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Research Article

Quality control of *Mallotus repandus* stem samples collected in Thailand; pharmacognostic, physical and chemical characteristics

Chutima Petchprayoon[1], Tharita Kitisripanya[1], Somnuk Bunsupa[1], Chaiwat Aneklaphakij[1], Natthinee Anantachoke[1], Veena Satitpatipan[1], Nutputsorn Chatsumpun[1], Pongtip Sithisarn[1,*]

1 Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

ABSTRACT

Mallotus repandus (Willd.) Müll. Arg. is a plant in the Euphorbiaceae family. The stem of this plant has been assigned in the Thailand National List of Essential Herbal Drugs 2023 as muscle pain-relieving agents. However, quality control including the identification, pharmacognostic, and physical and chemical properties of this plant is still required. The objective of this study was to evaluate the microscopic analysis, physical characteristics, and phytochemical screening of *M. repandus* (Willd.) Müll Arg. stem samples. *M. repandus* (Willd.) Müll. Arg. stem samples were collected from twelve different locations in Thailand and were evaluated for macroscopic, microscopic, physical, and phytochemical characteristics using the official methods in Thai Herbal Pharmacopoeia 2019. *M. repandus* had woody stems with no odor or taste. The main histological characteristics of the plant powder were the corks, which were composed of alternating layers of polygonal cells, large fibers with prism calcium oxalate crystals, medullary rays, and phloem parenchyma cells with a lot of large border pitted vessels and some amount of small round starch granules. Foreign matter contents were less than 1% weight per weight (w/w), with a loss on drying of less than 10% w/w. The total ash and acid-insoluble ash contents were less than 10% w/w and 1% w/w, respectively. The ethanol-soluble extractive and water-soluble extractive contents were in the ranges 3.13 ± 0.06 to 9.23 ± 0.09% w/w and 5.23 ± 0.20 to 11.42 ± 0.03% w/w, respectively. Thin layer chromatographic analysis of *M. repandus* stem samples showed specific chromatographic fingerprints with the chemical marker corresponding to bergenin. Phytochemical screening using color reaction suggested the presence of phenolics and coumarins. *M. repandus* stem macroscopic and microscopic characteristics, physical properties, as well as phytochemical properties were reported. These results are useful for further quality control of raw materials and finished products.

Keywords:
Mallotus repandus; pharmacognostic characteristics; microscopic characteristics; physical properties; chromatographic fingerprint

1. INTRODUCTION

Mallotus repandus (Willd.) Müll. Arg. is a shrub or woody climber in Euphorbiaceae. This plant is called as “pho khan or kho-khlan” in Thai. The stem of this plant has been assigned in the Thailand National List of Essential Herbal Drugs 2023 as muscle pain-relieving agents in the formula “Ya Pa Som Kho-Khlan”[1] and was also assigned in Thai Herbal Pharmacopoeia (THP) 2021 supplement 2023 in the category of analgesic and anti-inflammatory[2]

The stems of *M. repandus* has been generally sold in local herbal markets in Thailand as the ingredient in pain relieving formula[3]

***Corresponding author:**
***** Pongtip Sithisarn Email: pongtip.sit@mahidol.ac.th

while in Taiwan it has been used as anti-inflammatory agent, insecticide to stop itching and as agent to treat fever, rheumatic arthritis, snake bite, hepatitis, and liver cirrhosis[4,5].

There are some reports about phytochemicals in *M. repandus* including phenolics, terpenoids, and steroids[6]. Bergenin, a polyphenolic has been reported as a major compound in *M. repandus*[7]. Bergenin promoted various biological activities including antioxidant, anti-inflammatory, antimicrobial, immunomodulatory and hepatoprotective effects[8-13]. There was a development of HPLC method to quantitatively analysis of bergenin content in the stems of *M. repandus*[14].

Even though *M. repandus* stem has been assigned in the National official document including the Thailand National List of Essential Herbal Drugs 2023 and THP 2021 supplement 2023, and the methanol extract from the stem of this plant at the dose of 4,000 mg/kg showed no toxicity in animal acute toxicity model suggesting the LD_{50} higher than 4,000 mg/kg[15]. However, with the word "kho-klan", there are 2 plants species other than *M. repandus*; *Croton caudatus* Gleiseler and *Anamirta cocculus* (L.) Wight & Arn that share this local name[16]. There are some side effects and toxicities from receiving the different species of kho-klan such as irritation and allergic responses from *C. caudatus*[17] and effects to central nervous system from neurotoxin constituents such as picrotoxin, picrotin, methyl picrotoxate, dihydroxypicrotoxinin and picrotoxic acid in *A. cocculus*[18,19]. Moreover, with the raw materials appeared as stem crude drugs or powders, it is hard to identify or distinguish between plant species. Therefore, this study was set up in order to evaluate the microscopic analysis, physical characteristics, and phytochemical screening of *M. repandus* (Willd.) Müll Arg. stem samples collected from different locations in Thailand. The obtained results are useful for further quality control of plant raw materials and herbal products.

2. MATERIALS AND METHODS

2.1. Plant collection and preparation

M. repandus stem samples were purchased from various local markets in Thailand or collected from a nature park in 2018. The sources of *M. repandus* stem samples are shown in Table 1. The samples were botanically authenticated according to their botanical and taxonomical characteristics using the identification key described in Flora of Thailand (2007)[20]. The voucher specimens were deposited at the Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand. *M. repandus* stems were cleaned, sliced into thin sheets, dried in a hot-air oven (60 °C for 48 h) and then ground to the fine powder. The stem samples were investigated for macroscopic characteristics, while stem fine powders were used for microscopic, physical, and chemical analyses. All the chemicals and reagents used in this study were of analytical grade.

Table 1. Sources of *Mallotus repandus* stem samples.

Sample	Province	Region of Thailand
Sample 1	Nakhon Pathom no. 1	Central
Sample 2	Bangkok no. 1	Central
Sample 3	Bangkok no. 2	Central
Sample 4	Nakhon Pathom no. 2	Central
Sample 5	Lop Buri	Central
Sample 6	Bangkok no. 3	Central
Sample 7	Nakon Ratchasima	Northeastern
Sample 8	Nakhon Si Thammarat	Southern
Sample 9	Sakon Nakhon	Northeastern
Sample 10	Chanthaburi	Eastern
Sample 11	Phitsanulok	Northern
Sample 12	Chiang Mai	Northern

2.2. Macroscopic studies

The macroscopic characterization of *M. repandus* stem samples were evaluated according to the guidelines described by Evans (2009)[21] and Upton et al.[22] for shape, size, taste, fracture, color, and odor.

2.3. Microscopic studies and powder analysis

Using the methods from Evans (2009)[21] and Upton et al.[22], the *M. repandus* stem samples were crosscut and soaked in distilled water for 2 d. Then, the samples were manually sliced (transverse sections) and observed under a light microscope. Powdered samples were studied under a light microscope (Leica ICC*50* W; Leica Camera Inc.; USA). The powder was cleared in a chloral hydrate reagent. Then, the cleared powder was stained with aniline sulfate reagent. Some powder samples were directly stained with iodine solution without the clearing process. Then, small amounts of the stained powders were mounted on glass slides with glycerin water and examined under the light

microscope. The camera connected to the light microscope under the management of the Leica Application Suite *v*4.12 software (Leica Camera Inc.; USA) was used to record the cells in the plant powders.

2.4. Evaluation of physical properties[23]

The physical properties of *M. repandus* stem samples were evaluated using the methods in the Thai Herbal Pharmacopoeia 2019. Each *M. repandus* stem sample was performed in triplicate for every experiment.

2.4.1. Foreign matter

One hundred grams of *M. repandus* stem crude drugs were spread as a thin layer on a tray and any foreign matter was manually separated as completely as possible. Then the remaining sample was weighed and the percentage of foreign matter was calculated.

2.4.2. Loss on drying

A glass-stoppered weighing bottle was dried in a hot-air oven at 105 °C for 30 min, followed by cooling in a desiccator and accurately weighing. Two grams of each *M. repandus* stem powder were added into the weighing bottle then the stopper was replaced and the bottle containing the plant powders was accurately weighed. The loaded weighing bottle was placed in the hot-air oven with the stopper removed and left to dry in the oven at 105 °C for 2 h; then, the sample and its bottle were cooled in a desiccator and accurately weighed. The loaded weighing bottle and the stopper were dried again in the hot-air oven at 105 °C for 1 h, followed by cooling in the desiccator and accurately weighing. The drying and cooling processes were repeated until the difference between two consecutive weights of the loaded weighing bottle with the stopper was less than 0.50 mg/g plant powder. The percentage loss on drying was calculated.

2.4.3. Total ash

Two grams of each *M. repandus* stem powder were incinerated in a tared silica dish at a temperature not exceeding 450 °C in a furnace until free from carbon; then the silica dish was cooled in the desiccator and weighed. The percentage of total ash was calculated.

2.4.4. Acid-insoluble ash

Twenty-five milliliters of dilute hydrochloric acid were added into the silica dish obtained from the final step of the total ash evaluation; then, it was boiled in a water bath for 5 min after which, the solution was passed through ashless filter paper and washed with hot water until the filtrate was neutral. The obtained residue and ashless filter paper were transferred to the tared silica dish and incinerated at 500 °C in a furnace for 3 h. The silica dish was cooled in the desiccator and weighed. The percentage of acid-insoluble ash was calculated.

2.4.5. Ethanol-soluble extractive

Five grams of each *M. repandus* stem powder were macerated with 95% ethanol (100 mL) in a closed flask for 24 h, shaking frequently during the first 6 h and then allowed to stand for 18 h. The extraction solution was rapidly filtered and 20 mL of filtrate was evaporated in a tared evaporating dish in a water bath. The evaporating dish containing the dried extract was dried at 105 °C in a hot-air oven until a constant weight was obtained. The percentage of ethanol-soluble extractive was calculated.

2.4.6. Water-soluble extractive

Five grams of each *M. repandus* stem powder were macerated with chloroform water (100 mL) in a closed flask for 24 h, shaking frequently during the first 6 h and then allowed to stand for 18 h. The extraction solution was rapidly filtered and 20 mL of filtrate was evaporated in a tared evaporating dish in a water bath. The evaporating dish containing the dried extract was dried at 105 °C in a hot-air oven until a constant weight was obtained. The percentage of water-soluble extractive was calculated.

2.5. Evaluation of chromatographic fingerprint using thin-layer chromatography

Three hundred milligrams of each *M. repandus* stem powder were extracted by sonicating with 2 mL of methanol for 3 min. The extraction solution was left at room temperature overnight then the supernatant was transferred to the new containers for thin-layer chromatography (TLC) analysis. Ten microliters of each of the *M. repandus* stem extract solution or standard bergenin (Sigma, USA) were applied as a band (1 cm in length) on a precoated silica gel 60 GF254 aluminum sheet, using the mixture of ethyl acetate, formic acid, acetic acid, and water in the ratio of 100:11:11:27 v/v/v/v as the solvent system. The TLC plates were detected under white light, ultraviolet (UV) light at 254 and 366 nm, and white light after spraying with 10 % w/v phosphomolybdic acid in ethanol and heated on a hot plate (105 °C for 10 min). The TLC visualization was undertaken using a mounted digital camera and the CAMAG manual winCATS software (Camag, Muttenz, Switzerland).

2.6. Phytochemical analysis based on color reaction

2.6.1. *M. repandus* stem extract preparation

One gram of each *M. repandus* stem powder was refluxed with 20 mL of 95% v/v ethanol on a water bath (95 °C) for 10 min and then filtered. The filtrate was transferred to new containers for phytochemical analysis[24].

2.6.2. Phenolic compounds (ferric chloride test)

M. repandus stem extract (2 mL) was added with 2-3 drops of dilute ferric chloride solution; the formation of blue or green coloring indicated the presence of phenolic compounds[24].

2.6.3. Flavonoids (Shinoda's test)

M. repandus stem extract (2 mL) was added with magnesium ribbons (5 mg) and five drops of concentrated hydrochloric acid were then added. The appearance of a pink, red or orange color after a few minutes confirmed the presence of flavonoids[24].

2.6.4. Tannins (gelatin salt test)

M. repandus stem extract (2 mL) was added with 2-3 drops of gelatin solution (1% weight per weight; w/w) containing 10% w/w sodium chloride solution; the formation of a white precipitate indicated the presence of tannins[24].

2.6.5. Anthraquinone glycosides (modified Borntrager's test)

M. repandus stem extract (2 mL) was boiled with 0.5 N potassium hydroxide (20 mL) and 3% hydrogen peroxide (1 mL) in a water bath for 10 min and then filtered. The filtrate was cooled and acidified using glacial acetic acid and then partitioned with an equal volume of chloroform. The chloroform layer (2 mL) was separated and shaken with ammonia TS (test solution; 2 mL). Pink-red coloring in the ammonia layer indicated the presence of anthraquinone glycosides[24].

2.6.6. Coumarins (Photo effect test)

M. repandus stem extract (1 drop) was spotted on a filter paper and then 10% w/v sodium hydroxide solution (1 drop) was dropped next to the spotted extract to allow the overlapping of these two spots. The reaction was observe under ultraviolet light (365 to 366 nm). The formation of more intense blue or green fluorescence in the overlapping region indicated the presence of coumarins[24].

2.6.7. Alkaloids (Dragendorff's test and precipitation test)

M. repandus stem extract (2-3 drops) was spotted on a filter paper and dried; then, 1-2 drops of Dragendorff's spray reagent were dropped on the spotted extract. The formation of orange coloring indicated the presence of alkaloids. *M. repandus* stem extract (2-3 drops) was also tested for precipitation by separately adding two drops of different reagents (Dragendorff's, Mayer's, Marmer's, tannic acid, Wagner's and Valser's reagents). The formation of orange or white precipitate after the reaction was observed[24].

2.6.8. Steroids and triterpenes (Libermann-Burchard's test)

M. repandus stem extract (2 mL) was evaporated in an evaporating dish in a water bath; then, 7-8 drops of acetic anhydride were added followed by 2-3 drops of concentrated sulfuric acid. The formation of color changes from pink to red to purple to blue to dark bluish green coloring indicated the presence of steroids, while the formation of pink or red-purple coloring indicated the presence of triterpenes[24].

2.6.9. Saponins (froth test)

Five hundred milligrams of *M. repandus* stem powder were extracted with 10 mL of hot water by shaking vigorously for 1 min and then filtered. The 1 mL of the filtrate was transferred to a clean, stoppered test tube and diluted with distilled water to 10 mL. The solution was shaken vigorously for 15 s. The test tube was allowed to stand for 10 min and then the height of the honeycomb froth was measured. The presence of at least 1 cm of honeycomb froth that persisted for 10 min indicated the presence of saponins[24].

2.7 Data analysis

Statistical analyses were performed on triplicate sub-samples and all results were presented as the mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Macroscopic studies

M. repandus stem was observed as an indistinct odor, tasteless, cylindrical stem, transverse or oblique pieces with the size of 2-5 cm long and 1-4 cm in diameter. The external bark was brown, dark brown, or greyish brown color with some lenticels. The internal wood was smooth off-white, light yellow or light brown

color. The macroscopic characteristics of *M. repandus* stem are shown in Figure 1.

The observed macroscopic characteristics corresponded with the macroscopic described in Thai Herbal Pharmacopoeia 2021 supplement 2023[2].

3.2. Microscopic studies and powder analysis

The powder drug analysis and macroscopic and microscopic of all twelve *M. repandus* stem samples promoted the same macroscopic and microscopic characteristics including the cell characteristics and layers in transverse section and the cells and crystals in powder drug analysis.

A transverse section of *M. repandus* stem presented a periderm that was composed of cork layers of rectangular brownish cells. The cortex was composed of broad zone of parenchyma cells. Some parenchyma cells contained starch granules. The stele was composed of phloem and xylem layers. Pith was composed of parenchyma cells, some of which contained yellow substances or rosette aggregate crystals of calcium oxalate (Figure 2). Powdered drug of *M. repandus* stem was composed of dark color corks, consisting of stacked round or polygonal cells in surface view. The corks in sectional view were had several layers of rectangular cells. The thin-walled parenchyma cells in surface view were large, globoid, and loosely packed cells. The thin-walled parenchyma cells in sectional view appeared as large rectangular in shape. Numerous border pitted vessels presented as long, large, lignified cells. Numerous long, lignified edges of border pitted vessel were appeared in curve, spiral, or coil shape. Additionally, the large lignified spiral vessel was also found. Numerous groups of lignified fibers, characterized by their long, slender shape with lumen, were associated with prism crystals of calcium oxalate and medullary rays. The medullary rays were observed as round rectangular cells arranged in lines between the fibers. Large, lignified thick-walled sclereids were presented. Small and round shape of starch granules were moderately observed (Figure 3).

The observed microscopic characteristics and powdered drug analysis from this study corresponded with the macroscopic described in THP 2021 supplement 2023[2].

Figure 1. Macroscopic characteristics of *Mallotus repandus* stem crude drug.

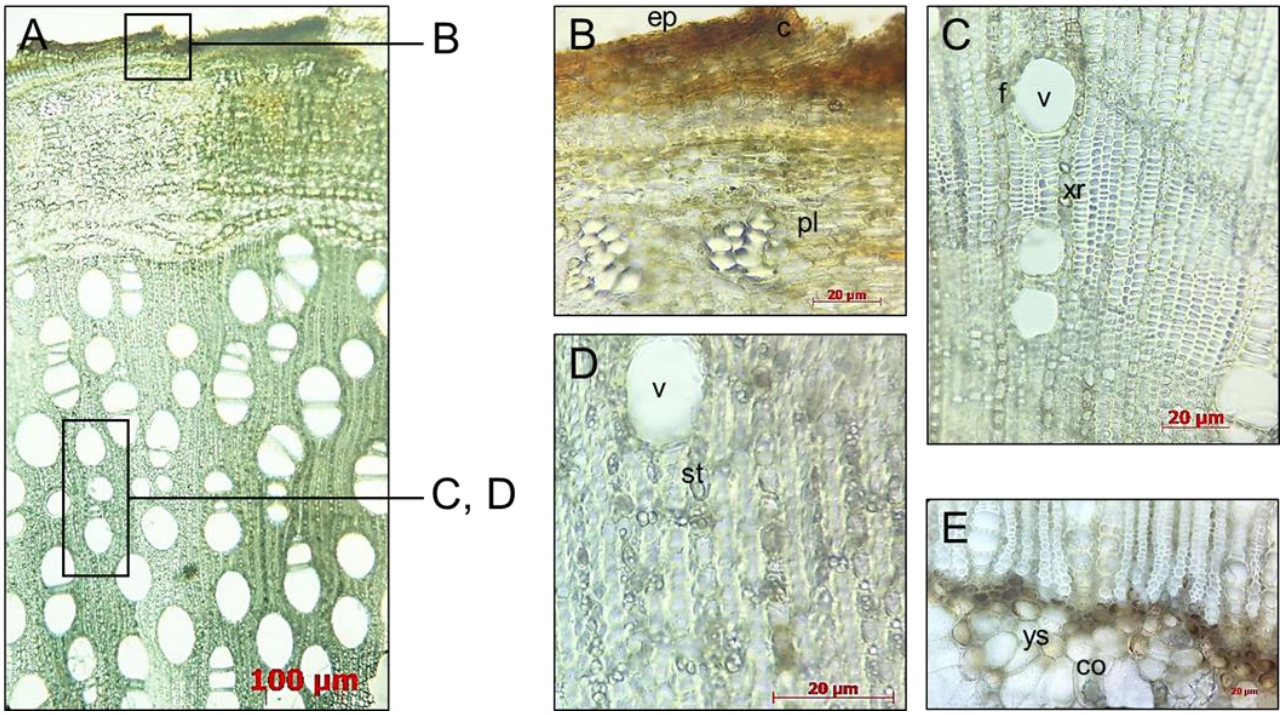

Figure 2. Transverse section characteristics of *Mallotus repandus* stem: (A) transverse section; (B) epidermis (ep), cork layers (c) and phloem layers (pl); (C) xylem ray (xr), vessels and xylem fibers (f); (D) vessels and parenchyma cells with starch granules (st); (E) parenchyma cells containing yellow substances (ys) and rosette aggregate crystals of calcium oxalate (co).

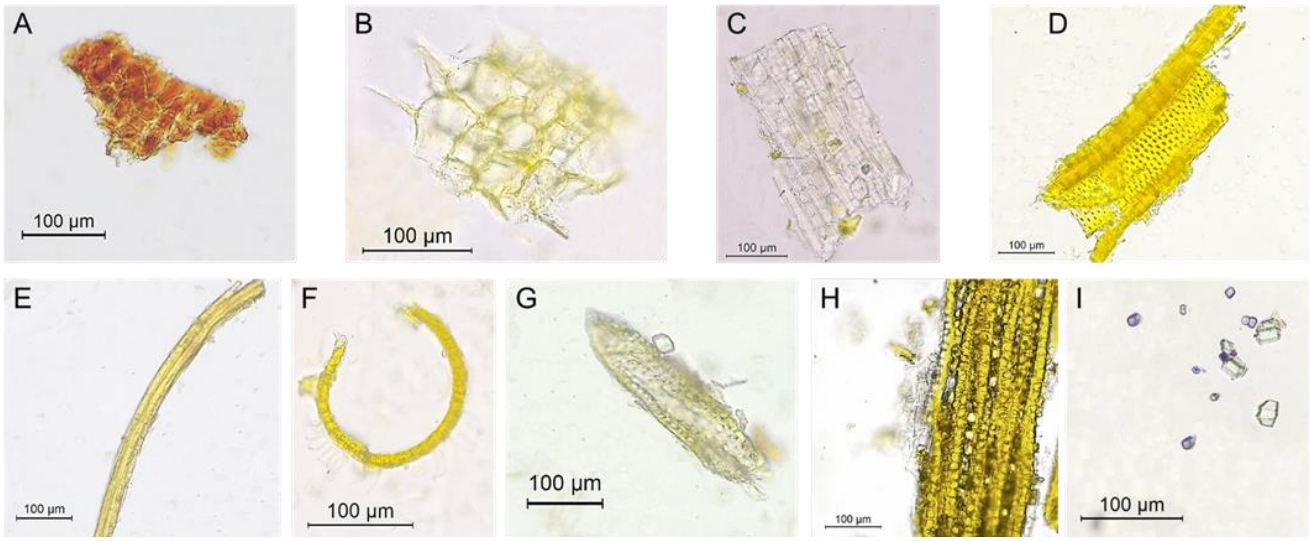

Figure 3. Microscopic characteristics of *Mallotus repandus* stem powders: (A) corks in surface view; (B) parenchyma cells in surface view; (C) parenchyma cells in sectional view with medullary ray; (D) large border pitted vessel; (E) fiber; (F) edge of border pitted vessel; (G) sclereid, (H) fibers with medullary rays and prism crystals of calcium oxalate; (I) starch granules (purplish blue color) with prism crystals of calcium oxalate; where A–H were stained with aniline sulfate solution and I was stained with iodine solution.

3.3. Evaluation of physical properties

The physical properties of all powders of the *M. repandus* stems collected from the 12 locations in Thailand are shown in Table 2. The foreign matter values in the plant materials were less than 1% w/w with the loss on drying less than 10% w/w. The total ash contents were less than 10% w/w, while the acid insoluble ash contents were less than 1% w/w. The ethanol-soluble extractive values varied substantially in the range 3–9% w/w, while the water extractive values were in the range 5–11% w/w.

The physical properties of *M. repandus* stems from this study corresponded to the limits indicated in the monograph of *M. repandus* in THP 2021 supplement2023 which assigned the limits of loss on drying not more than

9.0 % w/w, foreign matter not more than 2.0 % w/w, acid-insoluble ash not more than 1.0 % w/w, total ash not more than 8.0 % w/w, ethanol-soluble extractive not less than 3.0 % w/w, and water-soluble extractive not less than 5.0 % w/w[2].

Table 2. Physical properties of *Mallotus repandus* stem powders collected from 12 locations in Thailand.

Physical characteristic	Range (value ± SD)	Mean ± SD
Foreign matter (%w/w)	0.00 ± 0.00 – 0.05 ± 0.00	0.02 ± 0.02
Loss on drying (%w/w)	7.10 ± 0.04 – 8.63 ± 0.16	7.97 ± 0.41
Total ash (%w/w)	2.21 ± 0.08 – 7.19 ± 0.11	5.70 ± 1.55
Acid insoluble ash (%w/w)	0.12 ± 0.03 – 0.72 ± 0.12	0.34 ± 0.17
Ethanol-soluble extractive (%w/w)	3.13 ± 0.06 – 9.23 ± 0.09	6.01 ± 1.75
Water-soluble extractive (%w/w)	5.23 ± 0.20 – 11.42 ± 0.03	8.44 ± 1.68

3.4. Evaluation of chromatographic fingerprint based on thin-layer chromatography

All *M. repandus* stems collected from 12 locations in Thailand showed the same specific TLC fingerprints (Figure 4). Fifteen major chromatographic bands were observed after the plate was detected under UV 254 and 366 nm and after sprayed with phosphomolybdic acid spraying reagent and detected in white light. The chromatographic characteristics and hR_f (retention factor × 100) values of the chromatographic bands in *M. repandus* stems are shown in Table 3. There was a major chromatographic band at the hR_f value of 71-73, which appeared as dark quenching under UV 254 nm, pale blue fluorescence under UV 366 nm, and orange-brown after spraying with phosphomolybdic acid spraying reagent and detection in white light. This chromatographic band showed specific characteristics corresponded to standard bergenin in all detection methods.

However, the intensity of bands including bergenin in TLC profile of *M. repandus* from Chanthaburi (Figure 4, track 11) was lower than that observed in other *M. repandus* stem samples. This could potentially influence by several biotic and abiotic factors such as the age of the plant and the collecting location including the variations in climatic conditions and geography. The amounts of secondary metabolites in plant are strongly correlated with the environment[25]. A previous report suggested that there was an optimum period for harvesting of plant raw material due to the effects of climatic condition such as temperature and rainfall[26]. The collecting location was also found to affect to the production of phytochemicals in plants[27]. Moreover, the age of the plant was also found to influence phytochemical contents[28]. Bergenin content *in Rodgersia sambucifolia* was reported to positively correlate with annual mean temperature (AMT) and 1-12 month monthly mean temperature (MMT)[25]. Other studies reported that different plant parts affected the amounts of bergenin in plants[29-30].

In THP 2021 supplement 2023, the TLC analysis of *M. repandus* stems was described using different solvent system (dichloromethane : methanol; 7:3 v/v) with the same detection methods. There was also a chromatographic band corresponded to standard bergenin which showed the same chromatographic characteristics as the results in this study, but it appeared at a different hR_f value (73-78)[2].

Table 3. Chromatographic characteristics and hR_f (retention factor × 100) values of chromatographic bands of *Mallotus repandus* stems.

Band	hR_f	Chromatographic characteristic		
		UV 254 nm	UV 366 nm	10 % w/v Phosphomolybdic acid
1	10-11	Dark quenching	Pale blue	Blue
2	16-18	Dark quenching	Pale blue	Blue
3	21-24	Dark quenching	Blue	Yellow-brown
4	28-29	Dark quenching	-	Yellow-brown
5	35-39	Dark quenching	Blue	Yellow-brown
6	44-46	Dark quenching	Green	Pale brown
7	50-53	Dark quenching	Blue	Blue
8	56-58	Dark quenching	Blue	Pale blue
9	63-64	Dark quenching	Pale blue	Pale blue
10	68-70	Dark quenching	Yellow	Pale brown
11*	71-73	Dark quenching	Pale blue	Orange-brown
12	76-78	Dark quenching	-	Pale blue
13	79-81	Dark quenching	Blue	Pale blue
14	85-87	Dark quenching	Blue	Blue
15	89-91	Dark quenching	Blue	Blue

* bergenin band

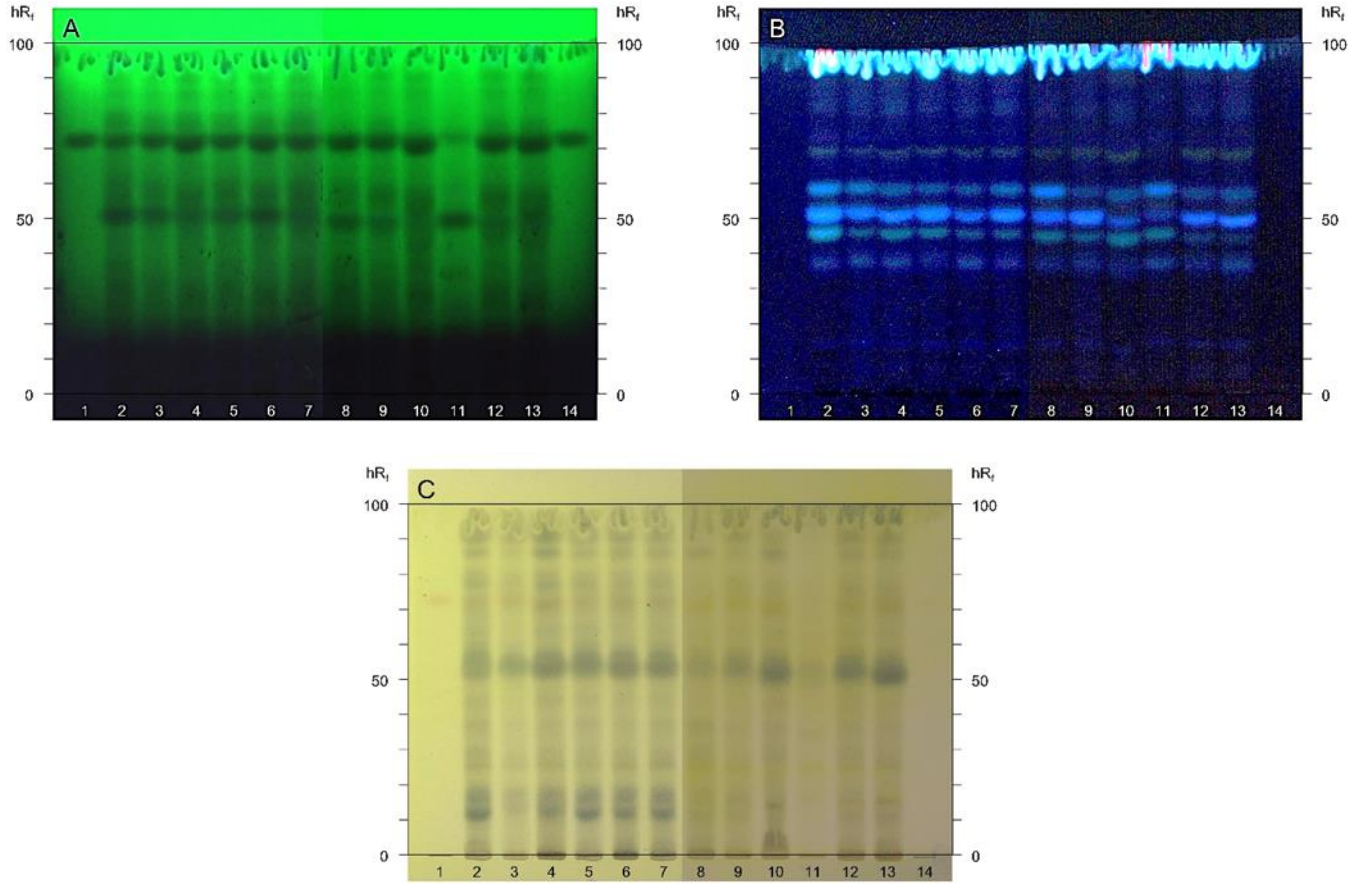

Figure 4. Thin layer chromatographic fingerprints of *Mallotus repandus* stems collected from 12 locations in Thailand, adsorbent: silica gel 60 GF254, solvent system: ethyl acetate–formic acid–acetic acid–water (100:11:11:27 v/v/v/v), detection: (A) ultraviolet 254 nm; (B) ultraviolet 366 nm; (C) white light after spraying with phosphomolybdic acid spray and heating on hot plate (105 °C for 10 min), where tracks are: 1 = standard bergenin, 2 = *M. repandus* stems from Nakhon Pathom no. 1, 3 = *M. repandus* stems from Bangkok no. 1, 4 = *M. repandus* stems from Bangkok no. 2, 5 = *M. repandus* stems from Nakhon Pathom no. 2, 6 = *M. repandus* stems from Lop Buri, 7 = *M. repandus* stems from Bangkok no. 3, 8 = *M. repandus* stems from Nakon Ratchasima, 9 = *M. repandus* stems from Nakhon Si Thammarat, 10 = *M. repandus* stems rhizomes from Sakon Nakhon, 11 = *M. repandus* stems from Chanthaburi, 12 = *M. repandus* stems from Phitsanulok and 13 = *M. repandus* stems from Chiang Mai,14 = standard bergenin.

3.5. Phytochemical analysis based on color reaction

Ethanol extracts of all *M. repandus* stem samples were phytochemically evaluated based on color reactions (Table 4). All extracts had positive results by producing a blue color after they were tested with a ferric chloride reagent, suggesting the presence of phenolic compounds. They also showed positive results in photo effect test, with the intense blue fluorescence suggesting the presence of coumarins. However, they showed negative results to Shinoda's test, a gelatin salt test, a modified Borntrager's test, Dragendorff's test and precipitation tests, Libermann-Burchard test and a froth test suggesting no presence of flavonoids, tannins, anthraquinone glycosides, alkaloids, steroid or triterpenoid structures, or saponins, respectively, in the *M. repandus* stems or if present, they were only available in small amounts.

Phytochemical screening promoted the results corresponded to previous studies with the presences of phenolics, terpenoids, steroids[6] and lactones[31]. Bergenin was found to be the major constituent[7,14].

Table 4. Phytochemical analysis based on color reaction of *Mallotus repandus* stems.

Test	Result
Phenolic compounds (ferric chloride test)	Positive (blue color)
Flavonoids (Shinoda's test)	Negative (no red or orange color)
Tannins (gelatin salt test)	Negative (no white precipitate)
Anthraquinone glycosides (modified Borntrager's test)	Negative (no pink color)
Coumarins (photo effect test)	Positive (blue fluorescence)
Alkaloids (Dragendorff's test and precipitation tests)	Negative (no orange color and no precipitate)
Steroids and triterpenes (Libermann-Burchard's test)	Negative (no red, purple, or dark bluish green color)
Saponins (froth test)	Negative (no honeycomb froth)

4. CONCLUSION

M. repandus stems samples collected from different provinces in Thailand were investigated for macroscopic and microscopic characteristics. The results suggest the present of the major organelles, including cork cells, parenchyma cells, lignified border pitted vessels, and lignified fibers with some prism crystals of calcium oxalate. The physical properties, such as foreign matter, loss on drying, total ash, acid-insoluble ash, ethanol-soluble extractive, and water-soluble extractive, were examined. Additionally, the analysis of phytochemical properties of the stems revealed the presences of phenolics and coumarins. The information obtained from this study should be beneficial for the quality control of raw materials and finished products of this plant in the future.

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Conflict of interest
The authors declare that they have no conflict of interest.

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Ethics approval
None to declare.

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