. The peak purity test confirmed that analyte chromatographic peak is not attributable with another compound. The peak purity index of RA of leaf (Figure 23) and stem (Figure 24) were 1.00.

Peak purity index : 1.00000
Single point threshold : 0.999118
Minimum peak purity index : 881

Table 39 Peak purity of rosmarinic acid of *M. cordifolia* leaf extract (Peak purity index: 1.00)

Peak purity index : 1.00000
Single point threshold : 0.999609
Minimum peak purity index : 391

Table 40 Peak purity of rosmarinic acid of M. cordifolia stem extract (Peak purity index: 1.00)

4.7.1.6 Robustness

The robustness was evaluated during the analysis of HPLC method when the temperature was varied from 34-36 °C. The results showed that in the area under the peak and retention time as present on the Table 17.

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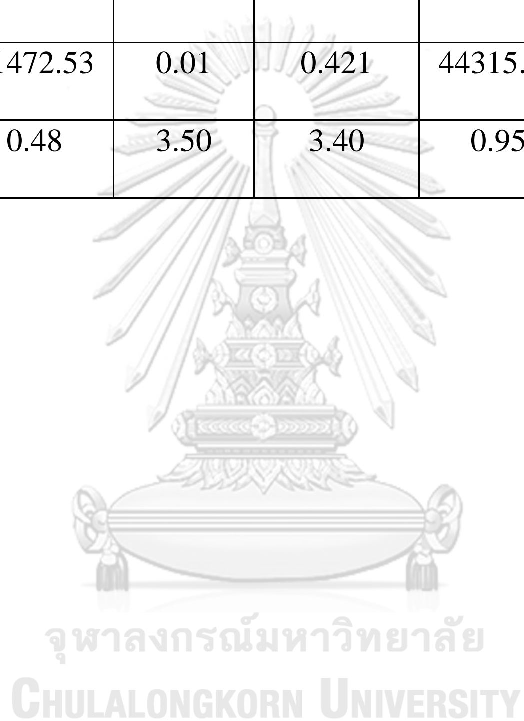
Table 41 Robustness of HPLC for RA quantitation in *M. cordifolia* leaf and stem

	***M. cordifolia* leaf extract**			***M. cordifolia* stem extract**		
	Peak area (AU)	**RA in extract (mg/ml)**	**Retention time (min)**	**Peak area (AU)**	**RA in extract (mg/ml)**	**Retention time (min)**
Temperature 34 °C	4466848	0.28	12.86	4704711	0.29	12.83
Temperature 35 °C	4500368	0.30	12.19	4698719	0.31	12.20

	M. cordifolia leaf extract			*M. cordifolia* stem extract		
	Peak area (AU)	**RA in extract (mg/ml)**	**Retention time (min)**	**Peak area (AU)**	**RA in extract (mg/ml)**	**Retention time (min)**
Temperature 36 °C	4506857	0.28	12.08	4625134	0.29	12.05
Mean	4491358	0.29	12.38	4676188	0.30	12.36
SD	21472.53	0.01	0.421	44315.43	0.01	0.414
%RSD	0.48	3.50	3.40	0.95	2.87	3.35



CHAPTER V

DISCUSSION AND CONCLUSION

The quality control of herbal plant can provide to guarantee the standard of raw material for manufacture traditional medicines, food, beverages, dietary suppletory as well as cosmetic products.

Plant morphological character is very important for plant authentication. For differentiation, characteristics of flower, leaf, stalk, seed and root forms were used as the keys. The genus *Mentha* belongs to the family Lamiaceae consisting of about 25-30 species have a subcosmopolitan distribution mainly found across regions of Eurasia, Africa, Asia, Australia, South Africa and North America (Brickell and J.D., 1997). *Mentha* is a taxonomically difficult genus because of extensive hybridization, vegetative propagation, polyploidization and cultivation (Šaric-Kundalic et al., 2009).

The plant morphology was described in the Flora of Java Vol. II Groningen that is a herbaceous aroma perennial plant growing 10–30 cm high with square stalk, propagating mostly by underground stolons. The leaves, which are arranged in opposite pairs are serrate 0.75–7.5 cm long and 0.75–7.5 cm wide, ovate or peltate shape. The small bell-shaped flowers; are generally in light-purple, crowded into spikes at the leaf axils. The fruits are small dry capsule and abort (Backer and Brink, 1965).

The dried leaf crude drug was brownish-green while the dried stem crude drug was light grayish-brown. (Backer and Brink, 1965)

The layer of epidermis was performed by sinuous ordinary epidermal cells i.e. wavy cell walls (Šaric-Kundalic et al., 2009). These features were observed only on the abaxial surface of leaves and were invariable for all mints. Diacytic stomata are most common in Lamiaceae (Naidu and Shah, 1980).

A transverse section of the leaf midrib presented non-glandular and uniseriate glandular trichomes. Midrib is performed by a large vascular bundle, with xylem

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facing the adaxial surface and phloem facing the abaxial surface. Palisade and lacunar parenchyma and uniseriate epidermis cells are performed to a symmetric mesophyll. One or two elongated cells on the adaxial surface of the leaf performed to palisade parenchyma while varying sizes of three to five cells layer are performed as lacunar parenchyma. The outer periclinal walls of the epidermal cells are thick and are covered by cuticles. (Bezerra et al., 2019).

The stem presented uniseriate epidermis can be found one or two layers of collenchyma. The vascular bundle consisted of four main xylem points, and externally to it was found the phloem, which gives the quadrangular shape to the stem (Bezara et al., 2019) as already reported in other species of Lamiaceae.

The trichomes are found both upper and lower sheath of the leaf. The uniseriate non-glandular and uniseriate glandular trichomes were found. The vesical of glandular trichome may secreted the chemical defense of a plant by possessing glands such as essential oil, terpenes, gums and tannins, which in contact with predators can trigger several reactions, repelling, provoking limb immobility or even toxicity and death which exude terpenes, phenolics, alkaloids or other substances which are olfactory or gustatory repellent (Naidu. and Shah, 1980).

Essential oils are lipophilic substances produced by specialized secreting tissues called glandular trichomes. glandular trichomes secreting essential oils are the base for the economic importance of several plant families, including the Lamiaceae. Anatomical taxonomy of *Mentha* specie is reported that contain both capitate and peltate glandular trichomes like other members of the Labiatae family (Maffei et al., 1986).

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However, *M. cordifolia* has a larger number of cell layers in the lacunar parenchyma, which is an important characteristic for the differentiation of species (Bezzara et al., 2019).

Quantitation of microscopic leaf constant number is often used for medicinal plant samples. Leaf constant numbers were used to identify between some closely species which cannot differentiated by general microscopy (Evans et al., 2009). It could be the first step to identify the plant (Thitikornpong et al., 2018).

The stomatal type is one of histological characteristic evaluation which used to differentiate plants species. The stomatal type is classified by their form and arrangement in the surrounding cells. Marsh mint leaves with stomata above and below, few above; diacytic type. The diacytic type is one of common stomata which can found in Lamiaceae. Most tree species have stomata only on the lower leaf surface. *M. cordifolia* leaf is amphistomatous, the stomatal were found on both side of the leaf. The lower epidermis contained more stomata than the upper epidermis.

Palisade cells are found in the mesophyll under the upper epidermis layer. The trichome is formed on both sides by the lower surface has more.

The quality specification due to the physico-chemical parameters indicated that the contents of acid-insoluble ash, total ash, loss on drying and moisture content of leaf crude drug should not be more than 1.98, 9.41, 7.06 and 10.50, and the contents of stem should not be more than 1.63, 8.40 ,7.04 and 10.01 % of dry weight, respectively. Ash values is the one parameter useful to reflex the purity and quality of crude drugs. It's useful to determine the inorganic substance; such as Ca, Na, K, Cl etc., in plant material after complete incineration. The acid insoluble ash was evaluated by boiling the total ash with 70g/l hydrochloric acid which could evaluate aluminum and silicon. Ash value is used as the parameter indicates the adulteration, contamination or substitutions in plant crude drug. To evaluate loss on drying content, both of water and volatile mater were came out, while the water content evaluation can carry out only water which is in the plant crude drug. The moisture in the environment of storage can affect to the plant crude drug quality. In the other hand, the excessive of water content in plant crude drug can be the suitable condition for growth of bacteria, fungi or activate enzyme which induce chemical degradation or microbial contamination. Thus, the limit of moisture content should be set for stability of plant crude drug. The ethanol-soluble extractive and water-soluble extractive values of leaf should not be less than 5.04 and 15.23 % of dry weight and stem should not be less than 5.20 and 16.60 % of dry weight, respectively. The evaluation of soluble extractive matter is used to determine the amount of active components when extract with specified solvent. The result presented that water soluble extractive value

was higher than ethanol soluble extractive value which indicated the high content of polar compounds. Ethanol and water are common solvents used for traditional medicine preparation. The volatile oil content found only in leaf should not less than 0.36 % of dry weight. The quality control of medicinal plant material also requires the determination of the phytochemical compound for ensuring the quality reliability of natural product obtained from plant sourced (Mukherjee et al, 2002). TLC fingerprint, the solvent system consisting toluene : ethyl acetate : formic acid (5 : 4 :1.2 v/v/v) and silica gel GFR254 were demonstrated for chemical fingerprints in standardization of *M. cordifolia* leaf and stem crude drug.

GC/MS analysis was used to separate the mixture of compounds in volatile oil. This method has high resolute power and high reproducibility. Two separate techniques; gas chromatography (GC) and mass spectrometry (MS), are successfully combined to form gas chromatography-mass spectrometry (GC-MS), the advantages become obvious. GC separate the compound mixture that are naturally volatile oil or that can be converted to volatile derivative. The molecules that have low molecular weight will elute out earlier while the molecules that have high molecule weight will elute out late due to the boiling point of substances. MS is detector instrument which each molecule was ionized by electron beam (70 eV) to produce the characteristic mass spectrum (Sneddon et al., 2007).

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Piperitenone oxide is the main chemical constituent in *M. cordifolia* leaf essential oil (73.22%). The previous study also reported that the peperitenone oxide content was also be the highest in arial part essential oil *M. cordifolia* (69%) of Turkey (Başer et al., 2012).

HPLC is a chromatographic technique which is adaptable, robust and widely used for the identification, quantification and purification of natural products. Reverse phase chromatography is the most commonly used in separation technique in HPLC. It is estimated that over 65% of all HPLC separations are carried out in the reversed phase mode (Boligon et al, 2014). RP-HPLC is suitable simplicity, versatility, and scope to handle compound of a diverse polarity and molecular mass such as plant secondary plant metabolites. RA is a phenolic compound containing conjugated double bonds which have strong UV absorbtion; thus, PDA is suitable detector for

analysis. The reverse phase HPLC column is widely used to separate phenolic compounds in plant extracts. Octadecilsilane (ODS or simply C_{18}) column is preferred for polar compound analysis. The highly polar substances may be irreversibly retained in the column and gradually changing the separation characteristics of the column. The usage of guard columns is necessary in the analysis of crude extract (Santos et al, 2017). Many natural product materials contain significant level of strongly binding components, such as chlorophyll and other endogenous materials that may in the long term compromise the performance of analytical columns. Therefore, the guard columns will significantly protect the lifespan of the analytical columns. (Boligon and Athayde, 2014). The chromatographic condition optimization as mobile phase, gradient elution procedure, flow rate, column temperature and wavelength detection were performed the good separation. Formic acid, phosphoric acid and acetic acid were usually employed to the aqueous phase to enhance the resolution, restrain the ionization and reduced the peak tailing of compounds. The most suitable mobile phase following previous study showed good resolution and symmetric peak shape were performed by two parts as solvent A ;0.2% phosphoric acid in water and solvent B ; methanol with isocratic program. The column temperature was hold at 30 °C for the duration of analysis. Based on data from literature, in this study the optimal detection wavelength for detect RA was set to be 330 nm (Geller et al, 2010). The mainly component of *M. cordifolia* ;RA is a hydroxycinnamic acid derivatives, are synthesized by shikimate partway. The HPLC results demonstrated that RA contents of *M. cordifolia* leaf and stem crude drugs in Thailand were 1.917 and 0.990 g/100g.

Pharmacognostic specification of *M. cordifolia* leaf and stem in Thailand were established. The chemical constituents of the volatile oil from *M. cordifolia* dried leaf were clearly revealed. For quantitative analysis, RP-HPLC of RA contents of *M. cordifolia* leaf and stem crude drugs were developed.

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REFERENCES

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Abedini A., Roumy V., Mahieux S., Biabiany M., Standaert-Vitse A., Rivière C., Sahpaz S., Bailleul F., Neut C., and Hennebelle T. 2013. Rosmarinic Acid and Its Methyl Esteras Antimicrobial Components of the Hydromethanolic Extract of *Hyptis atrorubens Poit.* (Lamiaceae). Evid Based Complement Alternat Med. 2013: 604536.

Abu-Hamdah S., Afifi F. U., Shehadeh M. and Khalid S. 2008. Simple Quality-Control Procedures for Selected Medicinal Plants Commonly Used in Jordan.Pharmaceutical Biology 43(1): 1-7.

Adams, R.P., Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. (essential oils). Vol. 3. 2001, Illinois USA: Allured Publishing Corporation. 9-456.

Amaral R.G., Fonsea .S., Silva T.K., Andrade L.N., Frana M.E., Barbosa-Filho J.M., Sousa D.P., Moraes M.O., Pessoa C.O., Carvalho A.A., and Thomazzi S.M. 2015.Evaluation of The Cytotoxic and Antitumour Effects of The Essential Oil from *Mentha x villosa* and Its Main Compound, Rotundifolone. Journal of Pharmacy and Pharmacology 67(3).

Amoah S.K.S., Sandjo L.P., Kratz J.M. and Biavatti M.W. 2015. Rosmarinic Acid – Pharmaceutical and Clinical Aspects. Planta Medica 2016; 82: 388–406.

Anusuya C. and Manoharab S. 2011. Antitumor Initiating Potential of Rosmarinic Acid in 7,12-Dimethylbenz(*a*)anthracene-Induced Hamster Buccal Pouch Carcinogenesis. Journal of Environmental Pathology, Toxicology and Oncology 30: 199-211.

Backer C.A., Brink R.C.B. Flora of Java Vol. II Groningen: N.V. Wolters-Norrdhoff.

CHULALONGKORN UNIVERSITY

1965:641pp.

Başer K.H.C., Kürkçüoğlu M., Demirci, Özek T. and Tarımcılar G. 2012. Essential Oils of Mentha Species from Marmara region of Turkey. Journal of Essential Oil Research 24:265-272.

Bezerra A.C., Barbosa L.S., Zura J.F.C., Oliveira A.M.F. and Azevedo C.F. 2019. Structural Characterization of Mint (*Mentha x villosa* Huds.) Stem and Leaf. Journal of Experimental Agriculture International 36(2): 1-6

Boligon A.A. and Athayde M.L. 2014. Importance of HPLC in Analysis of Plants Extracts. Austin Chromatography. 1(3).

Brickell, C. and J.D. Zuk, 1997. The American Horticultural Society: A-Z Encyclopedia of Garden Plants. DK Publishing Inc., New York.

Bum E.N., Taiwe G.S., Ngoupaye G.T. and Mbomo A. 2011. Antiepileptic Medicinal Plants Used in Traditional Medicine to Treat Epilepsy. Clinical and Genetic Aspects of Epilepsy.

Costa R.S., Carneiro T.C.B., Cerqueira-Lima A.T., Queiroz N.V., Alcântara-Neves N.M., Pontes-de-Carvalho L.C., Velozo E.S., Oliveira E.J. and Figueiredo C.A. 2012. *Ocimum gratissimum* Linn. and Rosmarinic Acid, Attenuate Eosinophilic Airway Inflammation in An Experimental Model of Respiratory Allergy to Blomia Tropicalis. International Immunopharmacology 13(2012): 126–134.

Chaowuttikul C., Palanuvej C. and Ruangrungsi N. 2020. Quantification of Chlorogenic Acid, Rosmarinic Acid, and Caffeic Acid Contents in Selected Thai Medicinal Plants Using RP-HPLC-DAD. Brazilian Journal of Pharmaceutical Sciences 56(2020)

Chulalongkorn University

Chen Y.Y., Tsai C.F., Tsai M.C., Hsu Y.W. and Lu F.J. 2017. Inhibitory Effects of Rosmarinic Acid on Pterygium Epithelial Cells through Redox Imbalance and Induction of Extrinsic and Intrinsic Apoptosis. Experimental Eye Research 160(2017): 96-105.

Chotchoungchatchai S., Saralamp P., Jenjittikul T. and Pornsiripongse S. 2012.

Medicinal Plants Used with Thai Traditional Medicine in Modern Healthcare Services:

A Case Study in Kabchoeng Hospital, Surin Province, Thailand. Journal of Ethnopharmacology 141(1): 193-205.

Ellis B.E. and Towers G.H.N. 1970. Biogenesis of Rosmarinic Acid in *Mentha*. Biochemical Journal 118(2): 291-297.

Evans W.C., Evans D., and Trease G.E. 2009. *Trease and Evans pharmacognosy*.

Fachel F.N.S., Schuh R.S., Veras K.S., Bassani V.L., Koester LS., Henriques A.T.,Braganhol E. and Teixeira H.F. 2018. An Overview of The Neuroprotective Potential of Rosmarinic Acid and Its Association with Nanotechnology-based Delivery Systems: A Novel Approach to Treating Neurodegenerative Disorders. Neurochemistry International journal 122: 47-58.

Fallarini S., Miglio G., Paoletti T., Minassi A., Amoruso A., Bardelli C., Brunelleschi S. and Lombardi G. 2009. British Journal of Pharmacology 157(2009): 1072–1084.

Fialová S., Tekelová D., Rendeková K., Klinčok J., Kolárik M., Kurucová K. and Grančai D. 2015. Phenolic Compounds Variation in Mentha L. Species in The

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Course of A Four-years Period. Acta Facultatis Pharmaceuticae Universitatis Comenianae LXII, 2015 (Suppl IX): 2-7.

Fialovaa S., Veizerova L., Nosalova V., Drabikova K., Tekelova D., Grancai D. and Sotnikova R. 2015. Water Extract of *Mentha x villosa*: Phenolic Fingerprint and Effect on Ischemia-Reperfusion Injury. Natural Product Communications 10(6):937-940.

Furtado R.A., de Araújo F.R.R., Resende F.A., Cunha W. R. and Tavares D. C. 2009. Protective Effect of Rosmarinic Acid on V79 Cells Evaluated by The Micronucleus and Comet Assays. Journal of Applied Toxicology 30(2010): 254-259.

GBIF Secretariat. 2019.*Mentha cordifolia* Opiz ex Fresen. [Internet]. GBIF Backbone Taxonomy. Checklist dataset [cited 2018 Sep 15]. Available from: https://doi.org/10.15468/39omei accessed.

Geller F., Schmidt C., Göttert M., Fronza M., Schattel V., Heinzmannc B., Werz O., Flores E.M.M., Merfort I. and Laufer S. 2010. Identification of Rosmarinic Acid as The Major Active Constituent in Cordia Americana. Journal of Ethnopharmacology 128(2010): 561–566

Hamaguchi T., Ono K., Murase A. and Yamada M. 2009. Phenolic Compounds Prevent Alzheimer's Pathology through Different Effects on the Amyloid-Aggregation Pathway. The American Journal of Pathology 175(6).

Hur Y.G., Suh C.H., Kim S. and Won J. 2006. Rosmarinic Acid Induces Apoptosis of Activated T Cells from Rheumatoid Arthritis Patients via Mitochondrial Pathway. Journal of Clinical Immunology, Vol. 27(1).Indian Pharmacopoeia

2010, Govt. of India, Ministry of Health and Family Welfare. Delhi: Indian Pharmacopoeia Commission, Ghaziabad, 2010.

Jang AH., Kim TH., Kim GD., Kim J.E., Kim H.J., Kim S.S., Jin YH., Park Y.S. and Park CS. 2011. Rosmarinic Acid Attenuates 2,4-dinitrofluorobenzene-induced Atopic Dermatitis in NC/Nga Mice. International Immunopharmacology 11 (2011): 1271–1277.

Jiang W., Chen X., Qu G., Yue X., Zhu H., Tian J. and Fu F. 2009. Rosmarinic Acid Protects Against Experimental Sepsis by Inhibiting Proinflammatory Factor Release and Ameliorating Hemodynamics. SHOCK 32(6): 608-613

Kim D., Kim B., Yun E., Kim J., Chae Y. and Park S. 2012. Statistical Quality Control of Total Ash, Acid-insoluble Ash, Loss on Drying, and Hazardous Heavy Metals Contained in The Component Medicinal Herbs of “Ssanghwatang”, A Widely Used Oriental Formula in Korea. Journal of Natural Medicines (2013) 67:27–35.

Kim D.S., Kim H.R., Woo E.R., Hong S.T., Chae H.J. and Chae S.W. 2005. Inhibitory Effects of Rosmarinic Acid on Adriamycin-induced Apoptosis in H9c2 Cardiac Muscle Cells by Inhibiting Reactive Oxygen Species and The Activations of c-Jun N-terminal Kinase and Extracellular Signal-regulated Kinase. Biochemical Pharmacology 70(2005): 1066–1078.

Kim H.K., Lee J.J., Lee J.S., Park YM. and Yoon T.R. 2008. Rosmarinic Acid Down-Regulates The LPS-Induced Production of Monocyte Chemoattractant Protein-1 (MCP-1) and Macrophage Inflammatory Protein-1α (MIP-1α) via the MAPK Pathway in Bone-Marrow Derived Dendritic Cells. Molecule and Cells

26(2008): 583-589.

Kupiec T. 2004. Quality-Control Analytical Methods: High-Performance Liquid Chromatography. International Journal of Pharmaceutical Compounding 8(3).

Lahlou S., Carneiro-Leão R.F.L. and Leal-ardoso J.H. 2002. Cardiovascular Effects of The Essential Oil of *Mentha x villosa* in DOCA-salt-hypertensive rats. Phytomedicine 9(8):715-720.

Lawrence B.M. 2007. Mint: The Genus Mentha. CRC Press Taylor & Francis Group.Latif Z, Sarker S. 2012. Isolation of natural products by preparative high performance liquid chromatography (prep-HPLC). In: Natural products isolation. 3rd ed. USA: Humana Press e Springer-Verlag

Lee H.J., Cho H.S., Park E., Kima S., Lee S.Y., Kim C.S., Kim D.K., Kima S.J. and Chun H.S. 2008. Rosmarinic Acid Protects Human Dopaminergic Neuronal Cells Against Hydrogen peroxide-induced Apoptosis. Toxicology 250(2008): 109–115.

Leung K.S.Y., Chan K., Lau A.C.K. and Lu G.H. 2006. Measurement Uncertainty for Total Ash and Acid-insoluble Ash Determination of Chinese Material Medica. *Accreditation and Quality Assurance* (2005) 10:289–294.

Li G., Jiang W., Tian J., Qu G., Zhu H. and Fu F. 2010. In Vitro and In Vivo Antifibrotic Effects of Rosmarinic Acid on Experimental Liver Fibrosis. Phytomedicine 17:282-288.

Maffei M., Gallino M. and Sacco T. 1986. Glandular Trichomes and Essential Oils of Developing Leaves in *Mentha viridis lavanduliodora*. Planta Med., 52, 187–193.

Matos F.J., Machado M.I.L., Craveiro A.A. and Alencar J.W. 1999. Essential Oil of *Mentha x villosa* Huds. From Northeastern Brazil. Journal of Essential Oil Research., 11, 41-44McPolin O. 2009. Validation of Analytical Methods for Pharmaceutical Analysis. Mourne Training Services.

Mehta S.J., Shah D.P., Mehta T.J., Patel P.M. and Patel N.M. 2011. Compendial Testing Method on Herbal Crude Drug – A Review. Asian Journal of Pharmaceutical Research 1(2): 49-52.

Monte F.J.Q. and Oliveira E.F. 2001. Pentacyclic Triterpinoids of *Mentha villosa*: Structural Identification and H1 and 13C Resonance Assignments. Quim. Nova 24(4) :491-500.

Moon D.O., Kim M.O., Lee JD., Choi Y.H. and Kim GY. 2010. Rosmarinic acid Sensitizes Cell Death through Suppression of TNF-a-induced NF-jB Activation and ROS Generation in Human Leukemia U937 Cells. Cancer Letters 288 (2010): 183–191.

Mukherjee P.K., 2002. Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. New Delhi, India: Business Horizons.

Naidu A.C. and Shah G.L., 1980. Observation on the Cotyledonary Stomata and Trichomes and Their Ontogeny in Some Genera of Lamiaceae. Phyton (Austria) 21 (1): 137—152

National Library of Medicine. Pubchem. Rosmarinic acid [Internet}. National Center for Biotechology Information; 2005 [cited 2018 Sep 15]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Rosmarinic-acid.

Osakabe N., Yasuda A., Natsume M. and Yoshikawa T. 2004. Rosmarinic acid

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Inhibits Epidermal Inflammatory Responses: Anticarcinogenic Effect of Perilla Frutescens Extract in The Murine Two-stage Skin Model. Carcinogenesis 25(4): 549-57.

Osakabe N., Takano H., Sanbongi C., Yasuda A., Yanagisawa R., Inoue K. and Yoshikawa T. 2008. Anti-inflammatory and Anti-allergic Effect of Rosmarinic acid (RA); Inhibition of Seasonal Allergic Rhinoconjunctivitis (SAR) and Its Mechanism. Biofactors. 21(1-4): 127-31.

Park D.H., Park S.J., Kim J.M., Jung W.Y., Ryu J.H. 2010. Subchronic Administration of Rosmarinic acid, A Natural Prolyl Oligopeptidase Inhibitor, Enhances Cognitive Performances. Fitoterapia 81 (2010): 644–648.

Panya A., Laguerre M., Lecomte J., Villeneuve P., Weiss J., McClements D.J. and Decker E.A. 2010. Effects of Chitosan and Rosmarinate Esters on the Physical and Oxidative Stability of Liposomes. Journal of Agricultural and Food Chemistry 58(2010): 5679-5684.

Patil S.G., Wagh A.S., Pawara R.C. and Ambore S.M. 2013. Standard Tools for Evaluation of Herbal Drugs: An Overview. The Pharma Innovation – Journal 2(9): 60-65.

Petersen M. and Simmonds M.S.J. 2002. Molecules of Interest Rosmarinic acid. Phytochemistry 62: 121-125.

Perez-Fons L., Garzon M.T. and Micol V. 2010. Relationship between the Antioxidant Capacity and Effect of Rosemary (Rosmarinus officinalis L.) Polyphenols on Membrane Phospholipid Order. Journal of Agricultural and Food Chemistry 58(2010): 161–171.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Pitakpawasutthi Y., Palanuvej C. and Ruangrungsi N. 2018. Microscopic Leaf Constant Numbers of *Chromolaena odorata* in Thailand. Pharmacognosy Journal10(6):S95-S99

PubChem, Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004. Rosmarinic acid. [Internet]. PubChem Compound Summary for CID 5281792, [cited 2018 Sep. 15]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Rosmarinic-acid

Wiersema J.H. (2019). *Mentha × villosa* Huds. GRIN Taxonomy. US National Plant Germplasm System.. [Internet] Fl. Angl. Ed. 2, 1:205. 1778 [cited 2018 Sep 15]. Available from: https://www.gbif.org/species/101379811

Roskov Y., Ower G., Orrell T., Nicolson D.. Baily N., Krirk P., Bourgoin T., DeWalt E., Decock W. Nieukerken E.V. and Penev L. 2020. Species Details : *Mentha x villosa* Huds. Catalogue of Life: 2020-09-01 Beta [Internet} [cited 2020 Sep 20]. Available from: http://www.catalogueoflife.org/col/details/species/id/0b843036b541df3371e1b971fb8b9e2a.

S.L. Patwekar, R.S. Sakhare and Nalbalwar N.N. 2015. HPLC Method Development and Validation- A General Concept. International Journal of Chemical and Pharmaceutical Sciences 6(1).

Sanbongi C., Takanowz H., Osakabe N., Sasa N., Natsume M., Yanagisawaw R., Inouez K., Sadakane§ K., Ichinose§ T. and Yoshikawa T. 2004. Rosmarinic Acid in Perilla Extract Inhibits Allergic Inflammation Induced by Mite Allergen, in A Mouse Model. Blackwell Publishing Ltd, Clinical and

CHULALONGKORN UNIVERSITY

Experimental Allergy 34:971–977.

Saric-Kundalic B., Fialova S., Dobes C., Olzant S., Telkelova D.,Grancai D., Reznicek G. and Saukel J. 2009. Multivariate Numerical Taxonomy of Mentha Species, Hybrids, Varieties and Cultivars Scientia Pharmaceutica Journal

Sarker S.D. and Nahar L. 2015. Applications of High Performance Liquid Chromatography in the Analysis of Herbal Products. Evidence-Based Validation of Herbal Medicine : 405-425

Shikov A.N., Pozharitskaya O.N., Makarov V.G., Wagner H., Verpoorte R. and Heinrich M. 2014. Medicinal Plants of the Russian Pharmacopoeia; Their History and Applications. Journal of Ethnopharmacology 154: 481-536.

Shimojo Y., Kosaka K., Noda Y., Shimizu T. and Shirasawa T. 2010. Effect of Rosmarinic Acid in Motor Dysfunction and Life Span in a Mouse Model of Familial Amyotrophic Lateral Sclerosis. Journal of Neuroscience Research 88: 896–904.

Sihanat A., Rungsihirunrat K., Palanuvej C. and Ruangrungsi N. 2016. Characteristics and Number of Trichome of Leaves from Sellected Cassia spp. In Thailand. Buentin of Health, Science and Technology BHST 14(1) : 10-20

Simon T.K. 2018. Systematic Significance of Palisade Ratio In Pharmacognostic Applications. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)13(2):1-8

Sneddon J., Masuram S. and Richert J.C. 2007. Gas Chromatography-Mass Spectrometry-Basic Principles, Instrumentation and Selected Applications for Detection of Organic Compounds, Analytical Letters, 40:6, 1003-1012

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Snyder L.R., Kirkland J.J., Glajch J.L. 1997. Practical HPLC method development. 2nd

ed. New York: John Wiley and Sons; Soares C.A. 2010. Medicinal plants from planting to harvesting. 1. Ed. São Paulo: Icon.;310.

Sotnikova R., Okruhlicova L., Vlkovicova J., Navarova J., Gajdacova B., Pivackova L.,

Fialova S. and Krenek P. 2013. Rosmarinic Acid Administration Attenuates Diabetes-induced Vascular Dysfunction of The Rat Aorta. Journal of Pharmacy and Pharmacology;65: 713–723.

Tekeľová D., Bittner F.S., Tóth J., 2016. Phenolic compounds in leaves insertions of *Mentha* × *villosa* Huds. cv. Snežná. Ceska Slov Farm. Fall 65(4): 161-165.

The Government Pharmaceutical Organization. 2013. National List of Essential Medicines (2013): 36.

Thitikornpong W, Ongpipattanakul B, Palanuvej C, Ruangrungsi N. 2018. Pharmacognostic Specification and Mangiferin Content of Aquilaria crassna Leaves. Pharmacogn J. 2018;10(2):293-8

Thomas S., Patil D.A., Patil A.G. and Chandra N. 2008. Pharmaognostic Evaluation and Physicochemical Analysis of *Averrhoa carambola* L. Fruit. Journal of Herbal Medicine & Toxicology 2(2): 51-54.

Tistaert C., Dejaegher B. and Heyden Y.V. 2010. Chromatographic separation techniques and data handling methods for herbal fingerprints: A review. Analytica Chimica Acta 690 (2011) 148–161.

TN 11th Standard State Board School. Unit 4 Plant anatomy. Chapter 9 Tissue and Tissue System. Botany 11th standard - 11th Standard (Plus One) Tamil Nadu

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

State Board School Textbook [cited 2020 Oct 20]. Available from: https://www.pharmatutor.org/articles/evaluation-crude-drugs-mono-polyherbal-formulation?page=2%2C2

Villaseñor I.M. and Sanchez A.C. 2009. Menthalactone, a New Analgesic from *Mentha cordifolia* Opiz. Leaves. Verlag der Zeitschrift für Naturforschung, Tübingen.

Villaseñor I.M., Angelada J, Canlas A.P., 2002. Echegoyen D. Bioactivity Studies on Beta-sitosterol and Its Glucoside. Phytother Reseserch 16(5):417-21

Vostalova´ J., Zdarilova´ A. and Svobodova' A. 2010. Prunella Vulgaris Extract and Rosmarinic Acid Prevent UVB-induced DNA Damage and Oxidative Stress in HaCaT Keratinocytes. Archives of Dermatological *Research 302(2010): 171-181.*

WHO. 2011. Quality Control Methods for Herbal Materials. Geneva. 1-40.

Wiersema J.H.. 2011 *Mentha cordifolia*. [Internet]. Syll. Pl. 2:232. 1828 [cited 2018 Sep 15]. Available from: https://www.gbif.org/species/7383339.

Wiersema J.H. (2019). *Mentha × villosa* Huds. GRIN Taxonomy. US National Plant Germplasm System.. [Internet] Fl. Angl. Ed. 2, 1:205. 1778 [cited 2018 Sep 15]. Available from: https://www.gbif.org/species/101379811.

Xu Y., Xu G., Liu L., Xu D. and Liu J. 2010. Anti-Invasion Effect of Rosmarinic Acid via the Extracellular Signal-Regulated Kinase and Oxidation–Reduction Pathway in Ls174-T Cells. Journal of Cellular Biochemistry 111(2010):370–379.

Yao Y., Mao J., Xu S., Zhao L., Long L., Chen L., Li D. and Lu S. 2018. Rosmarinic

Chulalongkorn University

Acid Inhibits Nicotine-induced C-reactive Protein Generation by Inhibiting NLRP3 Inflammasome Activation in Smooth Muscle Cells. Journal of Cellular Physiology 234(2019): 1758-1767.

Zixin Z., Guixin C.. 1995. The Chemical Components in The Essential oils from *Mentha cordifolia*. Journal of Plant Resources and Environment;4(3):63-64

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APPENDICES

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APPENDIX A

Leaf constant number

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Table 42 Upper epidermal cell number, upper epidermal cell area, upper stomatal index, upper oil gland number, upper oil gland index, upper trichome number and upper trichome index of *M. cordifolia* leaves in Thailand

Field	Upper epidermal cell number			Upper stomatal cell number			Upper stomatal index			Upper oil gland number			Upper oil gland index			Upper trichome number			Upper trichome index			Upper epidermal cell area (µm2)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	500	492	460	20	12	12	3.79	2.27	2.40	8	20	24	1.52	3.79	4.80	0	4	4	0.00	0.76	0.80	2028.00	2068.52	2213.91
2	536	508	444	16	20	12	2.86	3.62	2.54	8	24	16	1.43	4.35	3.39	0	0	0	0.00	0.00	0.00	1889.67	2012.50	2280.25
3	532	504	496	12	20	16	2.14	3.68	3.01	12	16	16	2.14	2.94	3.01	4	4	4	0.71	0.74	0.75	1907.70	2024.13	2052.13
4	528	536	436	12	12	12	2.19	2.10	2.56	8	24	20	1.46	4.20	4.27	0	0	0	0.00	0.00	0.00	1913.94	1901.67	2325.58
5	524	480	448	12	12	16	2.19	2.36	3.28	8	12	20	1.46	2.36	4.10	4	4	4	0.73	0.79	0.82	1932.40	2111.33	2272.14
6	500	444	432	12	12	12	2.29	2.54	2.61	12	12	16	2.29	2.54	3.48	0	4	0	0.00	0.85	0.00	2024.00	2280.25	2342.81
7	552	436	460	16	12	12	2.76	2.59	2.44	12	16	16	2.07	3.45	3.25	0	0	4	0.00	0.00	0.81	1839.59	2321.58	2205.91
8	552	480	508	8	20	16	1.39	3.82	2.92	12	20	20	2.08	3.82	3.65	4	4	4	0.69	0.76	0.73	1835.59	2127.33	2008.50
9	544	484	416	12	20	12	2.10	3.85	2.65	12	16	20	2.10	3.08	4.42	4	0	4	0.70	0.00	0.88	1866.24	2102.12	2439.85
10	492	504	444	12	20	12	2.31	3.68	2.52	16	16	16	3.08	2.94	3.36	0	4	4	0.00	0.74	0.84	2060.52	2024.13	2284.25
11	480	408	428	8	16	12	1.60	3.64	2.61	8	16	20	1.60	3.64	4.35	4	0	0	0.80	0.00	0.00	2103.33	2482.98	2368.45
12	500	464	440	12	12	16	2.29	2.42	3.33	12	16	20	2.29	3.23	4.17	0	4	4	0.00	0.81	0.83	2024.00	2187.17	2312.73
13	520	504	484	12	12	20	2.21	2.24	3.82	12	12	16	2.21	2.24	3.05	0	8	4	0.00	1.49	0.76	1947.08	2016.13	2106.12
14	500	456	484	20	16	12	3.73	3.28	2.29	12	16	24	2.24	3.28	4.58	4	0	4	0.75	0.00	0.76	2036.00	2224.98	2106.12
15	520	488	452	12	16	16	2.21	3.03	3.31	8	20	16	1.47	3.79	3.31	4	4	0	0.74	0.76	0.00	1947.08	2089.18	2244.39
16	496	448	440	12	16	12	2.29	3.31	2.56	12	16	16	2.29	3.31	3.42	4	4	0	0.76	0.83	0.00	2044.13	2268.14	2300.73
17	588	472	448	12	12	16	1.96	2.40	3.28	12	16	24	1.96	3.20	4.92	0	0	0	0.00	0.00	0.00	1724.68	2146.64	2272.14

Field	Upper epidermal cell number			Upper stomatal cell number			Upper stomatal index			Upper oil gland number			Upper oil gland index			Upper trichome number			Upper trichome index			Upper epidermal cell area (µm2)		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
18	492	552	452	12	16	16	2.29	2.68	3.31	16	24	16	3.05	4.03	3.31	4	4	0	0.76	0.67	0.00	2064.52	1855.59	2244.39
19	552	468	468	12	16	12	2.10	3.17	2.40	8	20	16	1.40	3.97	3.20	0	0	4	0.00	0.00	0.80	1831.59	2172.75	2168.75
20	500	452	428	20	12	12	3.76	2.44	2.59	8	20	20	1.50	4.07	4.31	4	8	4	0.75	1.63	0.86	2032.00	2252.39	2372.45
21	492	408	428	16	12	16	3.08	2.78	3.45	12	12	16	2.31	2.78	3.45	0	0	4	0.00	0.00	0.86	2060.52	2474.98	2372.45
22	528	500	436	16	20	16	2.86	3.65	3.39	12	24	20	2.14	4.38	4.24	4	4	0	0.71	0.73	0.00	1925.94	2048.00	2329.58
23	544	496	432	12	12	16	2.11	2.27	3.45	12	12	16	2.11	2.27	3.45	0	8	0	0.00	1.52	0.00	1862.24	2048.13	2346.81
24	516	412	440	12	16	16	2.21	3.57	3.36	12	16	20	2.21	3.57	4.20	4	4	0	0.74	0.89	0.00	1965.98	2463.18	2308.73
25	524	408	456	12	12	20	2.17	2.75	4.07	16	16	16	2.90	3.67	3.25	0	0	0	0.00	0.00	0.00	1936.40	2478.98	2228.98
26	580	452	436	24	12	16	3.82	2.48	3.39	20	20	16	3.18	4.13	3.39	4	0	4	0.64	0.00	0.85	1772.14	2244.39	2329.58
27	436	492	468	12	12	16	2.61	2.27	3.15	12	20	20	2.61	3.79	3.94	0	4	4	0.00	0.76	0.79	2317.58	2068.52	2176.75
28	484	484	460	16	20	16	3.08	3.85	3.20	16	16	20	3.08	3.08	4.00	4	0	4	0.77	0.00	0.80	2102.12	2102.12	2213.91
29	516	464	436	12	20	20	2.19	4.00	4.24	16	16	12	2.92	3.20	2.54	4	0	4	0.73	0.00	0.85	1969.98	2191.17	2329.58
30	576	424	480	24	12	12	3.92	2.65	2.38	12	12	12	1.96	2.65	2.38	0	4	0	0.00	0.88	0.00	1772.11	2386.49	2107.33
Min	408.00			8			1.39			8			1.40			0			0			1724.68		
Max	588.00			24			4.24			24			4.92			8			1.63			2482.98		
Mean	480.71			14.58			2.85			15.69			3.09			2.31			0.45			2128.64		
S.D.	42.44			3.41			0.64			4.29			0.89			2.24			0.44			182.80		

Source1 = Nakhonpathom, Source2 = Nonthaburi, Source 3 = Srisaket

Table 43 Lower epidermal cell number, lower epidermal cell area, lower stomatal index, lower oil gland number, lower oil gland index, lower trichome number and lower trichome index of *M. cordifolia* leaves in Thailand

Field	Lower epidermal cell number			Lower stomatal cell number			Lower stomatal index			Lower oil gland number			Lower oil gland index			Lower trichome number			Lower trichome index			Lower epidermal cell area (μm^2)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	836	628	604	264	180	144	23.00	20.93	17.39	48	40	80	4.18	4.65	9.66	0	12	0	0.00	1.40	0.00	1508.17	1824.36	1879.63
2	820	644	508	272	180	92	23.94	20.45	14.20	40	48	48	3.52	5.45	7.41	4	8	0	0.35	0.91	0.00	1535.51	1788.80	2108.50
3	800	628	496	300	184	100	26.22	21.30	15.24	40	44	60	3.50	5.09	9.15	4	8	0	0.35	0.93	0.00	1594.00	1828.36	2176.13
4	812	560	552	236	128	120	21.61	17.58	16.57	40	36	52	3.66	4.95	7.18	4	4	0	0.37	0.55	0.00	1511.53	1953.71	1983.59
5	824	608	616	236	180	128	21.61	21.43	15.84	28	48	60	2.56	5.71	7.43	4	4	4	0.37	0.48	0.50	1481.59	1876.74	1815.38
6	852	648	532	264	160	124	23.00	18.69	17.03	32	44	72	2.79	5.14	9.89	0	4	0	0.00	0.47	0.00	1469.71	1751.21	2075.70
7	876	692	516	216	156	120	18.88	17.41	17.05	48	44	64	4.20	4.91	9.09	4	4	4	0.35	0.45	0.57	1409.55	1649.09	2125.98
8	836	596	560	216	164	120	19.71	20.10	16.30	40	52	52	3.65	6.37	7.07	4	4	4	0.36	0.49	0.54	1456.17	1897.85	1961.71
9	924	632	588	244	172	96	20.13	20.09	12.77	40	48	64	3.30	5.61	8.51	4	4	4	0.33	0.47	0.53	1370.25	1806.28	1864.68
10	872	652	516	224	172	132	19.51	19.82	18.44	48	44	64	4.18	5.07	8.94	4	0	4	0.35	0.00	0.56	1422.79	1749.74	2137.98
11	836	600	516	212	160	132	19.41	19.80	18.44	40	44	64	3.66	5.45	8.94	4	4	4	0.37	0.50	0.56	1452.17	1874.67	2137.98
12	908	648	572	244	164	132	20.13	18.89	17.28	56	52	56	4.62	5.99	7.33	4	4	4	0.33	0.46	0.52	1405.32	1763.21	1940.25
13	808	576	540	200	152	100	18.94	19.59	14.12	48	44	64	4.55	5.67	9.04	0	4	4	0.00	0.52	0.56	1485.62	1936.11	2019.85
14	772	656	528	224	168	96	21.46	19.09	13.95	48	52	56	4.60	5.91	8.14	0	4	8	0.00	0.45	1.16	1567.34	1748.39	2053.94
15	880	628	528	204	180	124	17.96	21.03	17.61	48	44	52	4.23	5.14	7.39	4	4	0	0.35	0.47	0.00	1392.36	1820.36	2069.94
16	828	528	496	244	136	88	21.48	19.32	13.92	56	40	48	4.93	5.68	7.59	8	0	0	0.70	0.00	0.00	1515.73	2069.94	2152.13
17	852	636	588	256	152	132	21.77	18.18	16.84	56	44	60	4.76	5.26	7.65	12	4	4	1.02	0.48	0.51	1497.71	1772.33	1896.68

Field	Lower epidermal cell number			Lower stomatal cell number			Lower stomatal index			Lower oil gland number			Lower oil gland index			Lower trichome number			Lower trichome index			Lower epidermal cell area (μm^2)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
18	920	660	424	252	184	96	20.19	20.44	16.90	72	52	44	5.77	5.78	7.75	4	4	4	0.32	0.44	0.70	1414.96	1755.15	2502.49
19	824	660	484	252	192	96	22.34	21.33	15.29	44	44	48	3.90	4.89	7.64	8	4	0	0.71	0.44	0.00	1517.59	1755.15	2210.12
20	796	640	580	264	188	128	23.57	21.27	16.41	56	52	68	5.00	5.88	8.72	4	4	4	0.36	0.45	0.51	1580.28	1806.50	1924.14
21	800	656	540	232	168	144	21.48	19.18	19.46	40	48	52	3.70	5.48	7.03	8	4	4	0.74	0.46	0.54	1530.00	1744.39	2051.85
22	808	620	476	252	160	132	22.66	19.14	19.88	44	52	52	3.96	6.22	7.83	8	4	4	0.72	0.48	0.60	1541.62	1828.90	2288.84
23	872	672	464	236	168	96	20.42	18.67	15.58	44	52	44	3.81	5.78	7.14	4	8	12	0.35	0.89	1.95	1430.79	1716.10	2307.17
24	860	608	556	224	180	100	19.58	21.43	13.89	60	48	60	5.24	5.71	8.33	0	4	4	0.00	0.48	0.56	1446.79	1876.74	1962.56
25	856	656	508	236	188	100	20.56	20.98	15.15	52	48	48	4.53	5.36	7.27	4	4	4	0.35	0.45	0.61	1460.22	1764.39	2120.50
26	820	656	556	236	168	112	21.22	18.92	15.22	52	60	60	4.68	6.76	8.15	4	4	8	0.36	0.45	1.09	1511.51	1756.39	1978.56
27	800	656	516	232	172	104	21.25	19.28	15.29	56	60	56	5.13	6.73	8.24	4	4	4	0.37	0.45	0.59	1542.00	1760.39	2101.98
28	800	600	488	252	180	96	22.74	21.63	15.09	52	44	52	4.69	5.29	8.18	4	8	0	0.36	0.96	0.00	1558.00	1898.67	2197.18
29	820	612	524	216	168	140	19.93	20.19	19.34	44	48	56	4.06	5.77	7.73	4	4	4	0.37	0.48	0.55	1483.51	1853.99	2108.40
30	800	596	516	232	160	104	21.25	19.70	15.20	56	56	56	5.13	6.90	8.19	4	0	8	0.37	0.00	1.17	1542.00	1893.85	2105.98
Min	424.00			88			12.77			28			2.56			0			0			1370.25		
Max	924.00			300			26.22			80			9.89			12			1.95			2502.49		
Mean	665.02			174.04			19.08			50.80			5.97			4.00			0.46			1793.52		
S.D.	134.53			54.44			2.65			8.84			1.75			2.68			0.33			262.45		

Source1 = Nakhonpathom, Source2 = Nonthaburi, Source 3 = Srisaket

Table 44 Palisade cell number and palisade ratio of *M. cordifolia* leaves in Thailand

Field	Palisade cell number			Palisade ratio		
	1	**2**	**3**	**1**	**2**	**3**
1	18	20	17	4.5	5	4.25
2	20	20	16	5	5	4
3	20	18	17	5	4.5	4.25
4	21	20	18	5.25	5	4.5
5	20	19	17	5	4.75	4.25
6	19	20	18	4.75	5	4.5
7	19	19	18	4.75	4.75	4.5
8	21	18	19	5.25	4.5	4.75
9	21	20	18	5.25	5	4.5
10	18	19	17	4.5	4.75	4.25
11	18	19	19	4.5	4.75	4.75
12	21	19	17	5.25	4.75	4.25
13	20	18	19	5	4.5	4.75
14	19	18	18	4.75	4.5	4.5
15	20	19	16	5	4.75	4
16	18	18	17	4.5	4.5	4.25
17	18	20	17	4.5	5	4.25
18	21	19	17	5.25	4.75	4.25
19	20	19	18	5	4.75	4.5
20	20	18	15	5	4.5	3.75
21	18	20	17	4.5	5	4.25
22	18	20	17	4.5	5	4.25
23	20	18	16	5	4.5	4
24	18	20	16	4.5	5	4
25	19	19	17	4.75	4.75	4.25
26	19	20	16	4.75	5	4

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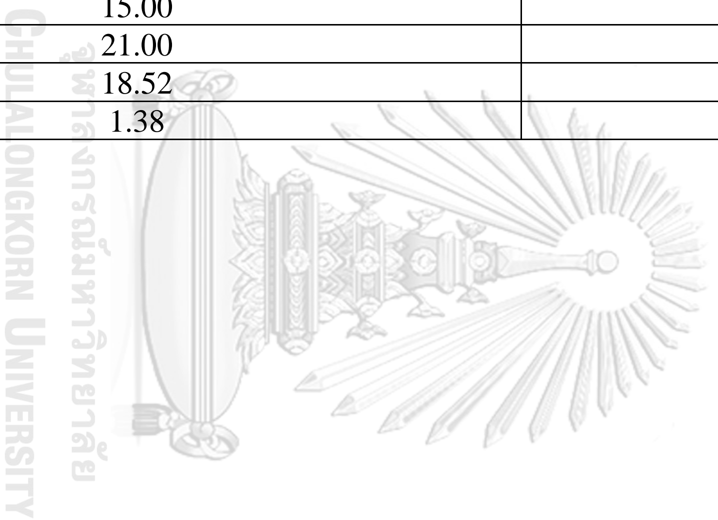
Field	Palisade cell number			Palisade ratio		
	1	**2**	**3**	**1**	**2**	**3**
27	21	18	16	5.25	4.5	4
28	18	19	18	4.5	4.75	4.5
29	18	20	17	4.5	5	4.25
30	18	19	17	4.5	4.75	4.25
Min	15.00			3.75		
Max	21.00			5.25		
Mean	18.52			4.63		
S.D.	1.38			0.35		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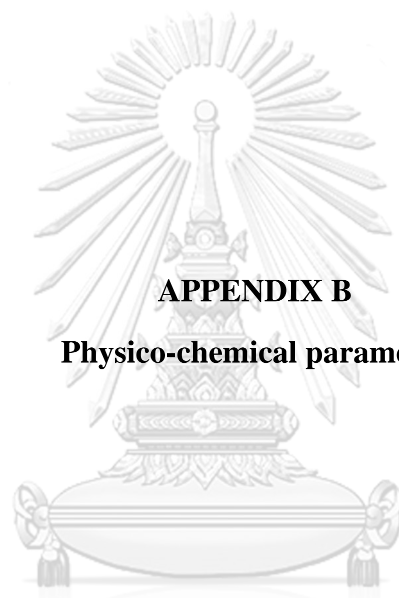

APPENDIX B

Physico-chemical parameter

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Table 45 Physico-chemical parameters of *M. cordifolia* leaf from 15 sources throughout Thailand

sources	No.	% by weight						
		Moisture content	Loss on drying	Total ash	Acid insoluble ash	Ethanol - soluble extractive	Water - soluble extractive	Volatile oil content
Ratchaburi	1	9.99	6.77	9.46	1.16	3.70	14.43	0.50
	2	9.98	6.70	9.56	1.10	3.44	14.25	0.51
	3	10.65	6.69	9.66	1.17	3.74	14.00	0.50
Petchabun	1	11.64	9.09	1.77	1.17	4.85	15.54	0.37
	2	11.65	9.25	1.76	1.22	4.78	15.23	0.36
	3	11.63	9.02	1.77	1.15	5.23	15.49	0.32
Sri sa ket	1	10.00	6.32	13.05	3.04	4.59	12.96	0.11
	2	9.98	6.22	13.24	3.17	4.57	12.33	0.12
	3	9.97	6.01	13.71	3.12	4.34	12.79	0.11
Lopburi	1	11.65	7.35	1.91	1.91	10.03	14.79	0.51
	2	11.65	7.71	1.85	1.85	9.53	14.07	0.52
	3	11.64	7.77	1.87	1.93	9.86	14.29	0.50
Nakhon Sawan	1	11.63	5.71	14.04	2.37	4.50	17.15	0.52
	2	11.63	5.88	14.16	2.36	4.40	16.63	0.50
	3	11.31	5.76	14.17	2.42	4.68	17.76	0.50
Kanchanaburi	1	8.65	6.29	1.85	1.85	4.76	14.42	0.18
	2	8.66	6.41	1.87	1.87	4.73	14.28	0.20
	3	9.31	6.36	1.78	1.81	5.07	14.89	0.19
Suphan Buri	1	9.98	8.20	1.51	1.41	6.52	14.93	0.50
	2	9.98	8.27	1.60	1.50	6.41	15.90	0.50
	3	10.64	8.10	1.65	1.38	6.97	15.15	0.45

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sources	No.	% by weight						
		Moisture content	Loss on drying	Total ash	Acid insoluble ash	Ethanol - soluble extractive	Water - soluble extractive	Volatile oil content
Prachinburi	1	10.65	7.40	1.70	1.70	4.15	15.95	0.29
	2	12.65	7.63	1.58	1.58	4.08	16.18	0.30
	3	11.64	7.70	1.66	1.71	4.22	16.99	0.31
Chachoengsao	1	9.97	7.00	13.65	2.27	3.76	15.46	0.25
	2	9.98	6.86	13.83	2.39	3.51	14.96	0.25
	3	10.63	6.66	13.77	2.49	3.77	14.92	0.22
Samuthprakarn	1	9.98	6.16	14.42	2.34	5.33	14.52	0.28
	2	10.65	6.12	14.67	2.32	5.22	15.87	0.29
	3	10.63	6.11	15.04	2.24	5.18	15.13	0.30
Nakhon Pathom	1	11.64	7.04	2.68	2.64	3.85	16.58	0.36
	2	11.64	7.47	2.72	2.52	3.67	16.02	0.33
	3	11.64	7.19	2.56	2.42	3.90	16.89	0.36
Bangkok	1	9.31	6.28	16.20	2.67	4.42	16.19	0.31
	2	9.32	6.11	15.33	2.55	4.67	16.72	0.33
	3	8.65	6.11	15.43	2.66	4.68	16.75	0.30
Saraburi	1	9.97	7.25	1.88	1.54	5.03	16.01	0.31
	2	10.64	7.14	1.72	1.66	5.04	15.93	0.33
	3	10.66	6.88	1.78	1.56	5.35	15.78	0.30
Nakhon Ratchasima	1	9.32	6.54	1.36	1.47	4.85	12.95	0.44
	2	9.32	6.69	1.47	1.59	5.14	13.28	0.47

sources	No.	% by weight						
		Moisture content	Loss on drying	Total ash	Acid insoluble ash	Ethanol - soluble extractive	Water - soluble extractive	Volatile oil content
	3	9.31	6.68	1.57	1.54	5.17	14.15	0.43
Petchaburi	1	9.99	7.60	2.03	1.97	4.82	15.45	0.53
	2	11.32	7.56	2.22	1.82	4.92	15.34	0.54
	3	10.64	7.23	2.04	1.98	5.16	15.87	0.53
Grand mean		10.50	7.06	9.41	1.98	5.04	15.23	0.36
Pooled SD.		0.41	0.14	0.18	0.07	0.18	0.42	0.02

Table 46 Physico-chemical parameters of *M. cordifolia* stem from 15 sources throughout Thailand

sources		No.	% by weight					
			Moisture content	Loss on drying	Total ash	Acid insoluble ash	Ethanol - soluble extractive	Water - soluble extractive
1	Ratchaburi	1	11.63	9.09	1.77	1.17	4.23	15.76
		2	11.46	9.25	1.76	1.22	4.05	15.27
		3	11.63	9.02	1.77	1.15	4.37	15.82
2	Petchabun	1	8.30	6.32	13.05	3.04	3.87	15.81
		2	8.54	6.22	13.24	3.17	3.57	16.53
		3	8.33	6.01	13.71	3.12	3.81	16.36
3	Sri sa ket	1	8.29	7.35	1.91	1.91	4.57	12.27
		2	8.27	7.71	1.85	1.85	4.59	12.46
		3	7.98	7.77	1.87	1.93	4.92	12.74
4	Lopburi	1	9.96	5.71	14.04	2.37	4.24	17.46
		2	9.98	5.88	14.16	2.36	4.19	17.71
		3	10.64	5.76	14.17	2.42	4.52	17.70
5	Nakhon Sawan	1	8.29	6.29	1.85	1.85	3.39	16.70
		2	8.31	6.41	1.87	1.87	3.51	16.66
		3	8.33	6.36	1.78	1.81	3.25	16.52
6	Kanchanaburi	1	9.94	8.20	1.51	1.41	5.56	16.16
		2	9.96	8.27	1.60	1.50	5.29	16.37
		3	10.59	8.10	1.65	1.38	5.08	16.24
7	Suphan Buri	1	9.98	7.40	1.70	1.70	6.71	15.85
		2	10.65	7.63	1.58	1.58	6.78	16.57
		3	10.64	7.70	1.66	1.71	6.61	16.27
8	Prachinburi	1	8.32	7.00	13.65	2.27	5.52	21.10
		2	8.32	6.86	13.83	2.39	5.10	20.40
		3	7.98	6.66	13.77	2.49	5.13	19.20
9	Chachoengsao	1	9.97	6.16	14.42	2.34	7.86	16.20

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	sources	No.	% by weight					
			Moisture content	**Loss on drying**	**Total ash**	**Acid insoluble ash**	**Ethanol - soluble extractive**	**Water - soluble extractive**
		2	9.98	6.12	14.67	2.32	7.68	17.24
		3	9.97	6.11	15.04	2.24	8.19	17.73
10	Samuthprakarn	1	15.00	7.04	2.68	2.64	4.43	17.04
		2	13.96	7.47	2.72	2.52	4.29	17.27
		3	14.99	7.19	2.56	2.42	4.55	16.84
11	Nakhon Pathom	1	8.21	6.28	16.20	2.67	4.39	17.48
		2	8.31	6.11	15.33	2.55	4.84	18.09
		3	8.65	6.11	15.43	2.66	4.63	17.80
12	Bangkok	1	9.23	7.25	1.88	1.54	5.57	15.88
		2	9.98	7.14	1.72	1.66	5.67	14.54
		3	9.98	6.88	1.78	1.56	6.04	15.37
13	Saraburi	1	13.31	6.54	1.36	1.47	6.68	16.56
		2	11.66	6.69	1.47	1.59	6.15	16.27
		3	11.66	6.68	1.57	1.54	6.37	15.85
13	Nakhon Ratchasima	1	9.27	7.60	2.03	1.97	4.66	16.98
		2	9.98	7.56	2.22	1.82	5.01	17.19
		3	9.96	7.23	2.04	1.98	5.04	17.71
14	Petchaburi	1	11.63	9.09	1.77	1.17	4.23	15.76
		2	11.46	9.25	1.76	1.22	4.05	15.27
		3	11.63	9.02	1.77	1.15	4.37	15.83
	Grand mean		10.01	7.04	8.41	1.63	5.20	16.60
	Pooled SD		0.38	0.14	0.18	0.05	0.20	0.49

Formulas:

$$Grand\ mean = \frac{\bar{x}_1 n_1 + \bar{x}_2 n_2 + \cdots + \bar{x}_k n_k}{n_1 + n_2 + \cdots + n_k}$$

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$$Pooled\ SD = \sqrt{\frac{\left((n_1-1)\times sD_1^2\right)+\left((n_2-1)\times SD_2^2\right)+\cdots+\left((n_k-1)\times SD_k^2\right)}{(n_1+n_2+\cdots+n_k)}}$$

Table 47 Rosmarinic acid content in *M. cordifolia* in Thailand

No.	Sources	Rosmarinic acid content (g/100g)					
		Leaf			Stem		
1	Ratchaburi	0.834	0.855	0.837	0.885	0.886	0.882
2	Petchabun	0.726	0.742	0.715	0.879	0.876	0.868
3	Si sa ket	1.406	1.431	1.441	0.381	0.383	0.382
4	Lopburi	0.945	0.960	0.965	0.829	0.831	0.832
5	Nakhon Sawan	2.260	2.253	2.260	1.311	1.309	1.315
6	Kanchanaburi	1.563	1.570	1.577	0.285	0.286	0.286
7	Suphanburi	3.550	3.557	3.573	1.833	1.812	1.807
8	Prachinburi	5.556	5.581	5.581	1.082	1.082	1.088
9	Chachoengsao	2.175	2.205	2.188	1.617	1.601	1.596
10	Samutprakan	0.840	0.827	0.824	0.652	0.652	0.653
11	Nakornpathom	1.517	1.505	1.511	1.071	1.069	1.070
12	Bangkok	1.207	1.208	1.214	1.219	1.225	1.222
13	Saraburi	2.471	2.471	2.470	0.833	0.834	0.834
14	Nakhon Ratchasima	1.573	1.576	1.567	1.089	1.080	1.088
15	Petchaburi	2.070	2.045	2.063	0.914	0.922	0.916
Average		1.917			0.990		
SD		1.265			0.406		

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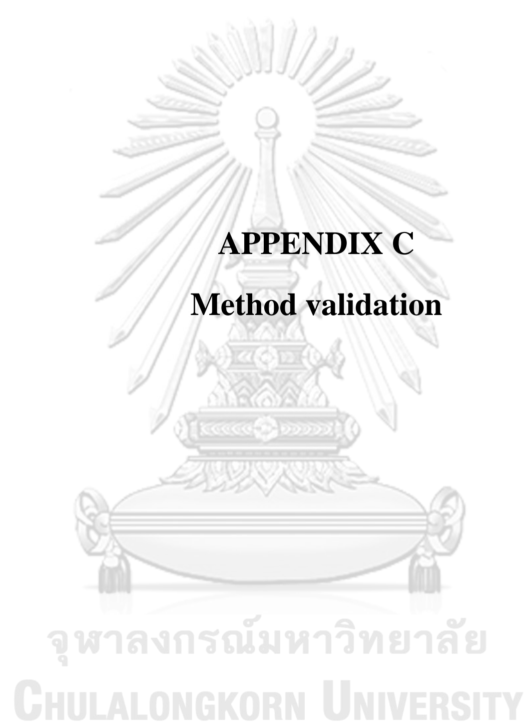

APPENDIX C

Method validation

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Table 48 LOD and LOQ of HPLC for RA quantitation of *M. cordifolia*

Injection volume	RA content (mg/ml)	Area (AU)			mean	y estimate	y obs-y est	(y obs-y est)^2
		1	2	3				
1	0.100	1437983	1456102	1516924	1470336.33	1439077.863	31258.47	977091967.6
2	0.200	2915340	2944459	3004098	2954632.33	2983475.496	-28843.16	831928032.6
3	0.300	4466061	4500231	4576182	4514158.00	4527873.129	-13715.13	188104763.5
4	0.400	5981449	6054652	6147489	6061196.67	6072270.762	-11074.10	122635587.5
5	0.500	7537973	7641132	7738022	7639042.33	7616668.395	22373.94	500593116.5
sum								2620353468
sd residual								29554.20708
Slope								15443976.333
LOD (mg/ml)								0.006315011
LOQ (mg/ml)								0.019136398

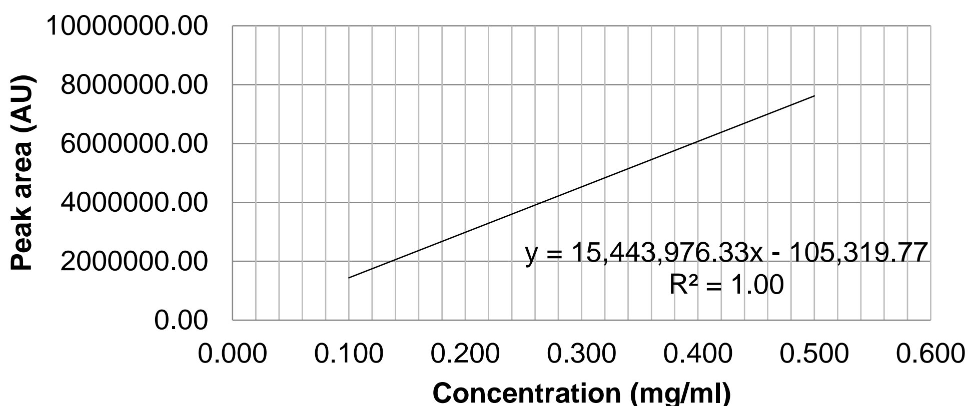

Table 49 The calibration range of HPLC for RA quantitation

Table 50 Accuracy and repeatability precision of HPLC for RA quantitation of *M. cordifolia* leaf

RA added (µg/ml)	RA found (µg/ml)			% Recovery			RA found (µg/ml)	% Recovery	% RSD
	1	2	3	1	2	3			Repeatability
-	62.211	62.368	59.188	-	-	-	61.256	100.000	-
50	114.010	113.929	110.245	101.603	101.390	100.968	112.728	101.320	1.908
200	287.232	289.293	291.049	109.542	110.262	112.293	289.192	110.699	0.661
400	472.570	467.317	466.858	102.241	101.070	101.670	468.915	101.661	0.677
Average								**103.420**	**0.182**

Table 51 Accuracy and repeatability precision of HPLC for RA quantitation of *M. cordifolia* stem

RA added (µg/ml)	RA found (µg/ml)			% Recovery			RA found (µg/ml)	% Recovery	%RSD
	1	2	3	1	2	3			Repeatability
-	91.936	91.323	88.167	-	-	-	90.475	100.000	-
50	140.533	139.790	137.492	99.012	98.915	99.511	139.271	99.146	1.138
200	305.741	304.750	302.472	104.729	104.609	104.964	304.321	104.767	0.551
400	491.191	488.504	493.507	99.849	99.426	101.094	491.067	100.123	0.510
Average								**101.009**	**0.733**

Table 52 Accuracy and intermediate precision of HPLC for RA quantitation of *M. cordifolia* leaf

RA added (µg/ml)	RA found (µg/ml)			% Recovery			RA found (µg/ml)	% Recovery	% RSD Intermediate precision
	1	2	3	1	2	3			
-	59.265	59.602	59.188	-	-	-	59.352	100.000	-
50	110.953	110.074	110.245	101.544	100.430	100.968	110.424	100.981	0.422
200	285.663	287.081	291.049	110.182	110.585	112.293	287.931	111.020	0.970
400	462.378	461.837	466.858	100.678	100.486	101.670	463.691	100.945	0.594
Average								103.236	0.662

Table 53 Accuracy and intermediate precision of HPLC for RA quantitation of *M. cordifolia* stem

RA added (µg/ml)	RA found (µg/ml)			% Recovery			RA found (µg/ml)	% Recovery	%RSD Intermediate precision
	1	2	3	1	2	3			
-	88.405	88.184	88.167	-	-	-	88.252	100.000	-
50	137.168	136.788	137.492	99.107	98.989	99.511	137.149	99.202	0.257
200	301.174	299.446	302.472	104.428	103.908	104.964	301.031	104.433	0.504
400	486.263	491.018	493.507	99.561	100.581	101.094	490.263	100.412	0.751
Average								**101.012**	**0.504**

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Table 54 Robustness of HPLC for RA quantitation of *M. cordifolia* leaf

	Area			**Mean**	**RA content in extract**			**Mean**	**Retention time**			**Mean**
Temperature 34 °C	4438219	4492949	4469376	4466848	0.276	0.280	0.278	0.2782	12.843	12.861	12.870	12.86
Temperature 35 °C	4502185	4496734	4502185	4500368	0.305	0.294	0.294	0.2977	12.188	12.183	12.188	12.19
Temperature 36 °C	4502452	4487106	4531013	4506857	0.284	0.283	0.285	0.2838	12.096	12.066	12.087	12.08
Grand mean	4491358				0.2866				12.38			
SD	21472.53				0.0100				0.421			
%RSD	0.48				3.50				3.40			

Table 55 Robustness of HPLC for RA quantitation of *M. cordifolia* stem

	Area			**Mean**	**RA content in extract**			**Mean**	**Retention time**			**Mean**
Temperature 34 °C	4682283	4735227	4696622	4704711	0.291	0.294	0.292	0.293	12.825	12.814	12.854	12.83
Temperature 35 °C	4681221	4706407	4708530	4698719	0.305	0.307	0.307	0.307	12.203	12.195	12.198	12.20
Temperature 36 °C	4716622	4670409	4488371	4625134	0.297	0.294	0.283	0.291	12.064	12.051	12.039	12.05
Grand mean	4676188				0.2967				12.36			
SD	44315.43				0.0085				0.414			
%RSD	0.95				2.87				3.35			

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