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Analytical Methods

Rapid screening and identification of antioxidants in aqueous extracts of *Houttuynia cordata* using LC–ESI–MS coupled with DPPH assay

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**ABSTRACT**

*Houttuynia cordata* Thunb., has been used traditionally as immune stimulant and anticancer agent. An aqueous extract of *H. cordata* tea showed high radical scavenging activity determined by off-line DPPH assays. Then, it was screened for its antioxidant components via an on-line DPPH radical scavenging technique coupled with a liquid chromatography–electrospray ionization mass spectrometer (LC–ESI–MS). Based on their mass spectra and fragmentation patterns; the antioxidant compounds were identified as quinic acid derivative, caffeic acid derivatives, procyanidin B, neo-chlorogenic acid, catechin, chlorogenic acid, crypto-chlorogenic acid and quercetin hexoside. LC–MS/MS in multiple reactions monitoring (MRM) mode was used to quantify these antioxidant compounds. Chlorogenic acid was found to be a major component in *H. cordata* tea.

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1. Introduction

*Houttuynia cordata* Thunb. (Saururaceae) is a pungent, heart-like leafed perennial herb native in Southeast Asia. It is called in Thai as *Kha-oo-tong* or *Plu-khao* which means fishy smelling vegetable. Despite its unpleasant smell, young leaves of *H. cordata* are popular vegetable in the Northern part of Thailand. It also has been used in traditional medicine for immune stimulation and as anti-cancer agent. *H. cordata* shows a variety of pharmacological activities such as anti-hypertension, and seems to have anti-edema, detoxicant, anti-inflammatory, anti-pyretic, anti-purulent and diuretic activities (Lu, Liang, & Wu, 2006; Probstle & Bauer, 1992). Additional activities of *H. cordata* against allergy, anaphylaxis, cancer and viral infection were reported (Chiang, Chang, Chen, Ng, & Lin, 2003; Kwon et al., 2003; Li et al., 2005). Hayashi, Kamiya, and Hayashi (1995) reported that it showed direct inhibitory activity against herpes simplex virus type I, influenza virus and human immunodeficiency virus type I (HIV-1) without showing cytotoxicity to the host. Moreover, Chiang et al. (2003) reported that *H. cordata* had selective activity against herpes simplex HSV-2. In the anti-viral aspect, *H. cordata* exhibited significant inhibitory activity on the severe acute respiratory syndrome (SARS) (Lau et al., 2008). *H. cordata* also acted as an antioxidant and antimutagenic agents (Chen, Liu, Chen, Chao, & Chang, 2003). In general, the chemical components of *H. cordata* comprise of six major types, namely; volatile oils, flavonoids, alkaloids, fatty acids, sterols and polyphenolic acids (Bansiddhi et al., 2003; Ch, Wen, & Cheng, 2007; Meng et al., 2007; Toda, 2005). In the beginning, most of the studies mainly focused on the chemistry of the essential oils which have been considered to be responsible for the claimed clinical efficacy (Hayashi et al., 1995). Recently, Meng et al. (2005) developed an HPLC–DAD–MS method for analysis of *H. cordata* and characterized its major active chemical constituents as chlorogenic acid, quercetin-3-O-β-D-galactopyranosyl-7-O-feruloyl-rutinoside, quercetin 3-O-α-L-rhamnopyranosyl-7-O-β-D-glucopyranoside, rutin, hyperin, isouqueretin, quercitrin, afzelin, quercetin, piperolactam A, and aristolactam B.

Antioxidants are usually found as complex mixtures in plant extracts. The on-line-HPLC coupled with the detection of antioxidative activity using post-column reaction of eluates with free radicals have been reported that they can be successfully applied for the identification and quantification of antioxidants in biological samples (Niederlander, van Beek, Bartasiti, & Koleva, 2008; Nuengchamnong & Ingkaninan, 2009). Beside these, a free radical spiking technique also showed good detection efficacy (Shui, Leong, & Wong, 2005). Our preliminary screening of four herbal teas indicated that *H. cordata* showed high antioxidant activity. However, so far there have been only few studies on the phenolic constituents responsible for the antioxidant activity of *H. cordata*. 

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The main objectives of this study were, therefore, to screen the antioxidants in *H. cordata* and to further identify and quantify those compounds using LC–MS coupled with DPPH assay.

### 2. Materials and methods

#### 2.1. Materials and reagents

The following herbal teas were examined: (1) *Orthosiphon grandidiflorus* Bolding (Lamiaceae), (2) *Houttuynia cordata* Thunb. (Sauraceae), (3) *Morus alba* L. (Moraceae), and (4) *Vernonia cinerea* L. (Asteraceae). The samples were obtained from Bangkratum hospital, Phitsanulok, Thailand.

The standard compounds, catechin, kaempferol were purchased from Wako (Pure Chemical Industries Ltd., Japan), Chlorogenic acid, quercetin dihydrate, ascorbic acid (vitamin C), quinic acid, from JT Baker (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA). For the analysis using on-line LC–ESI–MS coupled with DPPH assay system.

#### 2.2. Sample preparation

For the off-line antioxidant activity determination, each sample (dry leaves) 250 mg was extracted with 2 × 10 ml 70% (v/v) methanol by sonication for 15 min then filtered through Whatman No. 1 filter paper (Kent, England). The filtrate was adjusted to a volume of 25 ml. The extracts were directly tested for antioxidant activity using DPPH assay.

For the analysis on-line LC–E SI–MS coupled with DPPH assay. *H. cordata* dry leaves (2 g, 1 sachet) were infused in boiling water (100 ml) for 10 min, filtered through Whatman No. 1. The filtrate volume was readjusted to 100 ml and further filtered through a 0.2 μm Nylon syringe filter (Chrom Tech, Inc., MN, USA) prior to injection to the LC–MS coupled with DPPH assay system.

#### 2.3. Off-line antioxidant activity determination

The potential antioxidant activity of a plant extract was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Different concentrations (10–10,000 μg/ml in 70% (v/v) methanol) of test samples were prepared. The reaction mixtures consisting of 75 μl of test samples and 150 μl of 0.2 mM DPPH in methanol were mixed in 96 well plates and incubated for 30 min. The absorbance (OD) was measured at 515 nm. The antioxidant activity was obtained from the following equation: antioxidant activity (%) = [(OD control–OD sample)/(OD control)] × 100. Percent antioxidant activity was plotted against log concentration (μg/ml).

The antioxidant activity of plant extracts was expressed as EC50, which was defined as the concentration in μg/ml. The measurement was performed in triplicate. Trolox, ascorbic acid and BHA were used as positive controls.

#### 2.4. LC–ESI–MS coupled with DPPH assay

The HPLC was coupled on-line to MS (line A) and a continuous flow DPPH assay (line B) as described in the previous study (Nuengthammong & Ingkaninan, 2009). The set up system was shown in Fig. 1. Line A; Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA) is coupled to a PE SCIEX API 4000 triple quadrupole tandem mass spectrometer (Applied Biosystem, Foster city, CA) equipped with electrospray ionization interface. The chromatographic separation was achieved by a phenomenon Gemini column (5 μm, 250 × 4.6 mm i.d.) (Phenomenex, Torrance, CA) protected with an ODS C18 guard column, operated at 25 °C. The mobile phase consisted of solvent A (1 ml formic acid in 1 l of deionized water) and solvent B (methanol). The elution program started from 90:10 solvent A: solvent B for 4 min, then changed to 80:20 solvent A: solvent B in 6 min, and linearly increased to 10:90 solvent A: solvent B in 30 min and the ratio of solvent A: solvent B was constant at 10:90 for 5 min then changed to 90:10 solvent A: solvent B in 5 min and was kept constant at 90:10 solvent A: solvent B for 5 min for reconditioning of the column. Mass spectra were recorded within 55 min. The injection volume was 5 μl. The flow rate was set at 600 μl/min. The Analyst 1.3.2 software was used for data acquisition and processing. The full scan mass spectra from m/z 100–1000 amu were acquired both in positive and negative ion modes. The optimum conditions of the interface were as follows: ESI-positive; ion spray voltage of 4500 V, curtain gas of 69 Kpa (10 psi), ion source gas 1 of 450 Kpa (65 psi), ion source gas 2 of 380 Kpa (55 psi). The interface temperature was set at 400 °C. The entrance and declustering potential were 10 and 80 V, respectively. ESI-negative; the condition was similar to ESI positive except the voltage was set in negative mode. Line B represents the continuous flow system for antioxidant activity detection. It consisted of an HPLC pump, LC20AD prominence (Shimadzu, Kyoto, Japan), home-made knitted reaction coil PEEK tubing with an inner diameter of 180 μm and a total reaction coil volume of 100 μl. The flow of 0.1 mM DPPH was set to 200 μl/min and the reduction was detected as a negative peak at 515 nm using the UV–VIS detector (SPD 20AV, Shimadzu, Kyoto, Japan). The residence time in the coil is 0.45 min. The LC solution software was used for data acquisition and processing. The polarity of the signal output was reversed in order to obtain positive signals. The system was operated at 25 °C. Line A coupled to line B with a 24 cm length of 0.17 mm i.d. PEEK tubing by a Y-connector. The eluent flow was split in a ratio of 8:2 between the MS ion source and DPPH line. For the characterization of antioxidant peaks, the fragment ions from their corresponding parent ions in negative mode were induced with collision gas (CAD) of 41 Kpa at 515 nm using the UV–VIS detector (SPD 20AV, Shimadzu, Kyoto, Japan). The LC–ESI–MS coupled with DPPH assay system.

![Image](image-url)
2.5. Peak identification

Peak identification was performed by comparison of the retention time, mass spectra and fragmentation patterns with reference compounds and published data.

2.6. Quantitative analysis

For quantitative analysis of the antioxidants in H. cordata herbal tea, the same column was used and the gradient system was changed to shorten analysis time. The system was employed from solvent A:B (90:10) to (10:90) in 10 min, followed by isocratic elution with solvent A:B (10:90) for 5 min and linear gradient elution from solvent A:B (10:90) to (90:10) for 2 min and equilibration with solvent A:B (90:10) in 3 min before injecting a new sample. The flow rate was set at 600 μl/min. The injection volume was 5 μl. The MS parameters were operated in negative multiple reactions monitoring (MRM) scan mode. Optimal operating parameter of ESI–MS with maximum signal intensity of molecular ions and fragment ions were obtained by direct infusion of the standard solution at concentration 10 μg/ml in methanol, using a Harvard syringe pump (Syringe Pump 11 plus, Harvard apparatus Inc., Holliston, USA) at a flow rate of 5 μl/ml. The optimum conditions of the interface were as follows: ion spray voltage of –4500 V, curtain gas of 138 Kpa (20 psi), ion source gas 1 of 380 Kpa (55 psi), ion source gas 2 of 310 Kpa (45 psi). The interface temperature was set at 400 °C. The entrance potential was –10 V. The dwell time per transition was set at 100 ms. The individual optimized parameters for detection of each compound are as follows. For chlorogenic acid, the transition at m/z 353.2 → 190.8 amu with DP, CE and CXP of –120, –22 and –30 V were set. Catechin was detected at two transitions. At m/z 288.9 → 245.0, DP, CE and CXP were set at –150, –22 and –12 V, respectively. At m/z 288.9 → 125.1 amu, DP, CE and CXP were set at –150, –30 and –20 V. For quercetin, the two transitions were set at m/z 300.9 → 151.0 amu and m/z 300.9 → 178.9 amu. The DP and CE for both transitions were set at –150 and –30 V. The CXP was set at –22 for m/z 300.9 → 151.0 and at –26 V for m/z 300.9 → 178.9 detection. Chlorogenic acid, catechin and quercetin in various concentrations were used for preparation of calibration curves. The curves were generated by linear regression based on the peak area. Analyses were performed in triplicate. Other standards were not available; hence neo-chlorogenic acid and crypto-chlorogenic acid were quantified as chlorogenic acid equivalents and also procyanidin B and quercetin hexoside were quantified as catechin and quercetin equivalents, respectively. The limit of detection (LOD) was determined as the analyte signal having a peak area equal to three times of that of noise (S/N = 3) using Analyst software.

3. Results and discussions

3.1. Off-line antioxidant activity determination

The antioxidant activities of four herbal teas were measured using DPPH assay on 96 welled microplate. This assay is based on a measurement of the scavenging ability of antioxidants towards a stable radical, DPPH. DPPH, which shows absorption at 515 nm, is reduced to the corresponding hydrazine when it reacts with hydrogen donors and this can be detected as a decrease of absorption (Brand-Williams, Cuvelier, & Berset, 1995). The antioxidant activities of the methanolic extracts of O. grandiflorus, H. cordata, M. alba and V. cinerea were expressed as EC50 values of 217.2 ± 12.6, 341.5 ± 17.2, 874.9 ± 83.4 and 886.9 ± 53.1 μg/ml, respectively. The positive controls BHA, ascorbic acid and Trolox gave EC50 values of 4.5 ± 2.1, 6.7 ± 0.2 and 7.9 ± 1.3. The results showed that the methanolic extracts of O. grandiflorus and H. cordata had the highest activity among all the teas tested.

3.2. LC–ESI–MS coupled with DPPH assay for the rapid identification of antioxidants

An aqueous extract of H. cordata was subjected to the on-line LC–ESI–MS coupled with DPPH assay. The DPPH based antioxidant activity profile (Fig. 2a) exhibited that at least ten compounds showed antioxidant activity peak. The deprotonated molecular ions peaks of standard quinic acid and kaempferol were found at 5.7 and 39.3 min, respectively. The delay time between the read out from the MS detector and the corresponding peak from the antioxidant activity was then calculated as 0.6 min. Negative ionization gave a better result than positive ionization (Fig. 2b). The negative ions of the major active compounds are listed in Table 1 and the identification of these compounds is proposed. The aqueous extract was used in this study as it will provide the same form of antioxidants as that present in the tea.

Peak 1 (tR = 5.8 min) was a standard quinic acid that mixed in the sample to a concentration of 100 μg/ml as a delay time marker for the two detectors. Peak 2 has a mass spectra and fragmentation pattern nearly identical to standard quinic acid but difference in retention time (tR 9.3 min). So, peak 2 was tentatively identified as quinic acid derivative.

Peaks 3–5 (tR = 12.3, 14.7 and 17.9 min) with m/z 371.2 and fragmentation at m/z 197.3 [caffeic acid-H+18], m/z 191.0 [quinic acid–H], m/z 179.0 [caffeic acid –H] and m/z 173.3 [quinic acid–H+H2O]. These m/z are characteristic of caffeic acid. Therefore, these three compounds were identified as caffeic acid derivatives.

Peak 6 (tR = 20.3 min) exhibited [M–H]– base ion at m/z 577.3. It gave secondary fragment at m/z 289.0 [M–288.3] due to the cleavage of the inter-flavanoid C–C linkages with losses of 288 amu and m/z of 425 [M–152] evolved from retro Diels–Alder fragmentation of the heterocyclic ring. The m/z of 407 [M–152–18]– resulted from water elimination of m/z 425. When comparing with standard procyanidin B2 (tR 23.13 min), the fragmentation pattern of this compound is nearly identical but the retention time was different. This compound might be one of procyanidin B group, but stereochemistry of this compound cannot be indicated by this MS technique.

Peak 8 (tR = 23.04 min) with m/z 289.0 [M–H]– and fragmentation at m/z 245.0 and 125.1 (cleavage of the C ring) was identified as catechin by the comparison of the authentic standard.

Peak 9 (tR = 24.8 min) with m/z 353.6 and fragmentation at m/z 191.0 [quinic acid–H], m/z 179.0 [caffeic acid –H] and m/z 173.3 [quinic acid–H2O]–. By comparing its mass spectra and tR with standard compound, this compound was assigned to be chlorogenic acid (5-O-caffeoyl quinic acid). Compounds 7 and 10 were most likely naturally occurring isomers of chlorogenic acid. According to their elution order, neo-chlorogenic acid (3-O-cafeoyl quinic acid) was eluted prior to crypto-chlorogenic acid (4-O-cafeoyl quinic acid) (Carini, Facino, Aldini, Calloni, & Colombo, 1998). Therefore, peaks 7 and 10 were assigned as neo-chlorogenic acid and crypto-chlorogenic acid, respectively (Fig. 3a).

Peak 11 (tR = 32.5 min) with a molecular ion at m/z 463.3 and fragment ions at m/z 300.0 and m/z 301.0 [M–162]– (loss of a hexose unit) was tentatively identified as quercetin hexoside. Standard quercetin was eluted at 37.2 min (Fig. 3c).

Peak 12 (tR = 39.3 min) with m/z 283 was kaempferol standard at concentration 100 μg/ml was used as delay time marker for the two detectors.

The general structures of the antioxidant compounds identified in H. cordata are shown in Fig. 4.
3.3. Quantitative analysis of antioxidants using LC–MS/MS

Antioxidant compounds in *H. cordata* can be quantitatively analyzed using LC–MS/MS in MRM mode. In order to shorten the run time, the elution profile was changed and the analysis was finished in 20 min. In our studies, chlorogenic acid, catechin and quercetin at various concentrations were used for the preparation of calibration curves. The regression equation of chlorogenic acid at concentrations of 5–100 µg/ml was $y = 4.37e + 0.05x - 2.3e + 0.06$; with $r$ value of 0.9985. The regression equation of catechin at concentrations of 1–100 µg/ml was $y = 2.36e + 0.04x + 7.86e + 0.03$; with $r$ value of 0.9991. The regression equation of quercetin at concentrations of 1–100 µg/ml was $y = 9.26e + 0.04x + 6.6e + 0.04$; with $r$ value of 0.9977. The limits of detection (LOD) with signal

Table 1
Identification of antioxidant compounds in water extracts of *H. cordata* by using their LC–ESI–MS–DPPH assay; data in negative ionization.

| Peak no. | $t_R$ (min) | ESI–MS (m/z) | Identification |
|----------|-------------|--------------|----------------|
|          |             | MS | MS/MS          |                |
| 1        | 5.8         | 190.9 | 127.3         | Quinic acid*   |
| 2        | 9.3         | 190.9 | 127.3, 111.1  | Quinic acid derivative |
| 3        | 12.3        | 371.2 | 197.3, 191.0, 179.0, 173.3 | Caffeic acid derivative |
| 4        | 14.7        | 371.2 | 197.3, 191.0, 179.0, 173.3 | Caffeic acid derivative |
| 5        | 17.9        | 371.2 | 197.3, 191.0, 179.0, 173.3 | Caffeic acid derivative |
| 6        | 20.3        | 577.3 | 425.0, 407.2, 289.0 | Procyanidin B** |
| 7        | 22.2        | 353.6 | 191.0, 179.0, 173.3 | Neo-chlorogenic acid |
| 8        | 22.7        | 289.2 | 245.0, 125.1  | Catechin**     |
| 9        | 24.8        | 353.6 | 191.0, 179.0, 173.3 | Chlorogenic acid** |
| 10       | 26.5        | 353.6 | 191.0, 179.0, 173.3 | Crypto-chlorogenic acid |
| 11       | 32.5        | 463.3 | 301.3, 300.0  | Quercetin hexoside |
| 12       | 39.8        | 285.0 | –             | Kaempferol*    |

* Standard compound added.
** Compared with standard compound.
to noise ratio of three were determined. LOD of chlorogenic acid, catechin and quercetin was 2.5, 2.5 and 5.0 ng per injection, respectively. Neo-chlorogenic acid and crypto-chlorogenic acid were quantified by using a linear regression line of chlorogenic acid. Procyanidin B and quercetin hexoside were also quantified as catechin and quercetin. The extracts mass in MRM quantification

Fig. 3. Chromatograms of (a) chlorogenic acid (upper pane) and H. cordata extract (lower pane) obtained from the extract mass in MRM mode at transition of m/z 353.2 → 190.8, (b) catechin (upper pane) and H. cordata extract (lower pane) obtained from the extract mass in MRM mode at transition of m/z 288.9 → 245.0, (c) quercetin (upper pane) and H. cordata extract (lower pane) obtained from the extract mass in MRM mode at transition of m/z 300.9 → 151.0.
of the standard compounds and compound equivalent in the *H. cordata* tea aqueous extracts were shown in Fig. 3. The amounts of antioxidant compounds found in *H. cordata* tea were shown in Table 2. Quinic acid derivative and caffeic acid derivatives were not quantified due to contain in a small amount.

The major antioxidative components in this plant were found to be chlorogenic acid and its derivatives. Several reports showed that chlorogenic acid exhibited antivirus, anticancer, anti-inflammation activities, and anxiolytic-like effects (Bouayed, Rammal, Dicko, Younos, & Soulimani, 2007; Jiang, Satoh, Watanabe, Kusama, & Soulimani, 2005; Jin et al. 2005; Nakamura et al. 1997). Some of these compounds such as chlorogenic acid and quercetin glycoside have already been reported in *H. cordata* (Meng et al., 2005). However, this is the first time that the presence of catechin, and procyanidin B were characterized using on-line LC–MS coupled with DPPH assay for the first time.

4. Conclusions

The preparative isolation of the active compounds of herbal extracts in adequate quantities for off-line spectral and biological analysis is a laborious and time consuming. In this study, the use of on-line rapid screening of the antioxidants in herbal tea and their subsequent identification are reported. Antioxidants in the aqueous extract of *H. cordata* mainly consisting of chlorogenic acids and its derivatives, catechin and procyanidin B were characterized using on-line LC–MS coupled with DPPH assay for the first time.

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References

Bansidhi, J., Techadamrongsin, Y., Anuluknapakorn, K., Sriwanthana, B., Boonruad, T., Chaiyarat, S., et al. (2003). *Houttuynia cordata* Thumb. Bangkok, Thailand: The War Veteran Organization Press.

Bouayed, J., Rammal, H., Dicko, A., Younos, C., & Soulimani, R. (2007). Chlorogenic acid, a polyphenol from Prunus domestica (Mirabelle), with coupled anxiolytic and antioxidant effects. *Journal of the Neurological Sciences*, 262, 77–84.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lett. Lebensmittel Wissenschaft und Technologie*, 28, 25–30.

Carini, M., Facino, R. M., Aldini, G., Galloni, M., & Colombo, L. (1998). Characterization of phenolic antioxidants from *Mate* (Itlex Paraguayensis) by liquid chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry. *Rapid Communication in Mass Spectrometry*, 12, 1813–1819.

Ch, M. I., Wen, Y. F., & Cheng, Y. (2007). Gas chromatographic/mass spectrometric analysis of the essential oil of *Houttuynia cordata* Thumb. by using on-column methylation with tetramethylammonium acetate. *Journal of AOAC International*, 90, 69–697.

Chen, S. Y., Liu, J. F., Chen, C. M., Chao, P. Y., & Chang, T. J. (2003). A study of the antioxidative and antiinflammatory agencutes of *Houttuynia cordata* Thumb. using an oxidized frying oil-fed model. *Journal of Nutritional Science and Vitaminology (Tokyo)*, 49, 327–333.

Chang, L. C., Chang, J. S., Chen, C. C., Ng, L. T., & Lin, C. C. (2003). Ant-Herpes simplex virus activity of *Bidens pilosa* and *Houttuynia cordata*. *American Journal of Chinese Medicine*, 31, 355–362.

Hayashi, K., Kamiya, M., & Hayashi, T. (1995). Virucidal effects of the steam distillate from *Houttuynia cordata* and its components on HSV-1, influenza virus, and HIV. *Planta Medica*, 61, 237–241.

Jiang, Y., Satoh, K., Watanabe, S., Kusama, K., & Sakagami, H. (2001). Inhibition of chlorogenic acid-induced cytotoxicity by CoCg3. *Anticancer Research*, 21, 3349–3353.

Jin, U. H., Lee, J. Y., Kang, S. K., Kim, J. K., Park, W. H., Kim, J. G., et al. (2005). A phenolic compound, 5-caffeoylquinic acid (chlorogenic acid), is a new type and strong matrix metallopeptase-9 inhibitor: Isolation and identification from methanol extract of *Eucynamus alatus*. *Life Science*, 77, 2760–2769.

Kwon, K. B., Kim, E. K., Shin, B. C., Seo, A. E., Yang, J. Y., & Ryu, D. G. (2003). *Herba houttuyniae* extract induces apoptotic death of human promyelocytic leukemia cells via caspase activation accompanied by dissipation of mitochondrial membrane potential and cytochrome release. *Experimental and Molecular Medicine*, 35, 91–97.

Lau, K.-M., Lee, K.-M., Koon, C.-M., Cheung, C. S.-F., Lau, C.-P., Ho, H.-M., et al. (2008). Immunomodulatory and anti-SARS activities of *Houttuynia cordata*. *Journal of Ethnopharmacology*, 118, 79–85.

Li, G. Z., Chai, O. M., Lee, M. S., Han, E. H., Kim, H. T., & Song, C. H. (2005). Inhibitory effects of *Houttuynia cordata* water extract on anaphylactice reaction and mast cell activation. *Biological and Pharmaceutical Bulletin*, 28, 1604–1608.

Lu, H. M., Liang, Y. Z., Yi, L. Z., & Wu, X. J. (2006). Anti-inflammatory effects of *Houttuynia cordata* injection. *Journal of Ethnopharmacology*, 104, 245–249.

Meng, J., Dong, X. P., Zhou, Y. S., Jiang, Z. H., Leung, S. Y., & Zhao, Z. Z. (2007). Studies on chemical constituents of phenols in fresh *Houttuynia cordata*. *Zhongguo Zhong Yao Za Zhi*, 32, 929–931.

Meng, J., Leung, K.-S.-Y., Jiang, Z., Dong, X., Zhao, Z., & Xu, L.-J. (2005). Establishment of *HPLC–DAD–MS* fingerprint of fresh *Houttuynia cordata*. *Chemical and Pharmaceutical Bulletin (Tokyo)*, 53, 1604–1609.

![Fig. 4. Structure of phenolic compounds identified in the *H. cordata* tea.](image-url)

Table 2

| Compound                  | Content (µg/g) |
|---------------------------|---------------|
| Chlorogenic acid          | 2808.7 ± 7.5  |
| Neo-chlorogenic acid      | 921.7 ± 2.9   |
| Crypto-chlorogenic acid   | 6035.3 ± 2.9  |
| Catechin                  | 28.0 ± 0.8    |
| Procyanidin B             | 52.4 ± 5.2    |
| Procyanidin B′            | 183.5 ± 0.6   |
| Quercetin hexoside        | 730.0 ± 5.0   |

*The stereochemistry of procyanidin B could not be identified.*
Nakamura, T., Nakazawa, Y., Onizuka, S., Satoh, S., Chiba, A., Sekihashi, K., et al. (1997). Antimutagenicity of Tochu tea (an aqueous extract of Eucommia ulmoides leaves): The clastogen-suppressing effects of Tochu tea in CHO cells and mice. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 388, 7–20.

Niederländer, H. A. G., van Beek, T. A., Bartasiute, A., & Koleva, I. I. (2008). Antioxidant activity assays on-line with liquid chromatography. Journal of Chromatography A, 1210(2), 121–134.

Nuengchamnong, N., & Ingkaninan, K. (2009). On-line characterization of phenolic antioxidants in fruit wines from family myrtaceae by liquid chromatography combined with electrospray ionization tandem mass spectrometry and radical scavenging detection. LWT – Food Science and Technology, 42, 297–302.

Probstle, A., & Bauer, R. (1992). Aristolactams and a 4,5-dioxoaporphine derivative from Houttuynia cordata. Planta Medica, 58, 568–569.

Shui, G., Leong, L. P., & Wong, S. P. (2005). Rapid screening and characterization of antioxidants of Cosmos caudatus using liquid chromatography coupled with mass spectrometry. Journal of Chromatography B, 827, 127–138.

Toda, S. (2005). Antioxidative effects of polyphenols in leaves of Houttuynia cordata on protein fragmentation by copper–hydrogen peroxide in vitro. Journal of Medicinal Food, 8, 266–268.