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Research article

Pharmacognostic characteristics, physical properties and chromatographic fingerprints of *Curcuma comosa* Roxb. rhizome samples collected in Thailand

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Abstract

Importance of the work: There is no monograph or specification of *Curcuma comosa* rhizomes in Thailand. The misuse of the wrong *Curcuma* species affects the safety and effectiveness of their herbal uses.

Objectives: To evaluate the physical, microscopic and phytochemical characteristics of *C. comosa* rhizomes.

Materials & Methods: Twelve *C. comosa* rhizome samples collected from different locations in Thailand were evaluated for physical, microscopic and phytochemical characteristics using the official methods.

Results: The *C. comosa* rhizomes had a specific light odor and a slightly astringent taste. The important histological features of the *C. comosa* rhizomes were the corks, which were composed of alternating layers of rectangular cells. The cortex was composed of numerous parenchyma cells, with some containing starch granules or oleoresin. The foreign matter contents were less than 1% weight per weight (w/w), with a loss on drying of less than 11% w/w. The total ash and acid-insoluble ash contents were less than 14% weight per weight (w/w) and 3% w/w, respectively. The mean (± SD) ethanol-soluble extractive and water-soluble extractive contents were in the ranges 7.63 ± 0.16–40.66 ± 0.29% w/w and 14.07 ± 0.15–24.58 ± 0.33% w/w, respectively. The mean (± SD) swelling index was 3.54 ± 0.44. Thin layer chromatographic analysis of *C. comosa* rhizome samples showed specific chromatographic fingerprints. Phytochemical screening using color reaction suggested the presence of phenolic and steroid compounds.

Main finding: The phytochemical profile of the *C. comosa* rhizome was reported along with the physical properties and the macroscopic and microscopic characteristics. These results can be utilized for the quality control of raw materials and finished products.

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Introduction

Thai local wisdom has been promoted by the national policy to develop medicinal products from plants efficiently and sustainably to serve both domestic and international demands (Kwankhao et al., 2020). The quality control of herbal material can be considered as a crucial step to ensure the efficiency and effectiveness of medicinal products. *Curcuma comosa* Roxb., which is called 'wan chak motluk' in Thai, is a shrub in the Zingiberaceae family. The rhizome of this plant is edible and has been traditionally used as a gynecological herbal medicine, including as an anti-inflammatory agent to relieve postpartum uterine pain after delivery and for enhancement of uterine involution (Jantaratnotai et al., 2006; Sodsai et al., 2007; Keeratinijakal et al., 2010). The hexane extract of *C. comosa* exhibited estrogenic-like functions in the uterus and vagina of ovariectomized rats (Piyachaturawat et al., 1995a, b). In addition, its hexane extract selectively increased alpha subtype estrogen receptors in the rat hippocampus (Su et al., 2011) and exhibited uterotrophic activity and induced cornification of the vaginal epithelium and keratinization of the mucosal surface of the vagina (Weerachayaphorn et al., 2011). *C. comosa* exhibited a preventive effect on bone loss induced by estrogen deficiency (Weerachayaphorn et al., 2011), as well as antioxidant activities (Niumsakul et al., 2007). In addition, one report suggested the existence of a superoxide dismutase homologue antioxidant protein in the rhizomes of *C. comosa,* which could be responsible for its antioxidant activity (Boonmee et al., 2011). Two major phytochemicals reported in the *C. comosa* rhizome were sesquiterpenes and diarylheptanoids (Suksamrarn et al., 2008; Xu et al., 2008). Diarylheptanoids were found to be active constituents in the methanolic extract from *C. comosa* rhizomes in the inhibition of melanogenesis in theophylline-stimulated murine B16 melanoma 4A5 cells (Matsumoto et al., 2013). One of the diarylheptanoids, diarylheptanoid (3*R*)-1,7-diphenyl-(4*E*,6*E*)-4,6-heptadien-3-ol (DPHD) was reported as the major active component to effectively promote human osteoblast function (Tantikanlayaporn et al., 2013). From traditional uses and scientific studies, the rhizomes of *C. comosa* have the potential to be used as dietary supplements or alternative remedies and have a high potential to replace estrogen, which has been reported to have side-effects (Keeratinijakal et al., 2010). *C. comosa* rhizomes have been extensively cultivated and produced as health supplements. However, to date, there has been no official identification and quality control of the rhizomes of *C. comosa*. In herbal markets, other *Curcuma* species that have a similar rhizome morphology, such as *Curcuma latifolia* Rosc. and *Curcuma elata* Roxb. could be misidentified as *C. comosa* (Keeratinijakal et al., 2010). Not only does *C. latifolia* have less estrogenic activity, but it is also very toxic. The hexane extract of *C. latifolia* was reported to exhibit enlargement of the spleen, liver and kidney in animal models (Soontornchainaksaeng and Jenjittikul, 2010). The misuse of the wrong *Curcuma* species affects the safety and effectiveness of the herbal uses. Therefore, the current study investigated and analyzed the botanical characteristics of the plant macroscopic, microscopic and physical properties of *C. comosa* rhizome samples collected from various sources in Thailand. The information from this study could be used in guidelines for a monograph or specifications of *C. comosa* rhizomes in the future.

Materials and Methods

Sample collection and preparation

Some *C. comosa* rhizome samples were purchased from various local markets in Thailand, while others were collected from a nature park in 2018. Sources of the *C. comosa* rhizome samples are shown in Table 1. The samples were botanically authenticated according to their botanical and taxonomical characteristics using the identification key described by Maknoi (2006) and the voucher specimens were deposited at the Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand. The rhizomes were cleaned, sliced into thin sheets, dried in a hot-air oven (60 °C for 48 h) and finely powdered. The cleaned rhizomes were investigated

Table 1 Sources of *Curcuma comosa* rhizome samples

Sample	Province	Thai floristic region
Sample 1	Sa Kaeo	Southeastern
Sample 2	Phetchabun no. 1	Northeastern
Sample 3	Nakhon Pathom no. 1	Central
Sample 4	Nakhon Pathom no. 2	Central
Sample 5	Bangkok	Central
Sample 6	Lop Buri	Central
Sample 7	Surat Thani	Peninsular
Sample 8	Buri Ram	Eastern
Sample 9	Phetchabun no. 2	Northeastern
Sample 10	Sakon Nakhon	Northeastern
Sample 11	Udon Thani	Northeastern
Sample 12	Ratchaburi	Southwestern

for macroscopic characteristics, while fine powder samples of the rhizomes were used for microscopic, physical and chemical analyses. All the chemicals and reagents used in this study were of analytical grade.

Macroscopic studies

The macroscopic characterization of the *C. comosa* rhizome samples were evaluated according to the guidelines described by Evans (2009) and Upton et al. (2011) for shape, size, taste, fracture, color and odor.

Microscopic studies and powder analysis

Using the methods described by Evans (2009) and Upton et al. (2011), the *C. comosa* rhizome 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. Powder samples were studied under a light microscope (Leica ICC50 W; Leica Camera Inc.; USA). The powder was cleared in a chloral hydrate reagent. Then, the cleared powder was stained with aniline sulfate reagent, while some *C. comosa* rhizome 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 cells in the plant powders were recorded using the camera connected to the light microscope under the management of the Leica Application Suite v4.12 software (Leica Camera Inc.; USA).

Evaluation of physical properties

The physical characteristics of the *C. comosa* rhizome samples were evaluated using the methods in the Thai Herbal Pharmacopoeia, 2016 (Department of Medical Sciences, 2016).

Foreign matter

The crude drugs of the *C. comosa* rhizome (100 g) were spread as a thin layer on a tray and any foreign matter was separated by hand as completely as possible. Then the remaining sample was weighed and the percentage of foreign matter was calculated.

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. The powders of each *C. comosa* rhizome (2 g) were added into the weighing bottle then the stopper was replaced and the bottle containing the plant powders was accurately weighed. Then, 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.

Total ash

The powders of each *C. comosa* rhizome (2 g) 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.

Acid-insoluble ash

Dilute hydrochloric acid (25 mL) was 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.

Ethanol-soluble extractive

The powders of each *C. comosa* rhizome (5 g) sample 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.

Water-soluble extractive

The powders of each *C. comosa* rhizome (5 g) sample 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.

Swelling index

The swelling index test was performed using a method described in the monograph of Thian Klet Hoi from the Thai Herbal Pharmacopoeia, 2016 (Department of Medical Sciences, 2016). A sample (1 g) of *C. comosa* rhizome powder was placed in a 25 mL stoppered cylinder. The powder was moistened with 1 mL of ethanol, 25 mL of water was added and the cylinder was closed. The cylinder was shaken vigorously every 10 min for 1 h and then allowed to stand for 90 min after which the cylinder was rotated on its vertical axis and then allowed to stand for another 90 mins. The volume occupied by the *C. comosa* powder was measured.

Evaluation of chromatographic fingerprint using thin-layer chromatography

Each *C. comosa* rhizome powder (300 mg) was extracted by sonicating with 2 mL of 100% volume per volume (v/v) 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 chromatograph (TLC) analysis using the methods of Soonthornchareonnon et al. (2008). Ten microliters of each of the *C. comosa* rhizome extract solutions were applied as a band (1 cm in length) on a precoated silica gel 60 GF_{254} aluminum sheet, using dichloromethane-methanol (9:1 v/v) as the solvent system. The TLC plates were detected under white light and ultraviolet (UV) light at 366 nm after they had been sprayed with anisaldehyde/sulfuric acid reagent 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).

Phytochemical analysis based on color reaction

Preparation of C. comosa rhizome extract

Each *C. comosa* rhizome powder (1 g) 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 (Farnsworth, 1966).

Flavonoids (Shinoda's test)

Magnesium ribbons (5 mg) were added to the *C. comosa* rhizome extract (2 mL) and five drops of concentrated hydrochloric acid were added. The appearance of a pink, red or orange color after a few minutes confirmed the presence of flavonoids.

Phenolic compounds (ferric chloride test)

Two-to-three drops of dilute ferric chloride solution were added in *C. comosa* rhizome extract (2 mL); the formation of blue or green coloring indicated the presence of phenolic compounds.

Tannins (gelatin salt test)

A gelatin solution (1% weight per weight; w/w) containing 10% w/w sodium chloride solution (2–3 drops) was added into the *C. comosa* rhizome extract (2 mL); the formation of a white precipitate indicated the presence of tannins.

Steroids and triterpenes (Libermann-Burchard's test)

C. comosa rhizome extract (2 mL) was evaporated in an evaporating dish in a water bath; then, 7–8 drops of acetic anhydride were added followed by 5–6 drops of concentrated sulfuric acid. The formation of pink, red, purple or blue-dark green coloring indicated the presence of steroids, while the formation of pink or red-purple coloring indicated the presence of triterpenes.

Anthraquinone glycosides (modified Borntrager's test)

C. comosa rhizome 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.

Alkaloids (Dragendorff's test)

One-to-two drops of *C. comosa* rhizome extract were spotted on a filter paper and dried; then, 1–2 drops of Dragendorff's spray reagent were dropped on the spotted filter paper. The formation of orange coloring indicated the presence of alkaloids. *C. comosa* rhizome extract (1–2 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 precipitate after the reaction was observed.

Saponins (froth test)

C. comosa rhizome powder (500 mg) was extracted with 10 mL of hot water by shaking vigorously for 1 min and then filtering. Then, 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.

Results and Discussion

Macroscopic studies

The *C. comosa* rhizome could be described as an oval-to-round, brown rhizome about 6–10 × 8–15 cm. The cut surface was a pale ochraceous color and the inside texture of the rhizome was fine. The rhizome had a specific light odor and a slightly astringent taste. The macroscopic characteristics of *C. comosa* rhizome are shown is Fig. 1. The observed macroscopic characteristics corresponded with Keeratinijakal et al. (2010) who suggested that the 'wan chak motluk' rhizome was large ovoid-to-ovate and spheroidal with a master rhizome diameter of 8–15 cm. The unique characteristics of *C. comosa* have been reported as the absence of fine spindles when the rhizomes were cut or broken, a young mango-like odor and a fine internal texture (Keeratinijakal et al., 2010). The Myanmar Herbal Pharmacopoeia, 2018 described similar botanical characteristics for the *C. comosa* rhizome, including an ovate, oblong-to-pear-shaped primary rhizome with a palmately branched secondary rhizome. The external color was yellow-yellowish-brown while the internal color was orange yellow-to-orange with a turmeric odor and a hot bitter taste (Department of Traditional Medicine, 2018).

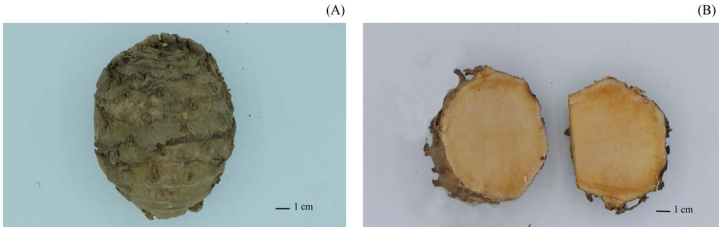

Fig. 1 Macroscopic characteristics of *Curcuma comosa* rhizome: (A) whole rhizome; (B) cross section

Microscopic studies and powder analysis

A cross section of the rhizome presented a soft, white, smooth texture, composed of corks with 4–6 layers of dark-colored rectangular cells. The endodermis of the cortex was composed of light-colored thin-walled cells aligned into lines. The cortex was composed of numerous globoidal, thin-walled, light-colored and dense parenchyma cells. Vascular bundles appeared as thin-walled, light-colored cells arranged in an ellipse (Fig. 2). The Myanmar Herbal Pharmacopoeia, 2018 provided a similar description of the *C. comosa* rhizome with a transverse section composed of similar cell components, including brownish epiblema cells, cork cells, a cortex composed of a broad zone of rounded-to-polygonal parenchymatous cells, cortical vascular bundles scattered in the cortical region, a single parenchyma layer of the endodermis, with a few layers of parenchymatous pericycle and oil, starch, tracheid, annular and spiral vessels. Yellow cell contents have been reported in the description of the *C. comosa* rhizome (Department of Traditional Medicine, 2018).

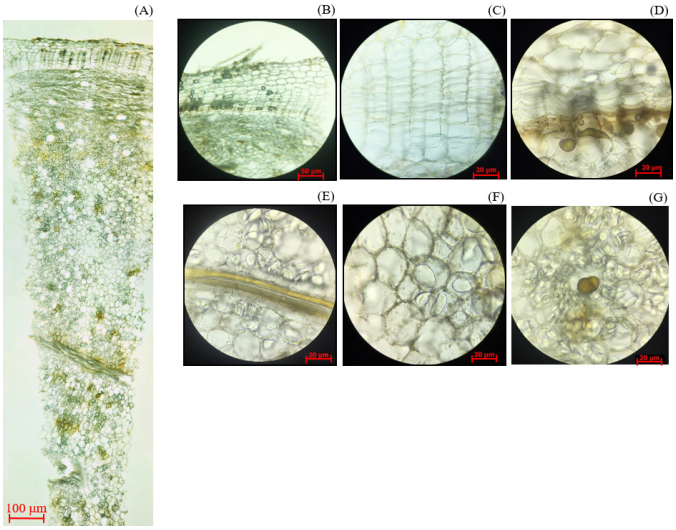

Fig. 2 Cross section characteristics of *Curcuma comosa* rhizome: (A) cross section; (B) epidermis, cork layers and root hair; (C) corks; (D) corks and phelloderms; (E) parenchyma cells with starch granules and reticulate vessels and fibers; (F) parenchyma cells with starch granules; (G) parenchyma cells with starch granules and oil

The powdered drug was composed of stacked, polygonal, lignified corks in surface view. The corks in sectional view were had several layers of suberized rectangular cells. Numerous thin-walled parenchyma cells in the surface view were large, globoid, loosely packed cells, with some containing starch granules or oleoresin. Numerous starch granules appeared as large, round or elliptical cells with crooked tips and lamellae. Reticulate vessels appeared as large, long rectangular,

non-lignified cells; some cells appeared with parenchyma cells. Border pitted and spiral vessels appeared as large, non-lignified cells. The lignified fibers were long and slender with a lumen (Fig. 3). There have not been many published reports on the microscopic characteristics of *C. comosa*. However, the current results corresponded with another report that suggested the *C. comosa* rhizome had large, round or elliptical cells with crooked tips and lamellaed starch granules, parenchyma cells, root hairs, helical and scalariform and pitted vessels (Khamthani et al., 2015). Similar microscopic information was indicated in the Myanmar Herbal Pharmacopoeia, 2018 in the monograph on the *C. comosa* rhizome, including polygonal cork cells in both the transectional and surface views, with fibers, annular and spiral vessels and pitted trachea (Department of Traditional Medicine, 2018).

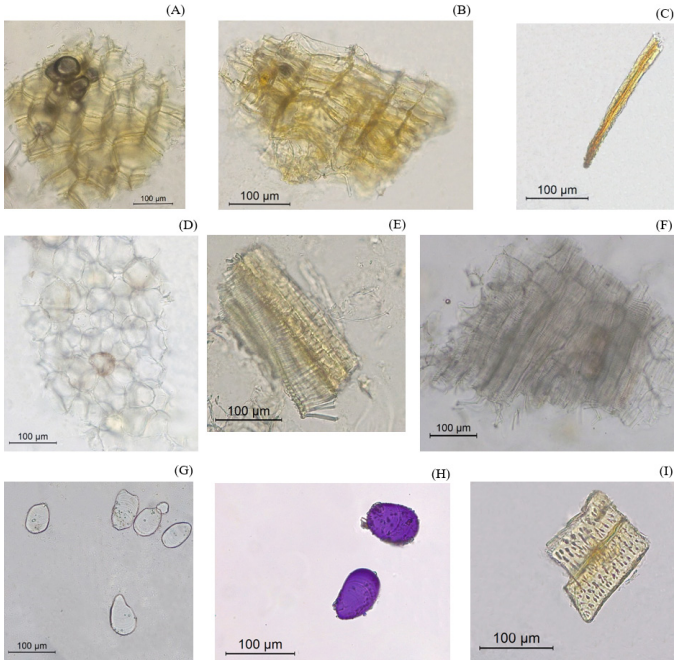

Fig. 3 Microscopic characteristics of *Curcuma comosa* rhizome powders: (A) corks in surface view; (B) corks in sectional view; (C) fiber; (D) parenchyma cells in surface view with starch granules and oleoresin; (E) reticulate and pitted vessels; (F) reticulate vessels with parenchyma cells; (G) starch granules; (H) starch granules; (I) pitted vessels, where A–G and I were stained with aniline sulfate solution and H was stained with iodine solution.

Evaluation of physical properties

The physical properties of all powders of the *C. comosa* rhizome collected from the 12 locations in Thailand are shown in Table 2. The foreign matter values in the plant materials were low (< 1% w/w) while the losses on drying were around 10% w/w. The total ash contents were less than 14% w/w, while the acid insoluble ash contents were less than 3% w/w. The ethanol-soluble extractive values varied substantially in the range 7–41% w/w, while the water extractive values were in the range 14–25% w/w. The swelling index was in the range 2.97±0.05–4.30±0.08. There has been no official monograph describing *C. comosa* rhizome raw material in Thailand until now. According to the Thai Herbal Pharmacopoeia, 2016, the foreign matter values in the current *C. comosa* rhizome samples corresponded with the values indicated in the official monographs of other *Curcuma* species, including the rhizomes of *C. longa* (‘khamin chan’) and *Curcuma* sp. (‘khamin oi’’) (< 2.0% w/w; Department of Medical Sciences, 2016). However, the indicated total ash value of the rhizomes of *C. longa* was lower than the values obtained from the *C. comosa* rhizome samples (< 8.0% w/w), while the acid insoluble ash contents of *C. longa* and *Curcuma* sp. (‘khamin oi’’) rhizomes were less than 1.0% w/w and 6.0% w/w, respectively (Department of Medical Sciences, 2016). The ethanol and water extractive values, which were provided only in the monograph of *C. longa*, but not for *Curcuma* sp. (‘khamin oi’) rhizomes, were lower than the results for the *C. comosa* rhizome samples in the current study (< 10.0 and 9.0% w/w, respectively), according to Department of Medical Sciences (2016). The major difference in the physicochemical properties was the volatile content between the rhizomes

Table 2 Physical properties of *Curcuma comosa* rhizome powders collected from 12 locations in Thailand

Physical characteristic	Range (value ± SD)	Mean ± SD
Foreign matter (%w/w)	0.00±0.00–0.15±0.00	0.03±0.05
Loss on drying (%w/w)	8.47±0.20–10.56±0.02	9.82±0.60
Total ash (%w/w)	5.16±0.03–13.56±1.10	7.37±2.05
Acid insoluble ash (%w/w)	1.12±0.12–2.46±0.14	1.75±0.38
Ethanol-soluble extractive (%w/w)	7.63±0.16–40.66±0.29	14.74±9.22
Water-soluble extractive (%w/w)	14.07±0.15–24.58±0.33	17.46±2.74
Swelling index	2.97±0.05–4.30±0.08	3.54±0.44

of *C. comosa* and other *Curcuma* rhizomes indicated in the Thai Herbal Pharmacopoeia, 2016. The volatile contents in the rhizomes of *C. longa* and *Curcuma* sp. ('khamin oi'') were indicated to be not less than 6.0% v/w and 4.0 %v/w, respectively (Department of Medical Sciences, 2016). In contrast, there was little or no volatile oil detected in the rhizome samples of *C. comosa* in this study. The water contents of the rhizomes of *C. longa* and *Curcuma* sp. ('khamin oi'') were not more than 10.0% v/w and 11.0% v/w, respectively (Department of Medical Sciences, 2016). To date, there has been no report regarding gum and mucilage in the *C. comosa* rhizome. Furthermore, according to the Thai Herbal Pharmacopoeia, 2016, there is no limitation to the swelling index in the monograph of *C. longa* and the *Curcuma* sp. ('khamin oi') monograph (Department of Medical Sciences, 2016). In the current study, the swelling index of the *C. comosa* rhizome was very low. Another report suggested that no gum and mucilage were found in the aqueous, methanol, hydroethanol, ethanol and acetone extracts of various cultivars of the *C. longa* rhizome (Salma et al., 2022).

Evaluation of chromatographic fingerprint based on thin-layer chromatography

All *C. comosa* rhizome powders collected from 12 locations in Thailand showed the same specific TLC fingerprints. Ten chromatographic bands were observed after the plate was sprayed with anisaldehyde/sulfuric acid spraying reagent and detected in white light and under 366 nm UV. The chromatographic characteristics and hRf (retention factor × 100) values of the chromatographic bands in *C. comosa* rhizomes are shown in Table 3. There was a major chromatographic band at the hRf value of 55, which appeared as dark brown after spraying with anisaldehyde/sulfuric acid spraying reagent and detection in white light, while appearing as light blue bands after they were sprayed with anisaldehyde/sulfuric acid spraying reagent and detected under UV at 366 nm. The next three major chromatographic bands could be detected at hRf values of 14, 28 and 78 and appeared as pale purple blue, pale purple and pale brown, respectively, after they had been sprayed with anisaldehyde/sulfuric acid spraying reagent and detected in white light, while they were undetectable or black and pale green bands after they were sprayed with anisaldehyde/sulfuric acid spraying reagent and detected under UV at 366 nm. In the Myanmar Herbal Pharmacopoeia, 2018 (Department of Traditional Medicine, 2018) study, the *C. comosa* rhizomes were analyzed for their phytochemicals using the TLC technique with a slightly different solvent system from the current study (dichloromethane:methanol 10:0.25 v/v). Four major chromatographic bands were reported after the TLC plate had been sprayed with anisaldehyde/sulfuric acid spraying reagent and detected in white light. They appeared as pink, pink, violet and pink bands at hRf values of 41, 71, 77 and 91, respectively. These chromatographic bands were reported as orange, greenish blue, not detectable and pink after the plate was sprayed with an anisaldehyde/sulfuric acid spraying reagent and detected under UV at 366 nm. These results indicated some correspondence between the TLC fingerprint of the *C. comosa* rhizomes from the current study and the information in the monograph from the Myanmar Herbal Pharmacopoeia, 2018 (Department of Traditional Medicine, 2018). However, the chromatographic bands, which appeared at hRf values higher than 78, could not be found in the TLC analysis of the *C. comosa* rhizomes in the current study. It was also remarked that the extraction solvent of the *C. comosa* rhizomes in the Myanmar Herbal Pharmacopoeia, 2018 (Department of Traditional Medicine, 2018) was ethyl acetate while methanol was used in the current study.

Table 3 Chromatographic characteristics and hRf (retention factor × 100) values of chromatographic bands of *Curcuma comosa* rhizomes

Band	hRf	Chromatographic characteristic	
		White light after anisaldehyde/sulfuric acid spray	Ultraviolet 366 nm after anisaldehyde/sulfuric acid spray
10	78	Pale brown	Pale green
9	67	Pale brown	Not detected
8	64	Pale brown	Pale blue
7	55	Dark brown (major)	Light blue (major)
6	40	-	Pale green
5	35	Pale brown	Not detected
4	28	Pale purple	Black
3	15	-	Pale green
2	14	Pale purple blue	Not detected
1	5	Pale brown	Not detected

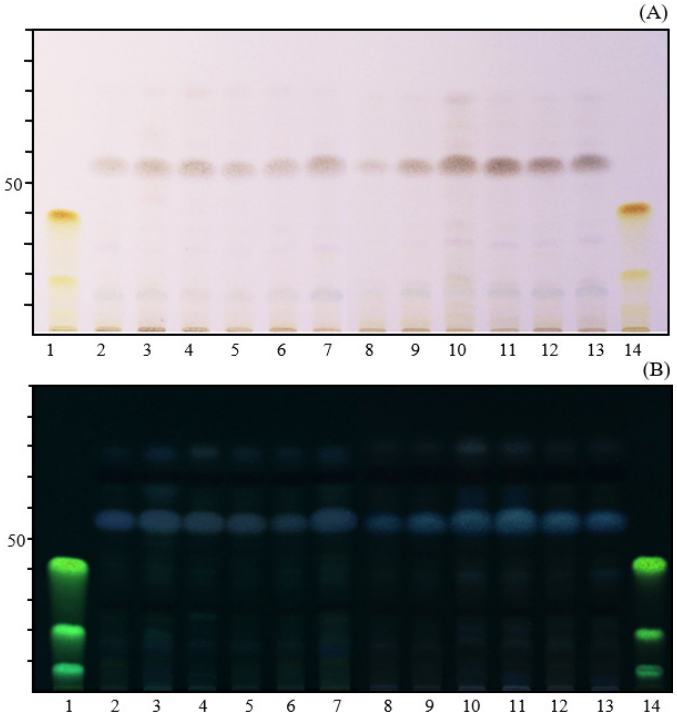

Fig. 4 Thin layer chromatographic fingerprints of *Curcuma comosa* rhizome powders collected from 12 locations in Thailand, adsorbent: silica gel 60 GF_{254} solvent system: dichloromethane-methanol (9:1 v/v), detection: (A) white light after spraying with anisaldehyde/sulfuric acid spray and heating on hot plate (105 °C for 10 min); (B) ultraviolet 366 nm after spraying with anisaldehyde/sulfuric acid spray and heating on hot plate (105 °C for 10 min), where tracks are: 1 = standard curcuminoids, 2 = *C. comosa* rhizomes from Sa Kaeo, 3 = *C. comosa* rhizomes from Phetchabun no. 1, 4 = *C. comosa* rhizomes from Nakhon Pathom no. 1, 5 = *C. comosa* rhizomes from Nakhon Pathom no. 2, 6 = *C. comosa* rhizomes from Bangkok, 7 = *C. comosa* rhizomes from Lop Buri, 8 = *C. comosa* rhizomes from Surat Thani, 9 = *C. comosa* rhizomes from Buri Ram, 10 = *C. comosa* rhizomes from Phetchabun no. 2, 11 = *C. comosa* rhizomes from Sakon Nakhon, 12 = *C. comosa* rhizomes from Udon Thani and 13 = *C. comosa* rhizomes from Ratchaburi, 14 = standard curcuminoids

Phytochemical analysis based on color reaction

Ethanol extracts of all *C. comosa* rhizome samples were phytochemically evaluated based on color reactions (Table 4). All extracts had positive results by producing a blue-green color after they were tested with a ferric chloride reagent, suggesting the presence of phenolic compounds. In addition, there were positive results from Libermann-Burchard's test, with the dark green color suggesting the presence of steroid structures. However, there was a negative response using Shinoda's test, a gelatin salt test, a modified Borntrager's test, a froth test, Dragendorff's test and precipitation tests. These results suggested that there were no flavonoids, tannins, anthraquinone glycosides, saponins or alkaloids, respectively, in the *C. comosa* rhizomes or they were available only in small amounts. Burapan et al. (2020) reported information about the phytochemicals in 23 Thai *Curcuma* species, with the *C. comosa* rhizome ethanol extract having a total phenolic content of 4.2±0.1 mg gallic acid equivalents per gram dried weight, while some sesquiterpenoids, such as germacrone, furanodienone and zederone, had around 30–81 mg/g dried weight and none of the three curcuminoids could be detected. 1,7-Diphenyl-(4*E*,6*E*)-4,6-heptadien-3-ol was also isolated and identified from *C. comosa* rhizome ethanol extract by Burapan et al. (2020). Another major group of compounds in the *C. comosa* rhizome is the diarylheptanoids that can be divided into non-phenolic and phenolic forms (Suksamrarn et al., 2008). One of the phenolic diarylheptanoids, (3*S*)-7-(3,4-dihydroxyphenyl)-1-phenyl-(1*E*)-1-hepten-3-ol (D-092) showed strong antioxidant activity determined using a DPPH assay (Jariyawat et al., 2009). A phenolic compound, namely 4,6-dihydroxy-2-*O*-(*β*-D-glucopyranosyl) acetophenone has been isolated and identified from the ethanol extract of the *C. comosa* rhizome (Niumsakul et al., 2007).

Table 4 Phytochemical analysis based on color reaction of *Curcuma comosa* rhizomes

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

This compound was also reported to exhibit antioxidant activity and cytotoxicity against human cervix adenocarcinoma cells (Niumsakul et al., 2007). Chokchaisiri et al. (2012) reported new flavonoids (curcucomosides A–D) along with some known flavonoids, including kaempferol 3-*O*-*α*-L-arabinoside, quercetin 3-*O*-arabinopyranoside and kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside from the aerial part of *C. comosa*; however, there have been no reports of flavonoids or saponin being identified in the rhizomes. Studies have reported both negative (Salma et al., 2022) and positive (Irshad et al., 2018; Salma et al., 2022) results in foam tests of aqueous extracts from the *Curcuma longa* rhizome, while negative results were reported for methanol, hydroethanol, ethanol and acetone extracts (Salma et al., 2022).

Conclusion

The macroscopic and microscopic characteristics of *C. comosa* rhizome samples collected from different provinces in Thailand were investigated and the results suggested that the main organelles were corks, parenchyma cells and starch granules with crooked tips and lamellae. Furthermore, evaluation of the physical and phytochemical properties of the rhizomes indicated that phenolic and steroid compounds were present. 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.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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