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Pharmacognostic specifications and the antioxidant activity of *Curcuma comosa* Roxb. crude drugs

Karakter Farmakognostik dan Aktivitas Antioksidan Simplisia Rimpang *Curcuma comosa* Roxb.

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ABSTRACT

Curcuma comosa Roxb. is popularly used to treat gynecological problems but has no official monograph in the Thai Herbal Pharmacopeia (THP). This study characterized the selected pharmacognostic and physicochemical specifications and antioxidant potentials of *C. comosa* crude drugs. The pharmacognostic and physicochemical properties of two kinds of crude drugs were characterized according to the WHO quality control methods for herbal materials. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, ferric reducing antioxidant power (FRAP), and total phenolic content (TPC) were evaluated as per the standard method. The microscopic observation showed relatively large-sized starch granules, cortical parenchyma, vessel, and sclerenchyma fibre. The thin-layer chromatography (TLC) profile demonstrated distinct separation with two major spots. The physicochemical evaluations specified as follow: moisture (8.87±1.37%), total ash (2.35±0.12%), acid-insoluble ash (0.80±0.08%), volatile oil (1.01±0.03%), water-soluble extractable (16.01±0.95%), and ethanol-soluble extractable (17.74±1.56%). The DPPH scavenging activity, FRAP, and TPC of the crude drugs were 765.56±80.50 mM Trolox equivalent (TE)/g dry weight (DW), 505.42±22.44 mM TE/g DW, and 46.09±2.27 mg Gallic acid equivalent (GAE)/g DW. This study specified quality parameters of *C. comosa* crude drugs that might serve as the reference for the quality control purpose.

Keywords: Antioxidant, crude drugs, *Curcuma comosa*, pharmacognostic specifications.

ABSTRAK

Curcuma comosa Roxb. merupakan tumbuhan obat yang banyak digunakan untuk penanganan tradisional masalah kandungan di Thailand. Hingga saat ini, monografi resmi dari simplisia dan ekstrak rimpang tumbuhan ini belum masuk dalam Farmakope Herbal Thai. Penelitian ini bertujuan untuk mengkarakterisasi spesifikasi farmakognostik terpilih dan mengevaluasi potensi antioksidan dari rimpang *C. comosa*. Spesifikasi farmakognostik dari rimpang *C. comosa* dikarakterisasi dengan metode kontrol kualitas bahan herbal standar WHO. Aktivitas penangkapan radikal 2,2-difenil-1-pikrilhidrazil (DPPH), *ferric reducing antioxidant power* (FRAP), dan kadar fenolik total dari rimpang *C. comosa* dievaluasi menurut metode standar masing-masing metode. Pengamatan mikroskopis menunjukkan granula pati berukuran besar, parenkim korteks, berkas pembuluh, dan serat sklerenkim. Profil kromatografi lapis tipis (KLT) menunjukkan pemisahan dua bercak utama yang jelas memisah satu sama lain. Nilai spesifikasi farmakognostik simplisia meliputi kadar air (8,87±1,37%), abu total (2,35±0,12%), abu tidak larut asam (0,80±0,08%), minyak atsiri (1,01±0,03%), kadar sari larut air (16,01 %) ±0,95%), dan kadar sari larut etanol (17,74±1,56%). Aktivitas penangkapan DPPH, FRAP, dan TPC masing-masing sebedsar 765,56±80,50 mM etara Trolox (ST)/g berat kering (BK); 505,42±22,44 mM ST/g BK, dan 46,09±2,27 mg setara asam galat (SAG)/g BK. Penelitian ini mengusulkan spesifikasi parameter mutu simplisia *C. comosa* yang dapat dijadikan acuan untuk pemastian mutu bahan baku obat herbal.

***Kata kunci**: Antioksidan, Curcuma comosa, karakter farmakognostik, simplisia.*

Introduction

Curcuma comosa Roxb. (Zingiberaceae) is commonly used for the traditional treatment of gynecological problems in Thailand. The estrogenic-related activities of this plant have been confirmed, with diarylheptanoids as the bioactive compounds (Chawalitpong et al., 2016; Thongon et al., 2017). *C. comosa* showed promising effects on the in-vivo model of estrogen-deficiency-induced hyperlipidemia and osteoporosis (Sutjarit et al., 2020; Vinayavekhin et al., 2016). Despite being popular and commercially available, *C. comosa* crude drug is not included in the Thai Herbal Pharmacopeia (THP).

The wide variation of quality of the crude drug is commonly reported. The growing environment and genetic variation are the factors manifested in the different phytochemical profiles and physicochemical properties of crude drugs (Chen et al., 2015; Qin et al., 2015; Yao et al., 2018). The crude drugs must be of good quality to exert efficacy and safety profile during use. Hence, the standardization process is essential to guarantee quality constantly. The monograph in herbal pharmacopeia specifies the quality of crude drugs by defining standards, constant parameters, absolute qualitative and quantitative values (Alam and Saqib, 2015).

As there is no official monograph of *C. comosa*, this study aimed to

characterize the selected pharmacopeial parameters of the crude drugs. The pharmacognostic characters (macroscopic and microscopic specifications and thin-layer chromatography (TLC) profile) and physicochemical characters (water, ash, and essential oil contents as well as water and ethanol soluble extractable matters) of *C. comosa* were characterized. In addition, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, ferric reducing antioxidant power (FRAP), and total phenolic content (TPC) of the water extract of the commercial crude drug were also evaluated in this study.

Research Method

Preparation of Plant Materials

The commercial *C. comosa* crude drugs were purchased from Vejpong Herbal Pharmacy (Bangkok, Thailand) in the form of powdered rhizomes. The fresh rhizomes of authentic plant materials (Figure 1A) were obtained from Chatuchak Plant Market (Bangkok, Thailand). A specimen (voucher identifier of Hartanti006) was deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University. Both plant materials were sold under the local name of *Wan-chak-mod-look*. The rhizomes were longitudinally sliced in thickness of around 3 mm and dried in the hot air oven (Memmert, Germany) at a temperature of 50^{o}C to obtain crude drugs (Figure IB). The crude drugs were pulverized into powders, passed through a 60-mesh sieve, and stored in the airtight container at room temperature until further analysis.

Experiments

1. Morphological characters evaluation

   The shape, color, odor, and taste of the crude drugs were organoleptically observed as the macroscopic characters. On the other hand, the microscopic characters of both crude drugs were observed by mounting the powdered crude drugs with chloral hydrate (Millipore Sigma, USA) or iodine TS (Millipore Sigma) on a slide. The diagnostic fragments were observed and captured under a camera-connected light microscope (Leica, Germany) (WHO, 2011).
2. TLC profile analysis

   The powdered crude drugs were extracted with ethanol in a ratio of 1:10 w/v under sonication for 10 min. The obtained filtrate was directly spotted onto a silica GF254 and separated by the mobile phase of toluene: ethyl acetate, 93:7 (v/v). TLC profile was recorded in a TLC visualizer (Camag, Switzerland) under UV light at 254 and 366 nm, and also the visible light after derivatization with the vanillin-sulphuric acid reagent
3. Physicochemical properties determination
   a. Water content determination

      Water content in both crude drugs was analyzed by the azeotropic method using toluene (Millipore Sigma) (WHO, 2011).

b. Ash contents evaluation

Total ash and acid insoluble ash contents were obtained after gradual ignition of crude drugs and re-ignition of the dilute hydrochloric acid-treated ash in a furnace (Nabertherm, Germany), as recommended in the WHO guideline (WHO, 2011).

c. Volatile oil content analysis

The volatile oil content in the crude drugs was determined by the water distillation method (WHO, 2011).

d. Water- and ethanol-soluble extractable matters analysis

The extractable matter of both crude drugs was determined by the cold maceration method with water and 70% ethanol (Millipore Sigma) as the solvent, respectively (WHO, 2011).

4. Antioxidant activities determination

a. Extract preparation

The commercial crude drug was extracted by a modified decoction method (Habibie et al., 2017). After being accurately weighed, the crude drug was boiled in the water (ratio of 1:100) for an hour. The filtrate was freshly used for antioxidant analysis.

b. DPPH radical scavenging activity analysis

The DPPH scavenging activity assay was conducted as a previous report with slight modification (Thaipong et al., 2006). In brief, the extract was mixed with 0.025 mg/ml DPPH methanolic solution in a ratio of 1:10 (v/v). After 30 min sits at room temperature protected from light, the absorbance was read at a wavelength of 517 nm using a UV – visible spectrophotometer (Thermo Scientific, USA). Various concentrations of Trolox were used for preparing standard curve, and DPPH scavenging activity was reported as mM Trolox equivalent (TE)/g dry weight (DW) crude drugs.

c. FRAP determination

FRAP was determined as the previously described method (Frond et al., 2019). In brief, the extract was mixed with FRAP reagent in a ratio of 1:19 (v/v). The mixture was stood at room temperature for 30 min, and the absorbance was read at 594 nm. Trolox was used as the standard and FRAP was reported as mM TE/g DW crude drugs.

d. TPC determination

TPC was determined using the modified Folin-Ciocalteau method (Tuekaew et al., 2014). Briefly, the extract was mixed with water and a Folin-Ciocalteau reagent (MilliporeSigma) ratio of 1:79:5 (v/v/v). After 5 min, 15 parts of saturated sodium carbonate was further added into each mixture.

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The mixture was stood at room temperature for two h, and the absorbance was read at 764 nm. The standard curve was prepared from various concentrations of gallic acid and TPC was reported as mg Gallic acid equivalents (GAE)/g DW crude drugs.

Data Analysis

All quantitative data were expressed as the mean value ± standard deviation (SD) from triplicate experiments. The water, ash, essential oil contents, and soluble extractable matters in both crude drugs were individually compared by the independent samples t-test. The correlation between TPC and both DPPH scavenging activity and FRAP was analyzed by Pearson correlation test. The values were considered significantly different at a p-value <0.05. Statistical analyses were conducted by IBM SPSS Statistics v.13 (IBM, USA).

Results and Discussion

Pharmacognostic Characters of the Crude Drugs

The rhizomes of *C. comosa* appeared as large primary rhizome with few branches, light brown outside and pale yellowish inside. Upon dried, the *C. comosa* crude drugs were thin pieces, round or ovate shaped, light in weight, stiff, fragile; externally brown, wrinkled; internally brownish yellow, unevenly curved, with a distinct odor, pungent and slightly bitter taste (Figure 1). The microscopic evaluation showed vessels, large cortical parenchyma, sclerenchyma fibre, and starch granules in both crude drugs (Figure 2). The TLC profile of both crude drugs was similar. After derivatization, two particular spots at hRf of 21 (teal) and 41 (yellow) were observed (Figure 3).

As the primary method for evaluating the identity aspect, morphological analysis is popularly performed to screen the quality of crude drugs (Ichim et al., 2020). The macroscopic morphological characters shown by *C. comosa* crude drugs in this study were not specific. They were commonly observable in some Curcuma species of the “Longa” group with large primary rhizome (Sirirugsa et al., 2007). The same phenomenon was also demonstrated by the microscopic morphological characters of the crude drugs. Those four diagnostic fragments were also commonly seen in other Curcuma species, including in *C. aeruginosa* and *C. caesia* (George et al., 2014; Verma et al., 2010). Hence, the macroscopic and microscopic morphology of *C. comosa* crude drugs alone could not be used to confirm the identity of the crude drugs.

TLC profile represents identity, purity, and content aspects of crude drug quality. It is widely used in for its simplicity and rapidness, among other chromatographic techniques (Mukhi et al., 2016).

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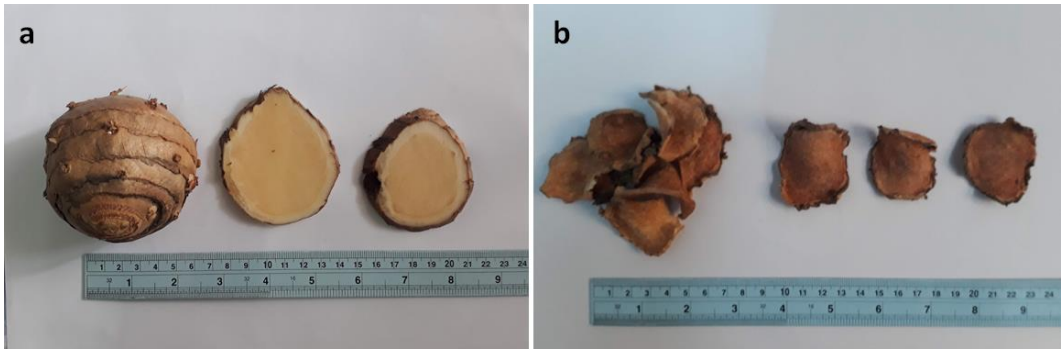

Figure 1. The fresh rhizomes (a) and the crude drugs (b) of *C. comosa*

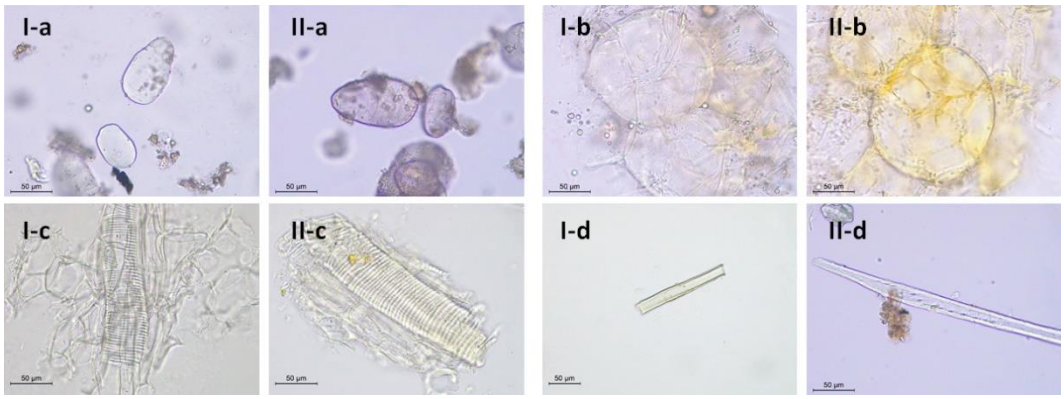

Figure 2. The diagnostic fragments of self-made (I) and commercial (II) *C. comosa* crude drugs, i.e., starch granules (a), cortical parenchyma (b), vessels (c), and sclerenchyma fibre (d). The scale bar is 50 µm

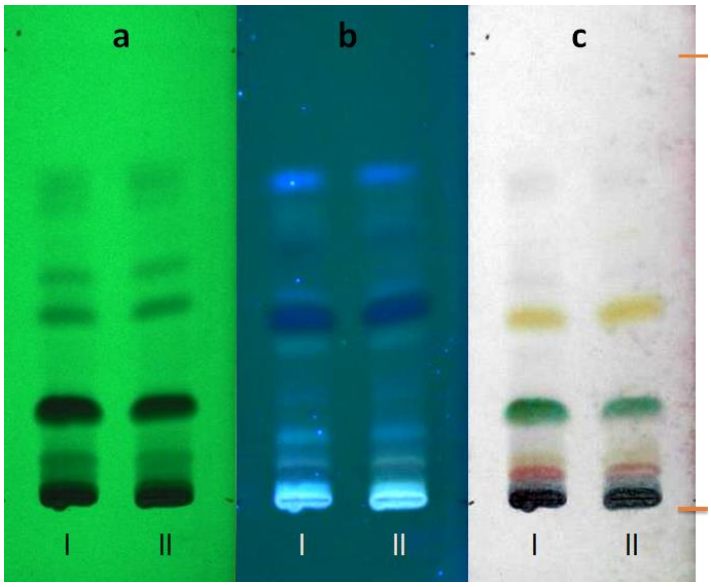

Figure 3. TLC profile of self-made (I) and commercial (II) crude drugs of *C. comosa* visualized under UV light at 254 nm (a), 356 nm (b), and visible light after spraying with vanillin-sulphuric acid reagent (c)

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The TLC profiles of the crude drugs with two major spots corroborated the report that *C. comosa* distinctively contained a very high level of diarylheptanoids among other Curcuma rhizomes (Burapan et al., 2020). Hence, combined with the morphology characters, the TLC profile provided helpful information to confirm the identity aspect of *C. comosa*.

The Physicochemical Characters of the Crude Drugs

Both *C. comosa* crude drugs contained water, total ash, and acid-insoluble ash content at a different level. The commercial crude drugs showed higher value in all three parameters. However, the solvent-soluble extractable matters showed the opposite trend, with the value was significantly lower in the commercial one. Nevertheless, the volatile oil content of both crude drugs was comparable. The water content of the *C. comosa* crude drugs were 8.87±1.37%. On the other hands, the total and acid-insoluble ash contents obtained in this study were 2.35±0.12 and 0.80±0.08%, respectively. The volatile oil content of the crude drugs was 1.01±0.03%, while their respective water-soluble extractable and ethanol counterpart was 16.01±0.95% and 17.74±1.56% (Figure 4).

The water and ash contents are the primary method to assess the purity aspects of the crude drug quality (Das et al., 2019). The moisture in the crude drugs might originate from the improper drying process or be taken up from the air during storage. It is associated with the risk of crude drug spoilage by microbial activity. The acceptable water content in crude drugs was generally set under 10% (Agarwal et al., 2014). Hence, *C. comosa* crude drugs in this study should be safe from the microbial-related decomposition risk.

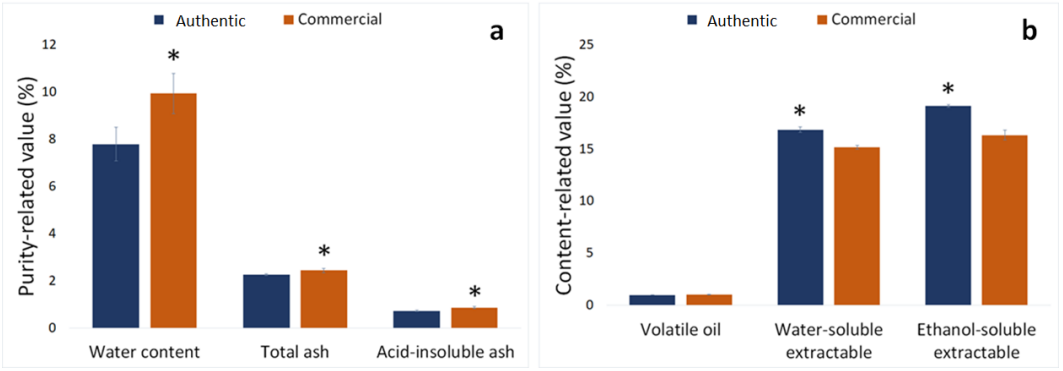

Figure 4. The purity-related (a) and content-related (b) physicochemical characters of *C. comosa* crude drugs. * indicated the crude drug with a statistically higher value of a given parameter

While total ash represented the naturally occurring and environmental-derived ashes, the acid-insoluble ash exclusively referred to the latter, mainly the sand and silica (WHO, 2011). Naturally, the limit of ash content in the rhizome-derived crude drugs was also relatively higher than those derived from other organs. For example, the Malaysian Herbal Monograph (MHM) set the total ash and acid-insoluble ash content in the *C. longa* and *C. zanthorriza* crude drugs were not more than 8 and 1% as well as 9 and 3%, respectively (Malaysian MoH, 2016). Hence, the ash contents obtained in this study should be applied as the standard value for *C. comosa.*

The volatile oil content and water- and ethanol-soluble extractable matters, on the other hand, represented the content aspect of crude drug quality. The essential oil content determined the quality of crude drugs prepared from rhizomes, and the standard was widely varied according to the species. THP set the standard for essential oil content of *C. longa* (*khamin-chan*) and *Curcuma* sp. (*khamin-oi*) was not less than 6 and 4% (Thai MoPH, 2018). Our result suggested that *C. comosa* crude drugs should contain not less than 1% of volatile oil.

The differences in the solvent-soluble extractable matters between both crude drugs represented the variation of phytochemical contents in a species. It is commonly reported, including in the closely related *C. aeruginosa* and *C. longa* (Safitri et al., 2017; Tanvir et al., 2017). The comparable water-soluble extractable and its ethanol counterpart in this study indicated the equal presence of polar and non-polar compounds in the plant.

The Antioxidant Potential of the Commercial Crude Drugs

The extract showed considerable free radical scavenging and ferric reducing capacities and contained low phenolic compounds at a low level. There was no correlation between TPC and DPPH scavenging activity (R = 0.158), while a weak correlation was observed between TPC and FRAP (R = 0.459) (Figure 5).

Similar to our result, the DPPH scavenging activity of Chiang Rai-originated *C. comosa* and the FRAP of that collected in Nakhon Pathom were previously reported (Niumsakul et al., 2007; Pintatum et al., 2020). Our result suggested that phenolic compounds were likely not the main constituents of *C. comosa*. It was consistent with a previous study on the relatively low TPC of *C. comosa* cultivated in Rayong (Thailand) (Burapan et al., 2020). The none-to-weak correlation between TPC and the antioxidant properties of *C. comosa* might indicate that the phenolic compounds were not the main contributor to the antioxidant activity of *C. comosa*. Other compounds, i.e., polypeptide and diarylheptanoid, were reported as the antioxidant compounds of *C. comosa* (Boonmee et al., 2011; Vattanarongkup et al., 2016).

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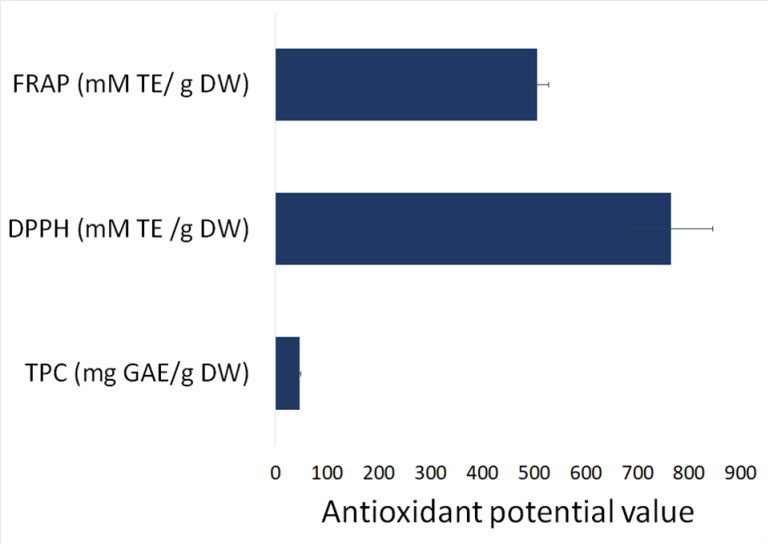

Figure 5. The profile of antioxidant capacity of commercial crude drugs of *C. comosa* water extract

Conclusion

This study provided the data on pharmacognostic, physicochemical, and antioxidant properties of *C. comosa* crude drugs that might be applied for quality control purposes. The morphological characters and the TLC profile might confirm the identity aspect, while the water and ash contents might ensure the purity aspect. Further, the volatile oil content and solvent-soluble extractable matters represented its content aspect. *C. comosa* showed considerably high DPPH scavenging activity and FRAP, which were none-to-weakly correlated to its TPC.

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