Pharmacognostic specification of *Moringa oleifera* Lam. leaves cultivated in Thailand

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Submitted 25 July 2013; accepted in final form 18 November 2013

Abstract

Moringa oleifera Lam. is a medicinal plant that belongs to the family Moringaceae, with a wide distribution in Thailand. All parts of this plant contain rich nutrients, making it an important food choice for both humans and livestock. Currently, Thai people eat its leaves to improve health, but there is a lack of any pharmacognostic specification data controlling its quality which will benefit both the seller and the consumer. In order to study pharmacognostic specification of *M. oleifera* Lam. leaves cultivated in Thailand, plant samples have been collected from 12 provinces distributed in 6 regions of Thailand. The evaluation was conducted according to the World Health Organization (WHO) guidelines for herbal standardization and Thai herbal Pharmacopoiea. Microscopic examination of this plant revealed characteristics of leaf tissue containing anomocytic stomata, parenchyma tissue containing calcium oxalate crystals, and several annular, reticulated and bordered pitted vessels. Starch or oil containing cells have not been found. Various factors were found to be constant for *M. oleifera* leaves, independent of harvest region: foreign matter (0.95%), total ash (11.77%), acid-insoluble ash (0.36%), moisture (10.14%), and loss on drying (11.34%). Further, ethanol and water extractive values were found to be 1.27%, and 7.83 % dry weight, respectively, meanwhile volatile content could not be detected. Additionally, thin-layer chromatographic fingerprinting of methanolic extract and water extract developed in suitable mobile phase system were also determined.

Keywords*: *Pharmacognostic specification, Moringa oleifera, leaves, macroscopic charateristic, microscopic characteristic, thin-layer chromatographic fingerprint

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บทคัดย่อ

มะรุมเป็นพืชสมุนไพรที่จัดอยู่ในวงศ์ Moringaceae พบปลูกกระจายอยู่ทั่วไปในประเทศไทย ทุกส่วนของมะรุมมีคุณค่าทางอาหารสูงจึงถูกนำมาใช้ประโยชน์เพื่อเป็นอาหารของมนุษย์และสัตว์ ปัจจุบันคนไทยนิยมบริโภคใบมะรุมเพื่อเสริมสุขภาพกันมากขึ้น แต่ยังขาดข้อมูลด้านข้อกำหนดทางเภสัชเวทเพื่อควบคุมคุณภาพที่จะเป็นประโยชน์ต่อทั้งผู้ผลิตและผู้บริโภคใบมะรุมต่อไป จึงเก็บตัวอย่างใบมะรุมจาก 12 จังหวัด ซึ่งกระจายอยู่ใน 6 ภาคของประเทศไทยเพื่อนำมาศึกษาข้อกำหนดของสมุนไพรใบมะรุม ใช้วิธีการประเมินสมุนไพรซึ่งระบุอยู่ใน World Health Organization (WHO) guidelines for herbal standardization และ Thai herbal Pharmacopoiea จากการศึกษาทางจุลทรรศน์พบว่าเนื้อเยื่อส่วนใบมะรุมประกอบด้วยลักษณะเฉพาะของปากใบชนิด anomocytic และมีเนื้อเยื่อ parenchyma ที่มีผลึกของ calcium oxalate สะสมอยู่ด้วย รวมทั้งมี vessel ชนิด annular, reticulated, และ bordered pitted vessel ทั้งนี้ไม่พบเซลล์ที่มีการสะสมแป้งหรือน้ำมันระเหยง่าย ค่าที่บ่งบอกลักษณะทางกายภาพของใบมะรุม ได้แก่ ค่าเฉลี่ยของสิ่งปลอมปน ปริมาณเถ้าทั้งหมด เถ้าที่ไม่ละลายในกรด ความชื้น และน้ำหนักที่หายไปเมื่อทำให้แห้ง เท่ากับร้อยละ 0.95, 11.77, 0.36, 10.14, 11.34 ของน้ำหนักแห้ง ตามลำดับ ส่วนค่าสารสกัดด้วยแอลกอฮอล์และสารสกัดด้วยน้ำเท่ากับร้อยละ 1.27 และ 7.83 ของน้ำหนักแห้งตามลำดับ และตรวจไม่พบน้ำมันระเหยง่าย และแสดงรายงานลายพิมพ์รงคเลขผิวบางของสารสกัดเมทานอลและสารสกัดน้ำซึ่งแยกองค์ประกอบโดยใช้วัฏภาคเคลื่อนที่ที่เหมาะสมด้วย

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1. Introduction

Moringa oleifera Lam. of the Moringaceae family is the most widely cultivated species of monogeneric family native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan, and cultivated throughout Asia, especially Thailand (Somali, Bajnedi, & Al-Faimani, 1984; Mughal, Ali, Srivastava, & Iqbal, 1999). It has many colloquial names such as the Drumstick tree, Horseradish tree, and Ben oil tree. In Thailand,

DOI : 10.14456/rjas.2013.7

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the moringa tree has been cultivated in every region where it goes by various names in different regions such as Ma-Rum (Central) and Pak-Nuea-Kai (North). It is considered one of the world's most useful trees, as almost all parts of this plant have been used for treatments various illnesses such as ascites, rheumatism and venomous bites, and also as cardiac and circulatory stimulants. Several parts of *M. oleifera* have been reported to show anti-tumor, anti-ulcer, anti-inflammatory, and anti-pyretic effects (Caceres, Saravia, Rizzo, Zabala, De Leon, & Nave, 1992; Singh & Kumar, 1999). *Moringa* leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Kumar, Mishra, Ghosh & Panda, 2010). However, the variation in the phytochemical constituents and nutritional values differ for a wide range of reasons, such as cultivated regions, growing conditions, nature of soil, seasonal changes, genetically different cultivars, storage conditions or due to the period of analysis (Jongrungruangchok, Bunrathep, & Songsak, 2010). Nutrients and mineral content of eleven different samples of *M. oleifera* cultivated in Thailand has revealed that various agro-climatic regions of Thailand contained an appreciable amount of nutrients and *M. oleifera* might be used as a good supplement for some nutrients such as protein, fiber and minerals (Jongrungruangchok, Bunrathep, & Songsak, 2010). Antioxidant activity and total phenolic content of *M. oleifera* leaves changed during the hot-air drying process, tending to decrease in the early stages of drying and then increase in the later stages. However the dried leaves still had at least 60% of the antioxidant activities compared to fresh leaves (Wangcharoen & Gomolmanee, 2013). A previous study has stated that *M. oleifera* leaves have medicinal properties and can be used in phytochemistry and pharmacological activities (Sabale, Patel, Paranjape, Arya, Sakarkar, & Sabale, 2008). *M. oleifera* leaves may be a potential novel adjuvant therapy for neuropathic pain management because the leaf extract can attenuate neuropathic pain in male Wistar rat diabetic conditions (Khongrum, Wattanathorn, Muchimapura, Thukhum-mee, Thipkaew, Wannanon & Tong-un, 2012). *M. oleifera* might offer benefits to diabetic patients. Its aqueous leaves extract revealed anti-hyperglecemic activity in normal mice and improved the glucose tolerance impairment in mildly diabetic mice (Luangpiom, Kourjampa & Junaimaung, 2013). The pharmacognostic study of bark cultivated in India has been reported (Sholapur & Patil, 2013), but any pharmacognostic evaluation of their leaves has not been reported. The present report details the physical properties represented for pharmacognostic specification of *M. oleifera* leaves cultivated in Thailand.

2. Objectives

The objective of the study was to perform the pharmacognostic specification of *M. oleifera* leaves cultivated in Thailand and to establish the basic knowledge of this plant which can be applied to authentication of a crude drug samples.

3. Materials and methods

3.1 Plant samples

Twelve samples of *M. oleifera* fresh leaves were collected in January – April 2011 from 6 regions around Thailand, which were Phang Nga, Phuket, Surat Thani, Ayutthaya, Ang Thong, Chanthaburi, Sa Kaeo, Kanchanaburi, Nakorn Pathom, Chiang Mai, Lampang, Surin provinces. All samples were further authenticated by comparison with the herbarium specimen (SN 202599 and SN 202600) at the Princess Sirindhorn Plant Herbarium Bangkok, Thailand. The leaves were put in an oven (60°C) to dry, then ground and kept in air-tight plastic containers at room temperature (30°C) for further analysis. The leaves from each region were assayed and analyzed individually in triplicate. Pharmacognostic evaluation was performed by World Health Organization (WHO) guideline standard methods (WHO 2007).

3.2 Macroscopic and microscopic examination

For macroscopic examination, each plant sample was identified by visual examination of physical properties such as texture, size, color, and visual inspection. For microscopic examination, the fresh sample and powered drug (ground and sifted through a 250 micron sieve) were inspected for cell and tissue characteristics under a microscope equipped with micrometer.

3.3 Foreign matter determination

Sample (50 g) was spread in a thin layer, and the pieces of foreign matter were sorted out by

visual inspection. The powdered of foreign matter was sifted through a 250 micron sieve. All portions of foreign matter were pooled and weighed.

All foreign matters had been removed before further pharmacognostic evaluation.

3.4 Total ash and acid-insoluble ash content determination

The ground sample (3 g, accurate weighed) were placed in a previously ignited and tarred crucible. The sample were spread in an oven layer and ignited by gradually increasing the heat to 500-600°C until white ash was obtained. The ash was then cooled in a desiccator and weighed immediately.

2M (70 g/l) HCl (25 ml) added to the crucible containing total ash. The crucible was then added with watch-glass, and the mixture was boiled gently for 5 minutes. The watch-glass was rinsed with hot water (5 ml), and this liquid was added into the crucible. The insoluble matter was collected on ashless filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to constant weight. The residue was allowed to cool in a desiccator, and weighed immediately.

3.5 Ethanol-soluble extractive determination

The ground sample (5 g, accurate weighed) was macerated with absolute ethanol (100 ml) in a closed conical flask in shaking bath for 6 hours and allowed to stand for 18 hours. The extracted was filtered rapidly to avoid loss of ethanol. The filtrate (20 ml) was evaporated to dryness in a tarred small beaker and then dried with heat to constant weight.

3.6 Water-soluble extractive determination

The ground sample (5 g) was macerated with distilled water (100 ml) in a closed conical flask in shaking bath for 6 hours and allowed to stand for 18 hours. The extracted was filtered rapidly to avoid loss of ethanol. The filtrated (20 ml) was evaporated to dryness in a tarred small beaker and then dried with heat to constant weight.

3.7 Loss on drying determination

The ground sample was weighed (5 g) in a small beaker and then dried at 105°C to constant weight.

3.8 Moisture content determination

The ground sample (50 g) in water-saturated toluene (200 ml) was subjected to Azeotropic method distillation. As soon as the water was completely distilled, the inside of the condenser was rinsed with toluene, and the distillation continued for 5 minutes. The heat was then removed, and the receiving tube was allowed to cool to room temperature. The water and toluene layers were allowed to separate, and then the volume of water was determined.

3.9 Volatile oil content determination

Volatile oil distillation was performed on the sample (100 g) in water (600 ml) using a Clevenger apparatus. The heat was removed, and the receiving tube allowed cooling to room temperature after the complete distillation. The volatile oil and water layers were allowed to separate, and then the volume of volatile oil was determined.

3.10 Thin-layer chromatographic identification

The ground sample (1 g) was macerated with methanol or distilled water (20 ml) for 12 hours, then the extract was filtered and evaporated. The residue was dissolved in methanol (0.5 ml) and then applied on TLC plate (Silica gel GF_{254} precoated plate). TLC plate was developed in suitable mobile phase, then allowed to dry in air, and examined under UV light at 254 nm and 365 nm, followed by spraying with anisaldehyde and sulfuric acid reagent, and heated in hot-air oven (120°C) for 10-15 minutes.

3.11 Statistical Analysis

The data are presented as group mean±SD. The calculations were performed using Microsoft Excel.

4. Results

Moringa oleifera is a deciduous tree or shrub, fast-growing, and drought resistant with an average height of 4-7 meters at maturity. Leaves have alternate arrangement and are tripinnately compound (20-40 cm). Leaflets are 0.5-1 cm broad and 1-3 cm long, ovate in shape. The ventral side of leaf is dark-green in color while the dorsal side is white-green. The flower is inflorescent, white, annual (Dec.-Jan.), 5-sepal, 5-petal, apopetalous, and bitter-sweet in taste. The fruit is located in a pod, is

dehiscent dried fruit, and green in color, 20-50 cm long and narrow in shape. Seeds are skinny 3-winged in triangle shape (Figure 1). By pharmacognostic studies, its leaf tissues contain characteristic stomata of anomocytic type, parenchyma tissue containing calcium oxalate crystals, and several annular, reticulated and bordered pitted vessels. Cells containing starch or volatile oil were not detected (Figures 2-4).

The average contents represented physical properties of *M. oleifera* leaves obtained from 12 provinces in 6 regions of Thailand are shown in Table 1. Volatile oil content could not be detected. The TLC fingerprint of methanolic extracts and water extracts were also performed by various ratios of organic solvents as suitable mobile phase system (Tables 2-5).

Table 1 The physical properties of *M. oleifera* leaves obtained from 12 provinces in Thailand

source	Average content*,**							
	foreign matter	total ash	acid-insoluble ash	moisture	loss on drying	volatile content	extractive value	
							ethanol	water
South:								
PNG	1.44 ± 0.02	9.62 ± 0.15	0.96 ± 0.01	10.06 ± 0.07	17.19 ± 0.12	ND	1.69 ± 0.06	7.85 ± 0.25
PK	1.61 ± 0.02	9.94 ± 0.15	0.47 ± 0.02	10.28 ± 0.46	10.72 ± 0.14	ND	0.87 ± 0.04	7.14 ± 0.30
STN	0.66 ± 0.25	12.74 ± 0.40	0.31 ± 0.02	10.21 ± 0.22	7.92 ± 0.21	ND	0.96 ± 0.03	8.39 ± 0.15
Central:								
AYT	1.33 ± 0.02	11.00 ± 0.02	0.65 ± 0.03	10.12 ± 0.21	12.64 ± 0.30	ND	1.26 ± 0.01	7.47 ± 0.37
AT	0.53 ± 0.29	10.19 ± 0.11	0.34 ± 0.02	9.84 ± 0.39	11.35 ± 0.42	ND	3.06 ± 0.06	11.74 ± 0.34
West:								
NPT	0.54 ± 0.18	12.42 ± 0.29	0.66 ± 0.02	9.79 ± 0.11	7.31 ± 0.34	ND	1.75 ± 0.02	8.65 ± 0.24
KCB	1.40 ± 0.02	12.70 ± 0.21	0.38 ± 0.02	10.09 ± 0.27	11.36 ± 0.17	ND	0.77 ± 0.01	8.23 ± 0.41
SK	0.54 ± 0.22	12.60 ± 0.29	0.49 ± 0.02	9.58 ± 0.31	7.32 ± 0.16	ND	0.63 ± 0.02	6.55 ± 0.29
North:								
CHM	0.43 ± 0.13	12.58 ± 0.62	0.13 ± 0.01	9.83 ± 0.35	13.88 ± 0.49	ND	0.56 ± 0.02	5.74 ± 0.08
LP	0.44 ± 0.31	11.16 ± 0.21	0.30 ± 0.02	10.54 ± 0.64	8.60 ± 0.19	ND	1.09 ± 0.05	6.14 ± 0.32
East:								
CTB	0.40 ± 0.08	11.49 ± 0.53	0.13 ± 0.01	10.10 ± 0.23	10.36 ± 0.21	ND	0.83 ± 0.03	8.48 ± 0.24
Northeast:								
SR	0.59 ± 0.05	12.61 ± 0.04	0.39 ± 0.02	11.59 ± 0.47	8.61 ± 0.28	ND	1.38 ± 0.04	8.66 ± 0.17

PNG = Phang Nga, PK = Phuket, STN = Surat Thani, AYT = Ayutthaya, AT = Ang Thong, NPT = Nakornpathom, KCB = Kanchanaburi, SK = Sa Kaeo, CHM = Chiang Mai, LP = Lampang, CTB = Chanthaburi, SR = Surin
* = % dry weight, ** = average ± standard deviation, ND = could not be detected

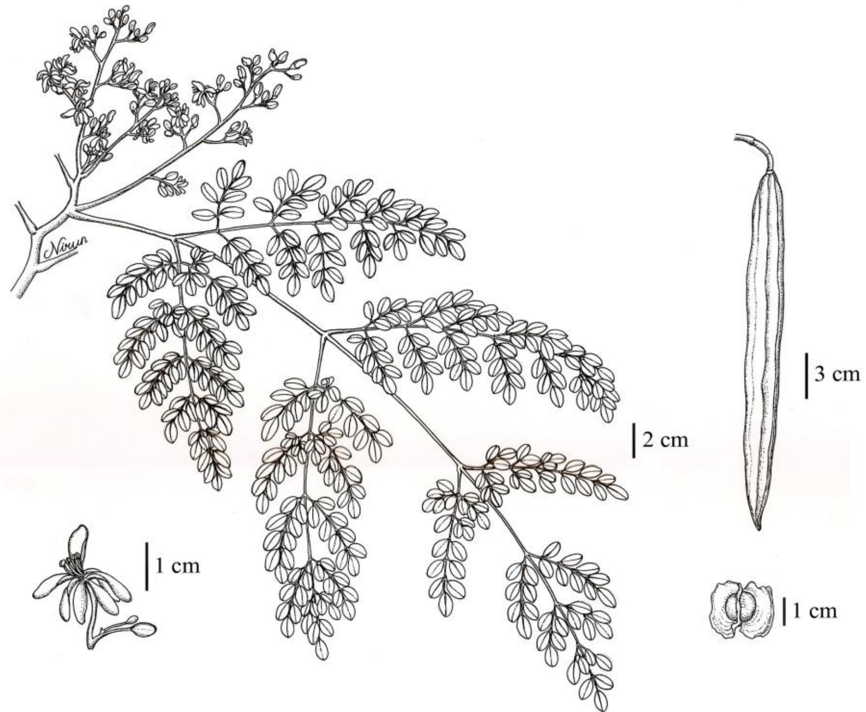


Figure 1 The characteristic of *Moringa oleifera* Lam. (whole plant)

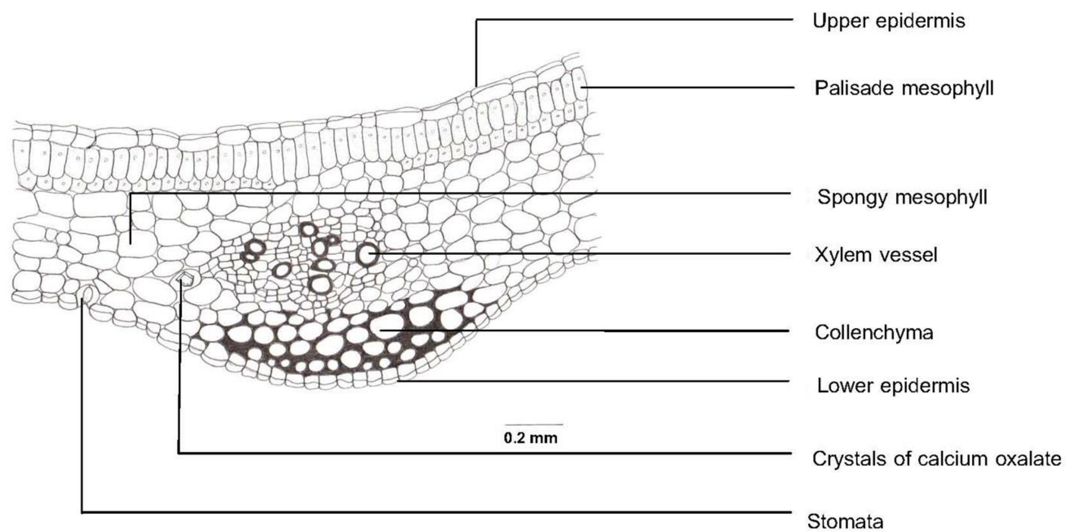


Figure 2 Transverse section of *Moringa oleifera* Lam. leaf

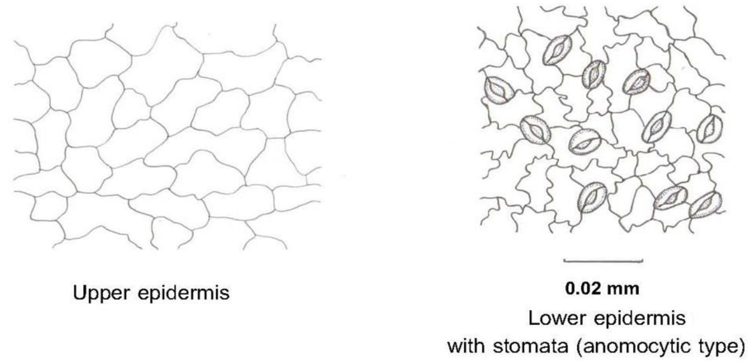
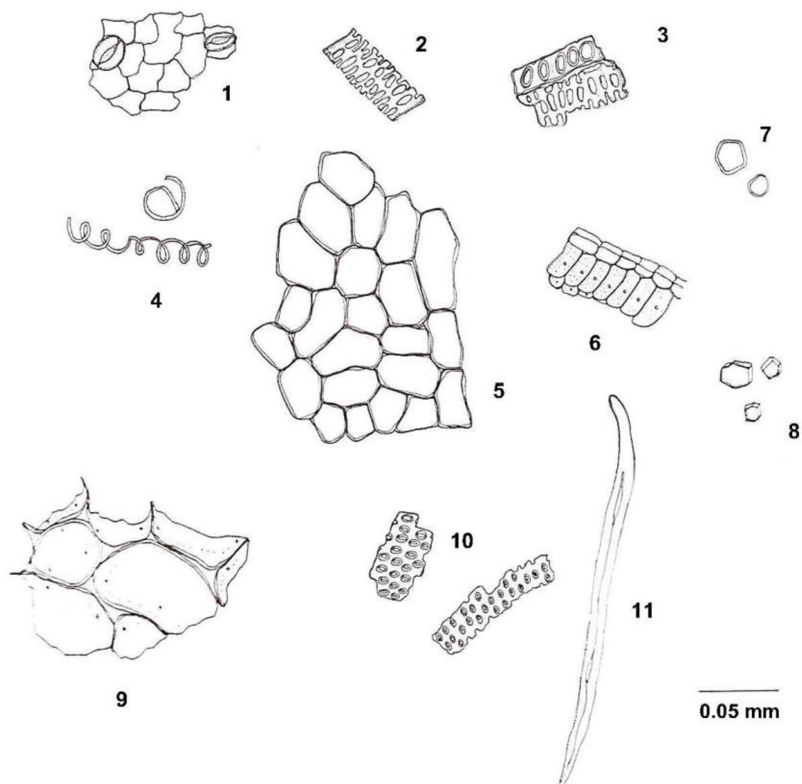


Figure 3 Section of *Moringa oleifera* Lam. leaf



1. Lower epidermis with stomata
2. Fragment of reticulated vessel
3. Annular vessel with reticulated vessel
4. Spiral vessel
5. Upper epidermis
6. Epidermal cell with palisade layer
7. Annular vessel
8. Crystals of calcium oxalate
9. Fragment of parenchyma
10. Fragment of bordered pitted vessel
11. Fragment of fiber

Figure 4 Powdered of *Moringa oleifera* Lam. leaves

Table 2 TLC fingerprint of *M. oleifera* leaf methanolic extract developed with toluene:ethyl acetate:diethylamine (7:2:1)

Spot no.	Rf value			
	Detection method			
	$UV_{254\ nm}$	$UV_{365\ nm}$	anisaldehyde/sulfuric acid spray reagent	
1	-	-	0.40	Dark blue
2	0.49	0.49	-	-
3	0.65	0.65	0.65	Dark blue
4	0.73	0.73	0.73	Green
5	-	-	0.82	Green
6	0.95	0.95	0.95	Green

Table 3 TLC fingerprint of *M. oleifera* leaf methanolic extract developed with toluene:ethyl acetate (3:1)

Spot no.	Rf value			
	Detection method			
	$UV_{254\ nm}$	$UV_{365\ nm}$	anisaldehyde/sulfuric acid spray reagent	
1	-	-	0.08	Purple
2	-	-	0.15	Green
3	-	-	0.27	Grey
4	0.34	-	0.34	Dark blue
5	-	-	0.51	Purple
6	-	-	0.60	Purple
7	0.63	0.63	-	-
8	-	-	0.67	Purple
9	0.69	0.69	0.69	Green
10	-	-	0.75	Green
11	-	0.80	0.82	Orange
12	0.89	0.89	0.89	Dark blue

Table 4 TLC fingerprint of *M. oleifera* leaf water extract developed with ethyl acetate:glacial acetic acid:water (10:1.1:2.6)

Spot no.	Rf value			
	Detection method			
	$UV_{254\ nm}$	$UV_{365\ nm}$	anisaldehyde/sulfuric acid spray reagent	
1	0.13	0.13	0.13	Brown
2	0.21	0.21	0.21	Brown
3	0.32	0.32	0.32	Brown
4	0.40	0.40	0.40	Brown
5	0.60	-	0.60	Brown
6	0.82	-	0.82	Yellow

Table 5 TLC fingerprint of *M. oleifera* leaf water extract developed with ethyl acetate:methanol:water (7.7:1.5:0.8)

Spot no.	Rf value			
	Detection method			
	$UV_{254\ nm}$	$UV_{365\ nm}$	anisaldehyde/sulfuric acid spray reagent	
1	0.18	0.18	0.18	yellow-brown
2	-	-	0.22	yellow-brown
3	0.25	0.25	0.25	yellow-brown
4	0.35	-	-	-
5	-	0.40	0.40	yellow-brown
6	0.53	0.53	0.53	yellow-brown
7	0.56	0.56	0.56	yellow-brown
8	-	0.61	0.61	yellow-brown

Table 6 Pharmacognostic specification of *M. oleifera* leaves

Content	Grand average ± 3SD*,**	Range (Max-Min)**
Foreign Matter	0.95 ± 0.03	1.61 ± 0.02 - 0.40 ± 0.08
Total ash	11.77 ± 0.50	12.74 ± 0.40 - 9.62 ± 0.15
Acid-insoluble ash	0.36 ± 0.02	0.96 ± 0.01 - 0.13 ± 0.01
Moisture	10.14 ± 0.38	11.59 ± 0.47 - 9.58 ± 0.31
Loss on drying	11.34 ± 3.72	17.19 ± 0.12 - 7.31 ± 0.34
Volatile content	ND	ND
Ethanol extractive	1.27 ± 0.05	3.06 ± 0.06 - 0.56 ± 0.02
Water extractive	7.83 ± 0.27	11.74 ± 0.34 - 5.74 ± 0.08

* = standard deviation, ** = % dry weight, ND = could not be detected

5. Discussion

The constant numbers indicating the quality of *Moringa oleifera* leaves presented for pharmacognostic specification were shown in Table 6. The contents of foreign matter, total ash, acid-insoluble ash, moisture, loss on drying should not be more than 0.95, 11.77, 0.36, 10.14, 11.34% dry weight, respectively, meanwhile the value of ethanol extractive and water extractive were not less than 1.27, and 7.83% dry weight, respectively. These constant numbers have been reported for the first time.

Various cultivating regions, such as agro-climate, growing conditions, nature of soil and seasonal change will affect the chemical compound contents in plants. As shown in ethanol extract value or water extract value (Table 6), the percentages vary from 0.56 ± 0.02 to 3.06 ± 0.06 or 5.74 ± 0.08 to 11.74 ± 0.34, respectively, even though the samples have been collected in the same period**.** It should be noted that the leaves had high moisture content. Dried plant samples, which are to be prepared for food supplementation, should be examined for microbial contamination.

6. Conclusion

This study exhibited the physical data that will be applied for pharmacognostic specification of *Moringa oleifera* Lam. leaves cultivated in Thailand. The constant values determined for *M. oleifera* leaves in this study, independent of harvest region, were: foreign matter (0.95%), total ash (11.77%), acid-insoluble ash (0.36%), moisture (10.14%), and loss on drying (11.34%). Further, ethanol extract and water extract values were found to be 1.27, and 7.83 % dry weight, respectively. The characteristics of leaf tissue contain stomata of anomocytic type, while parenchyma tissue containing calcium oxalate crystals, and several annular, reticulated and bordered pitted vessels were also revealed. Thin-layer chromatographic fingerprinting of methanolic extracts and water extracts developed in suitable mobile phase system were demonstrated. Together, these finding will be helpful in substantiation and authentication of crude drug samples.

7. Acknowledgements

The authors are grateful to the National Research Council of Thailand for a research grant.

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