Metabolomic profiling of the flower bud and rachis of *Tussilago farfara* with antitussive and expectorant effects on mice

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**Ethnopharmacological relevance:** Flower bud of *Tussilago farfara* L. is widely used for the treatment of cough, bronchitis and asthmatic disorders in the Traditional Chinese Medicine. However, due to the increasing demands, adulteration with rachis is frequently encountered in the marketplace. No report demonstrated the chemical and pharmacological differences between flower bud and rachis before.

**Materials and methods:** The water extracts were orally administrated to mice. Ammonia induced mice coughing model was used to evaluate the antitussive activity. The expectorant activity was evaluated by volume of phenol red in mice’s tracheas. Metabolites were identified directly from the crude extracts through 1D- and 2D-NMR spectra. A metabolic profiling carried out by 1H NMR spectroscopy and multivariate data analysis was applied to crude extracts from flower bud and rachis.

**Results:** Flower bud significantly lengthened the latent period of cough, decreased cough frequency caused by ammonia and enhanced tracheal phenol red output in expectorant evaluation. Principal component analysis (PCA) yielded good separation between flower bud and rachis, and corresponding loading plot showed that the phenolic compounds, organic acid, sugar, amino acid, terpene and sterol contributed to the discrimination.

**Conclusions:** These findings provide pharmacological and chemical evidence that only flower bud can be used as the antitussive and expectorant herbal drug. The high concentration of chlorogenic acid, 3,5-dicaffeoylquinic acid, rutin in flower buds may be related with the antitussive and expectorant effects of Flos Farfara. To guarantee the clinical effect, rachis should be picked out before use.

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### 1. Introduction

The flower buds of *Tussilago farfara* L. (Asteraceae), Flos Farfara, also locally known as “Kuandonghua” in China, are an important traditional Chinese medicine, widely used for the treatment of cough, bronchitis and asthmatic disorders (Committee for the Pharmacopoeia of People’s Republic of China, 2010). The extracts of Flos Farfara also exhibit various activities, such as antioxidant effect, antimicrobial activities, α-glucosidase inhibitory effect, and inhibitory effects on NO synthesis in LPS-activated macrophage and diacylglycerol acyltransferase activity (Gao et al., 2008; Park et al., 2008). Extensive phytochemical studies have revealed that Flos Farfara contains a number of diverse components including essential oils (Liu et al., 2006), sesquiterpenes (Ryu et al., 1999; Yaoita et al., 1999, 2001), flavonoids (Kim et al., 2006; Liu et al., 2007a; Wu et al., 2010), phenylpropanoids (Liu et al., 2007a; Gao et al., 2008; Wu et al., 2010), chromones (Wu et al., 2008), and pyrrolizidine alkaloids (Jiang et al., 2009).

Flos Farfara has been used in many Traditional Chinese Medicine (TCM) prescriptions, such as Juhongwan, Chunbeixueligao, Kuandongdingchuangtang. According to the theory of TCM, only the flower bud can be used as herbal drug. Due to the increasing demands of Flos Farfara, adulteration with rachis is frequently encountered in the marketplace (Fig. 1). However, to our knowledge, there is no report on the pharmacological and chemical differences between flower bud and rachis.

In Chinese pharmacopoeia, the quality of Flos Farfara was evaluated by the content of tussilagine. However, synergistic effects of different components are vital for the therapeutic efficacy of phyto medicine (Williamson, 2001; Liu et al., 2007b). To confine the analysis to a small range of metabolites may be misleading for quality evaluations of TCM. As a result, a non-targeted chemical fingerprinting, which can cover a wide range of metabolites, is necessary.
The term metabolome has been used to describe the observable chemical profile or fingerprint of the metabolites in whole tissues (Kim et al., 2005a). To obtain the most complete metabolomic profiles, it is necessary to use a wide spectrum of analytical techniques. Compared with the GC–MS or LC–MS based metabolomic studies, NMR has some unique advantages, such as rapid, non-selectiveness, reproducible, and stable (Kooy et al., 2009). In addition, detailed structural information of metabolites, including chemical shifts and coupling constants, can be directly obtained. This makes NMR an ideal choice for the profiling of the broad range of plant metabolites. Different multivariate data analysis tools, such as principal component analysis (PCA), are important tools for the analysis of the data obtained by NMR. Recently, NMR based metabolomic studies have been used in the quality evaluation of several kinds of herbal drugs, such as ginseng (Yang et al., 2006), Angelica acutiloba (Tarachiwin et al., 2008), Artemisia afra (Liu et al., 2010).

In this study, the antitussive and expectorant activities of flower bud and rachis were evaluated. Then a metabolic profiling was carried out, using 1H NMR spectroscopy and multivariate data analysis techniques. Diverse kinds of compounds, including sesquiterpenes, flavonoids, phenylpropanoids and fatty acids, were detected, and metabolic differences between flower bud and rachis were identified. This procedure suggested the possible metabolites responsible for the antitussive and expectorant properties of Flos Farfara.

2. Materials and methods

2.1. Plant materials

The flower buds of Tussilago farfara were purchased from WanshengHuangQi Co., Ltd. in Hunyuan, Shanxi, China and authenticated by Prof. Xue-Mei Qin from Modern Research Center for Traditional Chinese Medicine of Shanxi University. The rachises adulterated in the flower buds were picked out for the subsequent study. For metabolic profiling, ten flower bud samples and ten rachis samples having different lot numbers were evaluated. These samples were freeze-dried and ground to fine powders in a pestle and mortar, and then stored at −80 °C until analysis. Voucher specimens coded FB (flower bud) 201001-201010 and RA (rachis) 201001-201010 were deposited in herbarium of the Modern Research Center for Traditional Chinese Medicine of Shanxi University.

2.2. Solvents and chemicals

Analytical grade chloroform was purchased from Fengchuan Chemical Co. Ltd. (Tianjin, China) and analytical grade methanol was from Beijing Chemical Works (Beijing, China). Deuterated chloroform (CDCl₃, 99.8%D) containing tetramethylsilane (TMS, 0.03%, m/v) and methanol-d₄ (99.8% D) were obtained from Merck (Darmstadt, Germany). D₂O was bought from Norell (Landisville, USA). Sodium 3-trimethylsilyl-1H propionate (TSP) was from Cambridge Isotope Laboratories Inc. (Andover, MA), and NaOD was purchased from Armar (Dottingen, Switzerland). Pentoxyverine was obtained from CSPC Ouyi Pharmaceutical Co., Ltd. (Shijiazhuang, China). Ammonium chloride was from Beijing Chemical Works (Beijing, China) and phenol red from Tianjin Chemical Works (Tianjin, China).

2.3. Samples for antitussive and expectorant analysis

According to the traditional use, the flower buds and rachis were extracted with water by refluxing for three times (2 h each). The combined solution was filtered and concentrated under reduced pressure to afford the water extract.

2.4. Animals

ICR mice of either sex (19–24 g) were purchased from Beijing Vital Laboratory Animal Technology Company (license number SCXX-2006-0008). All animals were housed at room temperature (20–25 °C) and constant humidity (40–70%) under a 12 h light–dark cycle in SPF (Specific Pathogen Free) grade laboratory. The animal study was performed according to the international rules considering animal experiments and the internationally accepted ethical principles for laboratory animal use and care.

2.5. Antitussive effects against ammonia induced coughing

The antitussive activity was performed as previously described with minor modifications (Xu et al., 2005). After 3 days of adaptation, 60 mice were divided into 4 groups (n = 15) randomly and orally administered, including control group (distilled water), positive group (pentoxyverine 50 mg/kg), flower bud group (2.8 g/kg), rachis group (3.5 g/kg). The administration dose of extract was calculated according to the clinical dose of this plant and yield of extract. The administration had been lasted for 5 days and the mice were exposed to a 1000 ml special glass chamber with ammonium hydroxide (1 ml) cotton ball after 1 h of the last administration and the cough incubation period was recorded. After 1 min, the mice were taken out from the chamber and placed in a beaker and the frequency of cough within 2 min was observed and recorded.
2.6. Expectorant test

After 1 week of adaptation, 48 ICR mice were divided into 4 groups (12/group) randomly and orally administered, including control group (distilled water), positive group (ammonium chloride 1 g/kg), flower bud group (2.8 g/kg), and rachis group (3.5 g/kg). The procedures were performed as described previously (Engler and Szelenyi, 1984). All groups were treated with a single dose daily for 5 days and the last dose were given 30 min before intraperitoneal injection of phenol red solution (5% in saline solution, w/v, 0.1 ml/10 g body weight). Mice were sacrificed by cervical dislocation 45 min after application of phenol red. After dissected free from adjacent organs, the trachea was removed from the thyroid cartilage to the main stem bronchi and put into 2 ml normal saline immediately. After ultrasonic for 15 min, 2 ml NaHCO3 solution (5%, w/v) was added to the saline and optical density of the mixture were measured at 558 nm using UV–7501 UV–vis spectrophotometer (Wuxi Keda Instrument Co., Ltd., China).

2.7. Extraction of plant samples for metabolomics

2.7.1. Extracted with hot water

Plant material (10 g) was transferred into round-bottomed flask and extracted with water by refluxing for three times (2 h each). The combined solution was filtered and dried with a rotary vacuum evaporator. Then 50 mg dried extract was weighed into 2 ml centrifuge tube and added 800 µl mixture (1:1) of CD3OD and KH2PO4 buffer in D2O (adjusted to pH 6.0 by 1 N NaOD) containing 0.05% TSP and sonicated for 20 min. After centrifuging for 15 min at 13,000 rpm, the supernatant (600 µl) was transferred into a 5 mm NMR tube for NMR analysis.

2.7.2. Extracted with two phase system

Ground material (200 mg) was transferred into 10-ml glass centrifuge tube. 3 ml of 50% water–methanol mixture and 3 ml of chloroform were added to the tube followed by vortexing for 1 min and ultrasonication for 20 min. The material was then centrifuged at 3500 rpm for 25 min. The chloroform (lower) and aqueous methanol (upper) fractions were separated separately into a 25 ml round-bottomed flask and dried with a rotary vacuum evaporator. Chloroform fractions were dissolved in 800 µl CDCl3, and aqueous methanol fractions were dissolved in 800 µl mixture (1:1) of CD3OD and KH2PO4 buffer in D2O (adjusted to pH 6.0 by 1 N NaOD) containing 0.05% TSP. The supernatants (600 µl) of all the samples were transferred into 5-mm NMR tube for NMR analysis after 10 min of sonication and centrifuging for 15 min at 13,000 rpm.

2.8. NMR measurements

1H NMR, 2D J-resolved and 1H–1H-correlated spectroscopy (COSY), heteronuclear multiple bonds coherence (HMBC), heteronuclear single quantum coherence (HSQC) were recorded at 25 °C on a Bruker 600-MHz AVANCE III NMR spectrometer (600.13 MHz proton frequency). CD3OD and CDCl3 were used for internal lock purposes. Each 1H NMR spectrum consisted of 64 scans requiring 5 min acquisition time with the following parameters: 0.18 Hz/pixel, pulse width (PW) = 30° (12.7 µs), and relaxation delay (RD) = 5.0 s. A presaturation sequence was used to suppress the residual H2O signal with low power selective irradiation at the H2O frequency during the recycle delay. FIDs were Fourier transformed with LB = 0.3 Hz. The resulting spectra were manually phased and baseline-corrected, and calibrated to TSP at 0.00 ppm for water fractions and TMS at 0.00 ppm for organic fractions. Two-dimensional J-resolved NMR spectra were acquired with 2.0 s relaxation delay using 16 scans and spectral widths of 10,000 Hz in F2 and 78 Hz in F1. J-resolved spectra were tilted by 45° and symmetrized about F1. The COSY spectra were acquired with 2.0 s relaxation delay, 5411 Hz spectral width in both dimensions. The window function for COSY spectra was sine–bell (SSB = 0). The HMBC spectra were obtained with 1.5 s relaxation delay, using 8012 Hz spectral width in F2 and 133,200 Hz in F1. For HSQC spectra, the 1.5 s relaxation delay was used, 6099 Hz spectral width in F2 and 108,000 Hz in F1. All 2D spectra were calibrated at 0.00 ppm to TSP or MRS.

2.9. Data analysis

The 1H NMR spectra were processed using MestReNova (version 5.2.5, Mestrelab Research, Santiago de Compostella, Spain). For aqueous methanol fraction and aqueous extract, spectral intensities were scaled to TSP and reduced to integrated regions of equal width (0.04 ppm) corresponding to the region of δ 6.0–10.0. The regions of δ 4.70–5.02 and δ 3.30–3.38 were excluded from the analysis because of the residual signal of HDO and CD3OD, respectively. For the chloroform fraction, spectral intensities were scaled to TMS and reduced to integrated regions 0.04 ppm corresponding to the region of δ 10.02–5.0. The region between δ 7.22 and δ 7.30 was removed from the analysis because of the residual signal of CHCl3. Principal component analysis (PCA) with pareto scaling was performed with SIMCA-P11.0 (Umetrics, umea, Sweden). Relative amount of metabolites was evaluated based on the integrated regions (bucket) of the NMR spectra using Origin (8.0, OriginLab, USA).

The antitussive and expectorant results are expressed as mean ± standard error of mean (S.E.M.). Significance was evaluated using the Student’s t-test (Woodson, 1987). Values of p < 0.05 imply significance of the pharmacological effects in the experiments.

3. Results and discussion

3.1. Antitussive and expectorant effects

In this experiment, we used chemical stimuli (ammonium method due to its simple procedure that omitted of anesthetization and usually used in new drug development for traditional Chinese medicines. Therefore, the antitussive activity of the flower bud and rachis extracts were demonstrated in vivo experiment by prolonging the incubation time, and reducing coughing times in 2 min. The longer cough incubation period showed stronger effect of the drug on relieving cough and the less cough times exhibited its stronger antitussive effect. The antitussive effects of flower buds and rachis extracts on the ammonia liquor induced cough in mice were shown in Fig. 2. Positive and flower bud groups significantly prolonged latent period of cough compared with the control group. The data of cough frequency in 2 min were shown in Fig. 2B. It was observed that the cough frequency of flower bud group could be significantly reduced, comparable with that of the effect produced by pentoxyverine, a prototype antitussive agent. However, rachis group had no significant effect on the antitussive activity.

In the expectorant experiment, phenolsulphonphthalain was applied and discharged with sputum some minutes after i.p. injections into mice. Excretion of phenolsulphonphthalain would take place of the excretion of sputum because the agent was eliminated partly from trachea. The stronger eliminating phlegm activity and expectorant effect showed greater UV absorption. As shown in Fig. 3, ammonium chloride and flower bud groups could enhance tracheal red output, indicating the strong expectorant effect. For the rachis, no obvious effect was observed.
3.2. Chemical differences between flower bud and rachis in hot water extracts

All the hot water extracts of flower bud and rachis were subjected to $^1$H NMR analysis. The assignment of the $^1$H spectrum, see Fig. 4, was based on careful analysis of 1D and 2D NMR spectra, and further confirmed by comparing with the reported NMR data in literature. In the $^1$H NMR spectrum, the area between $\delta$ 0.50 and $\delta$ 4.00 corresponds to amino acids and organic acids. The region of $\delta$ 4.00–$\delta$ 5.50 is considered to be the carbohydrate region and the remaining part, i.e. $\delta$ 5.50–$\delta$ 10.02, is known as the aromatic region. The high signal intensities in the amino acid region were helpful to elucidate a number of amino acid signals, including isoleucine, leucine, valine, threonine, alanine, proline, glutamic acid, and aspartic acid. In addition, a number of signals were assigned to the organic acids, such as acetic acid, succinic acid, malic acid, citric acid. Other compounds, including 1-O-ethyl-$\beta$-D-glucoside, ethanolamine, choline, phosphatidylcholine, creatine, were also identified (Kim et al., 2010a; Liu et al., 2010; Lopez-Gresa et al., 2010; Lubbe et al., 2011). The signals in the carbohydrate regions were highly clustered and overlapped. This region showed the signals of the anomeric protons of $\beta$-glucose at $\delta$ 4.60 (d, $J=7.9$ Hz), $\alpha$-glucose at $\delta$ 5.20 (d, $J=3.7$ Hz), and sucrose at $\delta$ 5.42 (d, $J=3.8$ Hz) and 4.18 (d, $J=8.7$ Hz) (Kim et al., 2010a; Ali et al., 2011). The aromatic part of the $^1$H NMR spectra showed a series of signals of phenylpropanoids, including caffeic acid, chlorogenic acid, sinapic acid, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 3,4-dicaffeoylquinic acid (S. Table 1). The characteristic doublets of 16.0 Hz in the range of $\delta$ 6.39–$\delta$ 6.50 and $\delta$ 7.59–$\delta$ 7.70 (H-8’ and H-7’) and double triplets at $\delta$ 5.43, $\delta$ 5.49 and $\delta$ 5.67 were also evidence of these compounds (Verpoorte et al., 2007; Kim et al., 2010b; Liu et al., 2010). In the aromatic region, the flavonoids rutin and kampferol analogues were evident (Fig. 4). Maleic acid, fumaric acid, formic acid, p-hydroxybenzoic acid, adenine and adenosine were also identified in the aromatic region (Liu et al., 2010; Lopez-Gresa et al., 2010) (S. Table 1; S. Fig. 1).

Visual inspection of the NMR spectra indicated that flower bud had more phenylpropanoids (S. Fig. 2). However, it was not possible to get a consistent idea about the differences in the metabolite profile with simple visual inspection due to the intra-group variations. Therefore, multivariate data analysis tools were used to provide comparative interpretations and visualization of chemical differences between the flower bud and rachis.

All the obtained NMR data of flower bud and rachis were analyzed by principal component analysis (PCA). In the score plot, a clear separation was found between the flower bud and rachis, with the first two components explaining 71.6% of total variation (Fig. 5). The corresponding loading plot revealed that the rachis was characterized by high signals for the compounds of succinic acid, maleic acid, aspartic acid, sucrose. The flower bud showed higher aromatic compounds such as caffeic acid, chlorogenic acid, 3,5-dicaffeoylquinic acid and rutin. Some amino acids, including threonine, alanine, proline, and glutamic acid, as well as glucose, choline, phosphatidylcholine, acetic acid, formic acid, were also found to be higher in flower bud. Some unidentified signals were also found to largely contribute to the discrimination between flower bud and rachis. These signals mostly belong to sugars and are difficult to identify.

To confirm their biased distribution, the relative amounts (NMR signal buckets) are shown in Fig. 6. Independent student’s t-test indicated that chlorogenic acid, 3,5-dicaffeoylquinic acid, rutin were significantly higher in the flower buds. However, the rachis contained more malic acid, sucrose.

3.3. Metabolomic profiling using two-phase extraction method

Extraction with hot water is the traditional use in TCM (Han et al., 2010; Li et al., 2011). However, only the abundant polar metabolites, such as amino acids, organic acids, can be detected by NMR in the hot water extracts. Non-polar metabolites such as terpenoids are also believed to play an important role in the herbal materials. To evaluate the overall chemical differences...
3,5-dicaffeoylquinic creatine.


between flower bud and rachis, a different extraction method, the biphasic extraction, CHCl$_3$-MeOH-H$_2$O (2:1:1), was used. After extraction, two fractions, chloroform and aqueous methanol parts, were obtained and subjected to $^1$H NMR analysis and multivariate analysis, respectively (S. Fig. 3). The non-polar metabolites were concentrated in the chloroform fraction.

For the aqueous methanol fractions, the $^1$H NMR spectra were similar with those of hot water extracts (S. Fig. 4; S. Fig. 5), except that some minor differences in the aromatic and amino acid region. The differential metabolites between flower bud and rachis, revealed by the corresponding loading plot (S. Fig. 6), were also similar with those of hot water extracts (Fig. 5), except that two amino acids, isoleucine, leucine, were found to be higher in rachis.

For the chloroform fractions, six metabolites (Fig. 7) have been identified, including two sesquiterpenes, tussilagine and 7β-(3-Ethyl-cis-crotonyloxy)-1α-(2-methylbutyroloxy)-3(14)-dehydro-Z-notonipetranone (EMDNT), a triterpene, bauer-7-ene-3β, 16α-diol, two sterols, β-sitosterol and sitosterone, and fatty acids analogues. δ 0.78 (d, J = 6.6 Hz, CH$_3$-13), δ 0.98 (d, J = 6.6 Hz, CH$_3$-12), δ 1.08 (t, J = 7.3 Hz, CH$_3$-5′), δ 1.22 (d, J = 6.7 Hz, CH$_3$-15), δ 2.10 (s, OAC), δ 2.15 (s, CH$_3$-6′), δ 4.78 (s, H-10a), δ 5.14 (s, H-10b), δ 5.57 (br t, J = 2.3 Hz, H-7), and δ 5.63 (s, H-2′) were assigned to be tussilagine, a major sesquiterpene in Flos Farfara (Park et al., 2008). EMDNT, another structurally similar sesquiterpene, was at δ 0.88 (t, J = 7.4 Hz, CH$_3$-4′), δ 0.90 (d, J = 7.0 Hz, CH$_3$-13), δ 0.97 (d, J = 6.4 Hz, CH$_3$-12), δ 1.07 (t, J = 7.5 Hz, CH$_3$-5′), δ 1.12 (d, J = 7.1 Hz, CH$_3$-5′), δ 2.15 (s, CH$_3$-6′), δ 2.16 (d, J = 7.2 Hz, CH$_3$-15), δ 4.81 (s, H-10a), δ 5.17 (s, H-10b), δ 5.51 (d, J = 3.4 Hz, H-7), δ 5.53 (d, J = 4.0 Hz, H-1), δ 5.61 (s, H-2′), δ 6.38 (q, J = 6.4 Hz, H-14) (Park et al., 2008). In addition, resonances at δ 0.73 (s, CH$_3$-25) and δ 1.15 (d, J = 6.8 Hz, CH$_3$-29) were identified as bauer-7-ene-3β, 16α-diol, a triterpene in the plant (Santer and Stevenson, 1962;
Fig. 6. Student t-test for the relative amount of compounds based on mean peak areas of associated signals. (): chemical shifts of signals used for the student t-test. 25: chlorogenic acid (6.36), 27: 3,5-dicaffeoylquinic (6.47), 29: rutin (6.98), 11: malic acid (2.47), 20: sucrose (5.42).

Fig. 7. 600 MHz 1H NMR spectra of the chloroform fractions of Tussilago farfara. 35: 7β-(3-Ethyl-ciscrotonoxyloxy)-1α-(2-methylbutyroloxy)-3(14)-dehydro-Z-notonipetranone, 36: tussilagone, 37: bauer-7-ene-3β, 16α-diol, 38: β-sitosterol, 39: sitosterone, 40: fatty acid.
Yaoita and Kikuchi, 1998). The terminal methyl (δ 0.90), α-CH₂ (δ 2.3), β-CH₂ (δ 1.6), allylic CH₂ (δ 2.05), and bis-allylic CH₂ (δ 2.77), all the other protons of hydrocarbon chain (δ 1.2–1.3), and olefinic protons (δ 5.35) were detected as fatty acids analogues (Chatterjee et al., 2010). The metabolites were further confirmed by the 2D NMR spectra and purified standards.

PCA analysis of the chloroform fraction indicated that the rachis could also be clearly separated from the flower bud along PC1 (Fig. 8A). The loading plot suggests that in the flower bud, tussilagone, EMDNT, bauer-7-ene-3β, 16α-diol, are predominant; while in rachis, β-sitosterol, sitosterone, fatty acids are predominant (Fig. 8B).

4. Conclusions

For thousands of years, the flower bud was decocted for treating respiratory diseases. However, the adulteration with rachis is commonly encountered in the drug market for a long time. This study demonstrated that the rachis extract showed no obvious antitusive and expectorant activities, implying that it should not be used instead of flower bud.

Corresponding metabolomic approach based on 1H NMR spectroscopy coupled with multivariate data analysis revealed the chemical differences between flower bud and rachis. Some secondary metabolites, including chlorogenic acid, 3,5-dicaffeoylquinic acid, rutin were highly accumulated in flower bud, while the rachis showed higher concentration of primary metabolites, like malic acid, sucrose, fatty acids analogues. Chlorogenic acid, which is the active compound in many medicinal plants, has significant antibacterial and antiviral activities (Peluso et al., 1995; Zhang et al., 2001). Rutin, one of the major flavonoids found in a variety of plants, is also the active compound for many medicinal plants. Rutin has significant antioxidant, radic scavenging (Song et al., 2010), anti-inflammatory (Guardia et al., 2001; Kim et al., 2005b), anti-viral, and anti-bacterial (Pereira et al., 2008; Orhan et al., 2010) activities. No direct antitussive and expectorant effects of these compounds have been reported, however, synergistic effect may be existed for relieving the syndrome of cough and asthma. The high concentration of chlorogenic acid, 3,5-dicaffeoylquinic acid, rutin, tussilagone in flower buds may be related with the antitussive and expectorant effects of Flos Farfara.

The overall chemical differences between flower bud and rachis also revealed that the flower bud contained more tussilagone, EMDNT, bauer-7-ene-3β, 16α-diol. Tussilagone has been determined as the main active compound of Flos Farfara, and was used as marker compound for the quality evaluation of Flos Farfara in the Chinese Pharmacopoeia. Previous pharmacological studies have revealed that tussilagone exerts anti-inflammatory activities in murine macrophages by inducing heme oxygenase-1 (HO-1) expression (Hwangbo et al., 2009). The relationship of non-polar metabolites and the antitussive and expectorant activities remains unknown. And further studies should be conducted on systematic research for the active components of Flos Farfara for the antitussive and expectorant effects.

Previous studies showed that Tussilago farfara contained the toxic pyrrolizidine alkaloids (PAs), mainly senkirkine and traces of senecionine (Pu et al., 2004). The PAs can be hepatoxic, causing damage to the liver and may even cause liver cancer when minute quantities are consumed (Lin et al., 2007). However, no signals of PAs were found in the 1H NMR spectra of hot water extracts, aqueous methanol fractions or chloroform fractions (S. Fig. 7; S. Fig. 8), due to the low amount of PAs and also the low sensitivity of NMR compared with MS. Further study should be conducted to evaluate the PAs levels between flower bud and rachis by UPLC–MS.

This is the first time that a metabolic profiling analysis, using NMR and multivariate data analysis, was applied to Flos Farfara. The information obtained in this work is valuable for further phytochemical and activity research, and may be used to establish the analytical requirements for quality control purpose in the future.

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Appendix A. Supplementary data


References


