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Effects of drying pretreatments and extraction methods on biochemical activity of *Houttuynia cordata* Thunb.

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Abstract

Houttuynia cordata Thunb. (HCT) consists of various phytochemicals, with flavonoids being the key components. However, their quantity and quality are affected mostly by drying and extraction methods. This study aimed to evaluate the impact of drying and extraction methods on the phytochemical contents such as total phenolic content (TPC), total flavonoid contents (TFC) and the major components of flavonoids. In addition, antioxidant activities were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP). Freeze-drying (FD) and oven-drying (OD) methods were used for drying; aqueous (AE), ethanolic (EE), and ultrasonic assisted extraction (UAE) methods were used for extraction. The results indicated that FD had higher TPC, TFC, DPPH, and FRAP antioxidant activities than OD, irrespective of the extraction methods used. The highest TPC (29.40 ± 0.40 mg gallic acid equivalent (GAE)/ g dry wt.) and TFC (72.80 ± 0.95 mg quercetin equivalent (QE)/g dry wt.) were obtained in UAE. Likewise, UAE had the highest DPPH radical scavenging activity (69.94 ± 0.73%, 1 mg/ml) and FRAP reducing power (314.00 ± 1.51 μmol Fe^{2+}/g dry wt.). The HPLC analysis results confirmed the presence of four major flavonoids namely isoquercetin, rutin, quercitrin, and hyperoside. Their content ranges from 0.06 to 1.10 mg QE/g dry wt. of the sample plant. This study showed that both drying, and extraction methods had a substantial impact on the phytochemical contents and antioxidant activities of HCT, with FD and UAE being the best drying and extraction methods, respectively.

Keywords: Antioxidants, Flavonoids, Freeze-drying, *Houttuynia cordata* Thunb., Phenolics, Ultrasonic-assisted extraction

1. Introduction

In recent decades, the plant genus *Houttuynia* has piqued the interest of many researchers due to its therapeutic potential in the treatment of various ailments. *Houttuynia cordata* Thunb. (HCT) is an aromatic herbaceous perennial plant of the *Saururaceae* family that is native to China, Korea, Japan, and Southeast Asian countries [1]. In Thailand, HCT is found in the northern and north-eastern regions, and it is consumed as an edible salad as well as used in traditional medicine as an anticancer agent and immune stimulant [2]. HCT was also one of the herbs recommended for use in severe acute respiratory syndrome (SARS) prevention formulations by the Chinese Ministry of Health [3]. Furthermore, current pharmacological research has revealed that HCT has a wide spectrum of biological properties such as possessing antioxidant, anti-inflammatory, anti-viral, antibacterial, anti-cancer, and anti-diabetic activities [1,2,4]. Flavonoids are the main constituents of HCT that are thought to be responsible for the aforementioned pharmacological properties [3,5]. Flavonoids are a class of phenolic secondary metabolites ubiquitously present in plants, and most of their health benefits are related to their antioxidant and chelating properties [6]. The major flavonoids found in HCT are quercetin derivatives. Quercetin is a powerful antioxidant molecule that has a hydroxyl group in C3, a double bond between C2 and C3, a carbonyl group in C4, and a polyhydroxylated A and B aromatic ring [7]. Thus, the presence of this highly potent antioxidant flavonol, both

in glycoside and aglycone forms as its key constituents, makes HCT a uniquely promising source of bioactive compounds that can be incorporated into various food and pharmaceutical products [8,9]. Previous research has shown that the aerial parts (flowers > leaves > stems) contain higher flavonoids than the roots [10]. However, their isolation is influenced by a variety of factors such as drying methods, extraction solvent type, and extraction techniques employed [11]. Fresh or dried samples can be used to extract active plant constituents; nevertheless, due to the high moisture content of fresh plant materials, most phytochemicals deteriorate quickly after harvesting through enzymatic or microbiological reactions [12] and need some preservation methods. Drying is a simple and most widely used post-harvest technique for deactivating these degrading enzymes such as polyphenol oxidase, peroxidase, and tyrosinase, as well as for microbial decontamination and long-term preservation. Drying methods such as sun drying, oven drying, microwave drying, freeze-drying and shade drying have been adapted for medicinal plant drying. Nonetheless, previous research has shown that, depending on the properties of the plant material and the phytochemical substances present, drying pretreatment can either negatively or positively affect the chemical and biological activities of plant samples [13]. Therefore, to improve the medicinal potential of HCT, it is critical to evaluate the appropriate drying method that preserves or enhances the content of phytochemical components and their antioxidant activity. The extraction technique and solvent type used for extraction are other crucial parameters that affect the phytochemical content of herbal products. Flavonoids of HCT are moderately polar compounds that can be better extracted using polar solvents such as ethanol, methanol, chloroform, ethyl acetate or their mixture with water [14]. Nevertheless, ethanol or its mixture with water is the most preferred solvent for pharmaceutical or food applications, since it is non-toxic and environmentally friendly. Up to now, extraction methods such as maceration, soxhlet, microwave-assisted extractions, and ultrasound-assisted extraction are the most commonly used to extract flavonoids from plants [15]. Among these, ultrasound-assisted extraction has been effectively and extensively used due to its higher extract yield, time, and energy efficiencies, the fact that it is the most convenient method suitable for thermolabile compounds, and that it is environmentally benign [16]. Thus, the purpose of this study was to evaluate the impact of drying pretreatment and extraction methods on the antioxidant activity, total phenolic content, total flavonoid content, and individual flavonoid components of HCT.

2. Materials and methods

2.1 Plant material preparation prior to extraction

The aerial parts of HCT (fresh stem and leaves) were provided by a local producer (Khon Kaen province, Thailand). The samples were authenticated by Dr. Pornchai Kladwong, Department of Biology, Faculty of Science, Khon Kaen University, and a voucher specimen was kept at KKU herbarium (KKU No. 25656). The sample materials were thoroughly washed with deionized (DI) water and cut to sizes ranging from 10 to 50 mm and dried as follows. For oven drying (OD)**,** the leaf and stem of the HCT sample were dried separately in a ventilated air oven at 60°C for 12 h. For freeze-drying (FD), the leaf and stem of the HCT sample were placed separately in a refrigerator and frozen at -60°C overnight and then freeze-dried at -60°C under vacuum pressure of 0.05 mbar for 48 h. The FD and OD samples were then pulverized into a fine powder (30 mesh, 0.5 mm) using a blender and stored at 4°C until use.

2.2 Extraction of phenolics and flavonoids

Conventional extraction methods (aqueous and ethanolic extractions) were carried out following with some modifications [17]. For the aqueous extraction (AE), 5 g of the FD and OD powdered samples were extracted with 150 mL of DI water in a water bath shaker at 80°C for 30 min. For the ethanolic extraction (EE), 5 g of the FD and OD powdered HCT samples were extracted with 150 mL of 70% ethanol for 24 h in a water bath shaker at 35°C. The ultrasound-assisted extraction (UAE) was performed according to [18] with some modifications. Five grams of the FD and OD powdered samples were extracted with 200 mL of 60% ethanol in an ultrasonic bath at 70℃ for 30 min. All the extracts were then cooled and centrifuged for 10 min at 5000 rpm. The supernatants were collected and filtered through a Whatman paper no. 1 filter. The filtrate of EE and UAE were concentrated using a rotary evaporator at 40°C. Then all the filtrates of AE, EE, and UAE were lyophilized at -60°C for 48 h and then placed in glass bottles and kept at 4°C.

2.3 Determination of the extraction yield

The solvent effectiveness in extracting the phytochemical compounds of HCT was assessed by calculating its extraction yield as follows:

$$\text{Extraction yield (\%)} = \frac{\text{weight of sample extract}}{\text{weight of sample plant}} \text{ x } 100 \quad (1)$$

2.4 Determination of total phenolic contents (TPC)

The TPC of FD and OD powdered samples was determined using the Folin-Ciocalteu method as described by [19]. Briefly, 20 µL of the aliquot extracts (5 mg per ml in methanol) were mixed with 1.58 mL of DI water and 100 µL of FCR (10%, v/v). After 5 min, 300 µL of 20% (w/v) Na_2CO_3 solution was added to the mixture, then thoroughly mixed with vortex mixer and incubated for 2 h in the dark at 25°C. The absorbance was recorded with λ_{max} at 765 nm using a spectrophotometer. Gallic acid was used as a standard for the calibration curve at a concentration range of 50 to 500 mg/L and DI water was served as a blank. The results were expressed as mg gallic acid equivalent per gram (GAE/g) dry wt. Each analysis was done in triplicates.

2.5 Determination of total flavonoid content (TFC)

The TFC of FD and OD powdered samples were determined using the Al-colorimetric method according to [20]. In brief, 0.5 mL of each aliquot extract (5 mg per mL of methanol) was mixed with 2 mL of DI water and 0.15 mL of 5% $NaNO_2$ solution. Five minutes later, 0.15 mL of 10% $AlCl_3$ solution was added. After 6 min, 2 mL of 4% (w/v) NaOH solution was added, and the mixture was made up to 5 ml with DI water. The mixture was then vortexed and incubated for 30 min in the dark at 25°C before recording its absorbance in a spectrophotometer at 510 nm against DI water as a blank. Its calibration curve was constructed using quercetin as standard, and the results were expressed in mg quercetin equivalent per gram (QE/g) dry wt. Each analysis was done in triplicates.

2.6 Antioxidant DPPH assay

The DPPH free radical scavenging capacity of FD and OD powdered samples was assessed using the method described in with minor modifications [21]. A freshly prepared 1.8 mL of 0.1 mM DPPH solution was added to 200 µL (1 mg/mL concentration) of each extract solution, which was then mixed with vortex mixer and incubated in the dark at 25°C for 30 min. The absorbance was spectrophotometrically measured with λ_{max} at 517 nm, with methanol serving as a blank. Experiments were carried out in triplicates. The percentage inhibition (I, %) of the extract samples was calculated as follows:

$$\%I = [(A_{control} - A_{sample})/A_{control}] * 100 \quad (2)$$

where $A_{control}$ = absorbance of control and A_{sample} = absorbance of sample

2.7 Ferric reducing antioxidant power (FRAP) assay

The FRAP activity for FD and OD samples was performed following [22]. Every day, a new working solution was prepared and warmed in the oven at 37°C prior to use. Three hundred microliters of extract solution were added to 2.7 mL of FRAP reagent and thoroughly mixed. The content was then incubated for 30 min in the dark at 37°C, and the absorbance was spectrophotometrically measured with λ_{max} at 593 nm. Iron (II) sulphate solution was used as a standard. All the tests were performed in triplicates.

2.8 HPLC analysis of major flavonoids of HCT

The high performance liquid chromatography (HPLC) analysis was carried out according to [23], and the mobile phase used for gradient elution was composed of acetonitrile (solvent A) and 0.2% phosphoric acid in DI water at pH 2.6 (solvent B). The flow rate was 1 mg/ml, and the column temperature was 30°C. The detection wavelength was 360 nm, and the volume of the injection loop was 20 µL. Analytes were identified by comparing their retention times and ultraviolet-visible (UV-Vis) spectra to those of authentic external standards. Linear calibration curves of four external standards (isoquercetin, rutin, hyperoside, and quercitrin) were constructed, and the flavonoid content of the sample was calculated using a corresponding standard curve of the known flavonoids. All the tests were performed in triplicates.

2.9 Statistical analysis

Each experiment was carried out in triplicates, and the results were reported as mean (x) ± standard deviation (SD). A two-way analysis of variance (ANOVA) with Duncan's multiple range test was used to statistically determine a significant difference (*p*-value) between the means of the samples within a 95% confidence interval. A Pearson test was also used to examine the relationship between DPPH, FRAP antioxidant activity and TPC and TFC. All analyses were performed using a statistical package for the social science (SPSS) 28 (SPSS Inc., Chicago, USA).

3. Results and discussion

3.1 Effect of drying methods on moisture content (MC), drying time, and color

The results showed that the two drying pretreatment methods tested lowered the moisture content to less than 15%, which is the acceptable level of moisture content for post-harvest management of medicinal plants [24]. As shown in Table 1, the MC, drying time, and color of the two drying pretreatment methods vary significantly, with OD showing superiority over FD in terms of moisture content and drying time reduction. FD, on the other hand, proved to be more effective than OD at preserving the sample's original color as displayed in Figure 1. The vast difference in drying time can be attributed to the unique drying principle that underpins each technique.

Table 1 Drying time and moisture content of HCT for the two different drying methods.

Drying methods	Total drying time (h)	Final moisture contents (%), db
FD	48	9.81 ± 0.02^{a}
OD	12	8.06 ± 0.15^{b}

$^{a, b}$ represents a significant difference ($p < 0.05$) in % MC between the two drying methods. db. represents dry weight basis.

Figure 1 Appearance of HCT sample before and after drying. (A: fresh, B: freeze-drying, C: oven-drying).

3.2 Effect of drying pretreatment and extraction methods on extraction yield

The extraction yield was determined to indicate the solvent's effectiveness in extracting phenolic compounds, especially flavonoids from the HCT. As shown in Table 2, the highest extraction yield was obtained using UAE, while the lowest extraction yield was obtained using AE, regardless of the drying pretreatment used. The highest extraction yield found in the UAE can be attributed to the combined effect of ultrasonication and thermal treatment at elevated temperatures [25].

Table 2 The extraction yield, total phenolic content, total flavonoid content, and antioxidant activities of HCT.

Extraction methods	Drying methods	Extraction yield (%)	TPC (mg GAE/g dry wt.)	TFC (mg QE/g dry wt.)	Antioxidant activities	
					DPPH (% I)	FRAP (μmol Fe^{2+}/g dry wt.)
AE	FR	24.90 ± 1.73^{d}	9.18 ± 0.07^{b}	24.05 ± 0.34^{b}	30.33 ± 0.30^{c}	107.70 ± 2.08^{c}
	FD	19.40 ± 1.10^{b}	6.50 ± 0.55^{a}	16.23 ± 0.40^{a}	23.24 ± 0.65^{b}	58.80 ± 1.67^{b}
	OD	16.00 ± 0.40^{a}	5.30 ± 0.30^{a}	15.30 ± 0.62^{a}	16.80 ± 0.79^{a}	41.67 ± 1.53^{a}
EE	FR	26.43 ± 1.15^{e}	21.86 ± 0.38^{e}	74.20 ± 0.70^{g}	65.73 ± 0.64^{g}	358.10 ± 2.80^{h}
	FD	20.20 ± 1.70^{b}	17.00 ± 0.60^{d}	58.80 ± 1.06^{e}	60.89 ± 0.43^{f}	290.00 ± 1.00^{f}
	OD	20.67 ± 0.31^{b}	15.53 ± 0.73^{c}	45.00 ± 0.98^{c}	41.30 ± 0.12^{d}	266.33 ± 1.52^{d}
UAE	FR	29.10 ± 1.73^{f}	31.10 ± 0.11^{g}	87.90 ± 0.56^{h}	75.00 ± 0.40^{i}	384.20 ± 0.40^{i}
	FD	24.39 ± 0.02^{d}	29.40 ± 0.40^{f}	72.80 ± 0.95^{f}	69.94 ± 0.73^{h}	314.00 ± 1.51^{g}
	OD	22.80 ± 0.40^{c}	21.47 ± 0.50^{e}	47.33 ± 0.40^{d}	53.67 ± 0.72^{e}	276.00 ± 2.00^{e}

GAE = Gallic acid equivalent, QE = quercetin equivalent, I = inhibition Data expressed as mean ± SD (n = 3).
$^{a\,b\,c\,d\,e\,f\,g\,h\,i}$ each different small letters on the same column indicates a statistically significant difference $p < 0.05$, n = 3).

3.3 Effect of drying pretreatment and extraction methods on TPC and TFC

The TPC and TFC were determined spectroscopically to evaluate the effect of drying and extraction methods on the phytochemical contents of HCT. The results showed that both the drying pretreatment and the extraction methods employed had a substantial impact on the TPC and TFC of the HCT extracts. Regardless of the drying pretreatment, UAE showed the highest TPC and TFC values among the three extraction methods used, while AE showed the lowest TPC and TFC values as shown in Table 2. The UAE's high TPC and TFC gains could be

attributed to its high extraction efficiency via a physical mechanism, the so-called Sonothermal effect, in which the high intensity of ultrasonic waves and the heat disrupt and disintegrate the plant's tissue, allowing the solvent to access and extract the bioactive compounds effectively [26]. While the lower TFC values obtained in AE can be explained by the fact that water has a high polarity, making it an ineffective solvent for extracting phenolic compounds, particularly flavonoids, which are moderately polar to non-polar molecules [27]. Another explanation could be the deterioration of heat-sensitive bioactive compounds due to thermal degradation [28]. Prommajak et al. [18] reported a TPC of 36.40 ± 0.48 mg gallic acid g^{-1}dry wt., which is slightly higher than that of our study, while Tuyen et al. [29] reported a TFC of 71.11 ±6.76 mg rutin g^{-1}/dw which is within the range of our findings. Nevertheless, the TPC and TFC values may vary based on the different plant parts, the type of solvent, the extraction technique used, and the different units used to quantify the result. In terms of drying pretreatment, sample extracts of both drying pretreatments showed a significant loss in TPC and TFC compared to FR sample extracts, regardless of the extraction method used. The loss of TPC for AE, EE, and UAE extract samples were 29.20%, 42.20%; 22.30%, 29.20%; and 5.50%, 30.90% for FD and OD, respectively. While the loss of TFC for AE, EE, and UAE was 33.33%, 43.70%; 20.70%, 39.40%; and 17.20%, 46.20% for FD and OD, respectively. This loss can be attributed to processing conditions, most notably temperature and time. Previous research has shown that heat pretreatment can affect phytochemicals by impacting the permeability of cell structure, resulting in component migration and losses due to leakage or disintegration by a variety of chemical reactions involving enzymes, light, and oxygen [30]. Despite having higher TPC and TFC values than dried samples, fresh samples are more prone to enzymatic and microbiological contamination, making them unsuitable for large-scale industries that require adequate periodic storage to assure a stable supply of raw materials [31]. As a result, FD samples with lower chemical constituent loss are preferred for meeting the quality and quantity requirements of pharmaceutical industries by ensuring a consistent supply of raw materials. Furthermore, the drying process lowers the cost of production by reducing the weight and volume of the plant, lowering transportation, and storage costs. The results of this study support previous research findings that identified UAE as a viable extraction method [16,26] and FD as a suitable drying pretreatment method for preserving phenolic compounds [32,33].

3.4 Effect of drying pretreatment and extraction methods on antioxidant activity

3.4.1 DPPH Radical scavenging activity

The antioxidant compounds present in the aerial part of HCT scavenged free radicals by hydrogen donation resulted in the reduction of DPPH radical to DPPH-H. The DPPH radical scavenging activity was assessed as a percentage inhibition (% I) of the extracts at a concentration of 1 mg/ml. The results indicated that UAE exhibited the highest DPPH radical scavenging activity, whereas AE showed the lowest DPPH radical scavenging activity, irrespective of the drying pretreatment utilized. The DPPH scavenging activity ranges from 16.80 ± 0.79% to 75.00 ± 0.40% as shown in Table 2. The higher DPPH radical scavenging activity in the UAE can be attributed to higher overall phenolic and flavonoid content. Camder et al. [17] reported a DPPH radical scavenging effect of 75.30 ± 2.16% in methanolic leaf extract of HCT, which is similar to the results of our study. However, the DPPH radical scavenging activity of HCT varies based on the plant part, solvent type, and extraction technique used. The DPPH scavenging capacity for drying pretreated sample extracts was lower compared to the FR sample in all extraction methods used. The loss of DPPH scavenging activity for AE, EE, and UAE extractions was 23.40% and 44.50%; 7.30% and 37.10%; and 6.80% and 28.50% for FD and OD plant samples, respectively. Nevertheless, comparing the two-drying pretreatments, the FD pretreatment showed a lower loss in DPPH scavenging activity than the OD pretreatment. The relationship between DPPH radical scavenging activity and phytochemicals (TPC and TFC) was analyzed using the Pearson correlation. Pearson's r data analysis revealed a strong positive correlation between TPC, TFC, and DPPH radical scavenging activity with $r = 0.952$, $p = 0.01$ and $r = 0.980$, $p = 0.01$, respectively. This implies that the strong radical scavenging activity is strongly related to the presence of higher flavonoids and phenolic contents in HCT. This study demonstrated that UAE is a powerful extraction method for successfully extracting antioxidants present in aerial parts of HCT, which is consistent with prior research on other medicinal plants [16].

3.4.2 FRAP antioxidant activity

The reducing power of the aerial parts of HCT was determined using ferric reducing power assay (FRAP) which measures the reduction of ferric ion- 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) complex to ferrous ions-TPTZ complex under a low pH and temperature of about 37°C. The results revealed that UAE demonstrated the highest FRAP reducing capacity, followed by EE, whereas AE exhibited the lowest FRAP reducing capacity, irrespective of the drying pretreatment method used. The FRAP reducing power ranges from 41.67 ± 1.53 to 384.20 ± 0.40 µmol Fe^{2+}/g dry wt. as displayed in Table 2. Previous research indicated that the aerial parts of HCT contained significant levels of phytochemicals, primarily flavonoids, which had a stronger FRAP-reducing effect [10,17].

An earlier work by Park et al. [34] reported a FRAP antioxidant activity of 360.10 mg TE/g dry wt. in ethyl acetate extracts of HCT, which is comparable to the result obtained by UAE in our study. Compared to the FR sample, the drying pretreated sample showed a significant loss in FRAP reducing power. The loss of FRAP reducing power for UAE was 45.40% and 61.30% for AE, 19.00% and 25.30% for EE, and 18.20% and 28.00% for the FD and OD plant samples, respectively. This indicates that a higher loss of FRAP reducing activity was observed in samples obtained from OD pretreatment despite the extraction method used. Pearson correlation was also used to examine the link between FRAP antioxidant activity and phytochemicals (TPC and TFC), and it demonstrated a substantial positive association, with r = 0.92, $p = 0.01$ for TPC and r = 0.968, $p = 0.01$ for TFC. This suggests that flavonoids and phenolic compounds from the aerial parts of HCT play a major role in FRAP-reducing activity.

3.5 HPLC analysis of major flavonoids of HCT

A gradient RP-HPLC method was used to identify and quantify the major flavonoids of the aerial parts of HCT. The result of HPLC analysis revealed the presence of four major flavonoids namely rutin, hyperoside, isoquercetin, and quercitrin as displayed in Figure 2. Their content ranges from 0.06 ± 0.037 to 1.1 ± 0.022 mg/g dry wt. of plant sample as shown in Table 3. The highest rutin content was obtained in FD samples extracted either by EE or UAE. The highest contents of hyperoside and isoquercetin were found in FR. samples from EE, followed by FD samples from UAE. The highest contents of quercitrin were found in FR or OD samples from the UAE. Notably, no quantifiable amounts of quercetin, the aglycone form of these flavonol glycosides, were identified in any of the samples. The order of flavonoid contents obtained was as follows: quercitrin > hyperoside > rutin > isoquercetin. The individual flavonoid content values in this study's findings are within the range of those reported in earlier studies [9,35], and quercitrin and hyperoside were likewise identified as two of the major constituents present in high concentrations. This research reveals a high variation in individual flavonoid components with the type of drying pretreatment and extraction methods applied as shown in Table 3. Rutin, isoquercetin, and hyperoside are best preserved with FD pretreatment implying their high heat sensitivity, while quercitrin is best preserved with OD pretreatment implying its relatively better heat stability. The drying pretreatment has shown a pronounced effect, especially on hyperoside since it showed a higher decrease in its content than others. On the contrary, the quercitrin content in OD samples showed slightly higher content than in the fresh sample. The total flavonoid content for the four major flavonoids was slightly higher in FD samples than in FR. and OD samples, indicating that FD pretreatment had a slightly positive effect on the individual flavonoid component. Generally, the findings of this study suggested that UAE was the best extraction method and FD was the best drying pretreatment method for recovering the individual flavonoids in HCT.

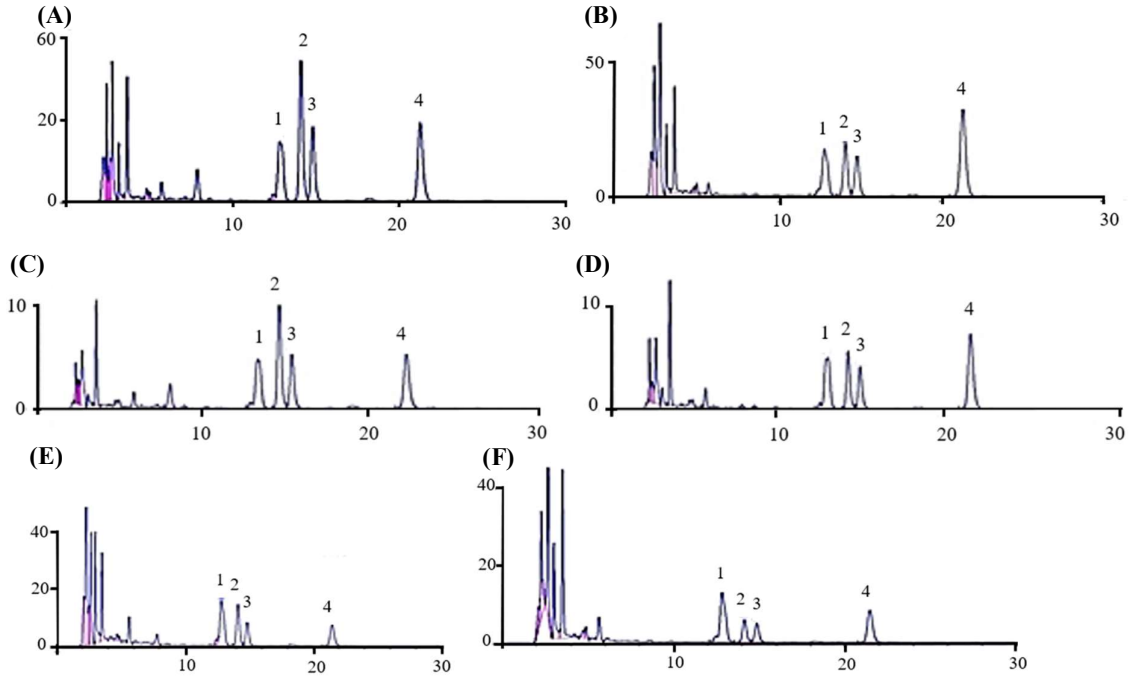

Figure 2 Chromatogram of the four major flavonoid glycosides identified from the aerial parts of HCT under different drying pre-treatment and extraction conditions at 360 nm: Aqueous extracts (A) Freeze-drying (FD) and (B) Oven-drying, Ethanolic extracts, (C) Freeze-drying (FD), (D) Oven-drying, (E) Freeze-drying (FD), and (F) Oven-drying. Peaks: 1, rutin; 2, hyperoside; 3, isoquercetin; 4, quercitrin; 5, quercetin.

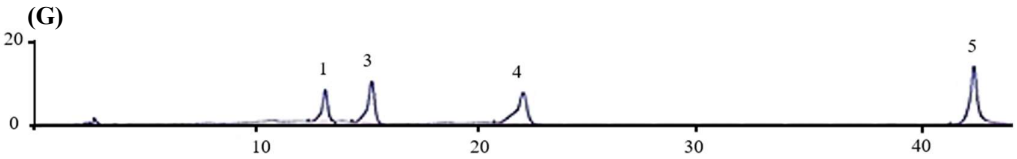

Figure 2 (continued) Chromatogram of the four major flavonoid glycosides identified from the aerial parts of HCT under different drying pre-treatment and extraction conditions at 360 nm: (G) standards. Peaks: 1, rutin; 2, hyperoside; 3, isoquercetin; 4, quercitrin; 5, quercetin.

Table 3 The contents of individual flavonoids of HCT for different drying pretreatment and extraction methods.

Extraction method	Drying Method	Flavonoid content (mg/g dry wt. of plant sample)			
		Rutin	Hyperoside	Isoquercetin	Quercitrin
AE	FR.	0.271 ± 0.008^{a}	0.270 ± 0.008^{b}	0.150 ± 0.004^{b}	0.550 ± 0.012^{b}
	FD	0.279 ± 0.045^{a}	0.110 ± 0.009^{a}	0.080 ± 0.025^{a}	0.120 ± 0.017^{a}
	OD	0.220 ± 0.067^{a}	0.080 ± 0.004^{a}	0.060 ± 0.037^{a}	0.130 ± 0.050^{a}
EE	FR.	0.640 ± 0.011^{b}	0.990 ± 0.001^{e}	0.570 ± 0.058^{e}	0.550 ± 0.015^{b}
	FD	0.940 ± 0.017^{d}	0.850 ± 0.033^{d}	0.540 ± 0.058^{e}	0.750 ± 0.075^{c}
	OD	0.800 ± 0.050^{c}	0.390 ± 0.062^{c}	0.310 ± 0.004^{c}	0.870 ± 0.012^{d}
UAE	FR.	0.580 ± 0.007^{b}	0.850 ± 0.009^{d}	0.460 ± 0.004^{d}	1.100 ± 0.006^{e}
	FD	0.830 ± 0.012^{c}	0.840 ± 0.013^{d}	0.540 ± 0.007^{e}	0.850 ± 0.014^{d}
	OD	0.760 ± 0.012^{c}	0.400 ± 0.008^{c}	0.360 ± 0.005^{c}	1.100 ± 0.022^{e}

Each value is expressed as mean ± SD (n = 3).
[a, b, c] All different small letters within a column for each compound are significantly different ($p < 0.05$).

4. Conclusion

This study has revealed the significance of drying treatments in preserving HCT phytochemicals, as well as the efficacy of various extraction methods in extracting these useful phytochemical compounds, particularly flavonoids. In general, both drying pretreatments and extraction methods have shown a significant impact on the TPC, TFC, and antioxidant capacities of HCT. According to this study, the best drying treatment for preserving phytoconstituents of HCT aerial part was FD pretreatment, and the most effective extraction method was UAE. This study also found that the individual flavonoid contents varied significantly depending on the drying pretreatment type used. Overall, this research found that the aerial part of HCT can be a promising source of antioxidant compounds that can be used as functional ingredients in pharmaceuticals, functional foods, dietary supplements, and cosmetic products if proper drying and extraction methods are used in post-harvest management practices and isolations. The result of this and other earlier studies indicated that the flavonoids present in the aerial part of HCT are found in glycoside form, which has less antioxidant activity than the aglycone form. Therefore, further research into the conversion of those flavonol glycosides to their aglycone quercetin via enzymatic biotransformation is recommended to enhance its antioxidant efficacy.

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