Changes in bioactive components related to the harvest time from the spicas of *Prunella vulgaris*

Yuhang Chen¹², Qiaosheng Guo¹, Zaibiao Zhu¹, and Lixia Zhang¹

¹Institute of Chinese Medicinal Materials, Nanjing Agricultural University, Nanjing, China and
²College of Pharmaceutical Sciences, Chengdu Medical College, Chengdu, China

**Abstract**

**Context:** *Prunella vulgaris* L. (Labiatae) is a perennial plant common in China and Europe and is rich in rosmarinic acid (RA), ursolic acid (UA), and oleanolic acid (OA). The dried spica of *P. vulgaris* has been used as traditional medicine in China for over a hundred years. To our best knowledge, no study has been conducted to determine the influence of harvesting time on concentrations of bioactive compounds of *P. vulgaris*.

**Objective:** In the current study, changes in the bioactive compounds present in spicas were investigated at five harvest times over 2 months.

**Materials and methods:** Plant material were collected at five fixed dates: 5th May, 20th May, 7th June, 15th June, and 25th June and assayed for chemical contents by high-performance liquid chromatography (HPLC).

**Results:** Among the different harvest times, the highest levels of RA (56.81 mg·g⁻¹), UA (2.77 mg·g⁻¹), and OA (0.91 mg·g⁻¹) were found on 5th May, whereas the lowest levels of RA (1.66 mg·g⁻¹), UA (2.27 mg·g⁻¹), and OA (0.43 mg·g⁻¹) were observed on 25th June.

**Discussion and conclusion:** As each medicinal product has its own content requirement for different bioactive components, the optimum harvest time might be determined according to the accumulation dynamics of target compound in dried spicas of *P. vulgaris*. These results may be useful for determining the optimal harvest time when bioactive components are at the maximum level, which is in early May.

**Keywords:** Spicas, harvest time, rosmarinic acid, ursolic acid, oleanolic acid

**Introduction**

*Prunella vulgaris* L. (Labiatae), also known as the “self-heal,” is a perennial plant common in China and Europe. Prunellae Spica, the dried spica of *P. vulgaris* is a standard medicinal material in the Chinese Pharmacopoeia (Board of Pharmacopoeia of P. R. China, 2010) that has been used in China for over a hundred years (Chen et al., 2010). Because of its good performance and few side effects, as confirmed by a long-term clinical application (Pinkas et al., 1994; Cheung & Zhang, 2008), the dried spica has been widely used either alone or with other herbal ingredients to treat sore throats, fevers, goiters, hypertension, tuberculosis, and mammary gland hyperplasia (Board of Pharmacopoeia of P. R. China, 2010).

In addition to its pharmaceutical uses, the spicas of *P. vulgaris* are manufactured as a refrigerated beverage, and the fresh leaves are consumed as a vegetable dish in southeast China (Chen et al., 2010).

Previous phytochemical studies on the biologically active components of *P. vulgaris* have been isolated and reported to mainly contain triterpenoids, phenolics, flavonoids, tannins, caffeic acids, and anionic polysaccharide prunelline (Ryu et al., 2000; Wang et al., 2000; Jiang et al., 2008). Rosmarinic acid (RA), one of the major phenolics, suppresses lipoperoxidation (Laranjinha et al., 1994), scavenges superoxide radicals (Osakabe et al., 2002), and exhibits anti-inflammatory (Osakabe et al., 2004) and antioxidant (Psotova et al., 2003) bioactivity.
Bioactive component changes in *Prunella vulgaris* spicas

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RA has also been used as the standard for quality control of *Prunellae Spica* by the Chinese Pharmacopoeia (Board of Pharmacopoeia of P. R. China, 2010). Of the triterpenes, ursolic acid (UA; Figure 1) and oleanolic acid (OA; Figure 1) have beneficial and notable effects on hepatoprotective, antifungal, anti-inflammation, antitumor, and antihyperlipidemia activities (Liu, 1995). There is increasing evidence that UA is the main active substance in antidiabetic medicines due to its ability to lower blood sugar, urine sugar, water intake, and urine volume, whereas OA is the main compound that protects the liver (Liu et al., 2003).

Because of its pharmacological and industrial importance, demand for *P. vulgaris* has increased steadily in recent years. The wild population of *P. vulgaris* cannot meet this growing need, and therefore, it has been proposed since the 1990s that *P. vulgaris* be cultivated in China (Chen et al., 2011). At present, approximately 10 million kg of *P. vulgaris* is required for prescriptions or export from China each year (Guo et al., 2010). Hence, production protocols for cultivating uniform and high-quality *P. vulgaris* are needed to meet the market demand.

An important aspect of *P. vulgaris* production is the timing of harvest to maximize yields and content of the desirable compounds. The spica maturity stage (mid-June) traditionally has been harvest time (Liu et al., 2010; Wang
et al., 2011) because it provides the highest spica yields and best marketing attributes (Xu, 2001); however, large variations in the amounts of the major bioactive components at this stage result in different optimal harvest times (Wang et al., 1993; Zhang et al., 2007). In previous studies, variations due to cultivation patterns, such as planting date and growth years, have received little interest (Jiang et al., 2009). Furthermore, only the yield (i.e., spica dry matter) or content of single active components was considered (Wang et al., 1993, 2011), and different harvesting dates were used in these studies. Our previous studies have shown that early May was considered an optimal harvest time of *P. vulgaris* in ancient China (Chen et al., 2010). In addition, *P. vulgaris* used in some studies was wild material from different regions, and growth conditions and cultivation methods were not clearly recorded, producing inconsistent results (Luo et al., 2005).

Therefore, information about the content variations of the major bioactive compounds in spicas of *P. vulgaris* in relation to harvest time is needed. It is also important to determine the optimal harvest time of desirable compounds for growers. In our study, we determined RA, UA, and OA content at five harvest times over 2 months.

**Materials and methods**

**Plant materials**

Seeds of *P. vulgaris* were collected from Queshan County in the Henan Province of North China on June 10, 2008 and authenticated by Professor Qiaosheng Guo of the Institute of Chinese Medicinal Materials, Nanjing Agricultural University, Jiangsu Province, P.R. China. Twenty seeds of approximately the same size were sown in separate pots on October 7, 2008 and germinated in the greenhouse. After 1 month, the seedlings were transplanted to the experimental field located in a flat land site (117°23′E, 31°24′N, 48.64 altitude) of Lujiang County, Anhui Province, P.R. China. The spacing was 30 cm between rows and 25 cm between plants within a row. The field was cultivated using conventional commercial methods from October 2008 to June 2009. The material represented 20 plants were randomly collected at five fixed dates: 5th May, 20th May, 7th June, 15th June, and 25th June. After collected, the whole plants were dissected into spica and other tissues. Spicas were oven dried to a constant weight at 60°C, weighed, ground, and passed through a 0.3 mm sieve. RA, UA, and OA were analyzed by high-performance liquid chromatography (HPLC).

**Chemicals and reagents**

HPLC-grade methanol was purchased from Fisher Scientific Co. (Fair Lawn, NJ). Deionized water was purified by the Milli-Q system (Millipore, Bedford, MA, USA). Analytical-grade phosphoric acid and ethanol were from Nanjing Liudu Fine Chemicals Co. Ltd. (Nanjing, China). Three authentic standards of RA, UA, and OA were purchased from the National Institute for Control of Biological and Pharmaceutical Products (Beijing, China).

**Apparatus and analytical conditions**

HPLC was performed with a chromatographic pump (LC-20AT), manual sample injector, and a diode array detector (SPD-M20A, Shimadzu, Kyoto, Japan). The HPLC fingerprint was performed on a C18 column (Shimadzu, ODS-C18, 4.6 mm × 250 mm, 5 µm) at 25°C with a sample injection volume of 10 µL. The data were collected with the model N2000 chromatography workstation (Zhejiang University, Hangzhou, China).

The chromatographic conditions used to detect the UA and OA components were as follows: The detection wavelength was 210 nm for the analysis. The mobile phase, consisting of methanol and 0.5% ammonium acetate (90:10, v/v), was at a flow rate of 0.6 mL·min⁻¹, and the column temperature was maintained at 25°C. The chromatographic conditions for the RA component were as follows: the mobile phase consisting of methanol and 0.01% aqueous phosphoric acid (52:48, v/v), the detection wavelength was 330 nm, the flow rate was 1.0 mL·min⁻¹, and the column temperature was maintained at 25°C.

![Figure 3. HPLC chromatogram fingerprint of the RA from P. vulgaris spicas and RS (reference standards). (3) RA.](image)
Making the calibration curves of active constituents

Stock solutions of RA, UA, and OA were prepared in deionized water and diluted with 75% methanol to six different concentrations to construct the calibration plots. The linearity of each standard curve was confirmed by plotting the peak area (y) against the corresponding concentration (x, µg·mL⁻¹) of the analytes. The regression equations are listed in Table 1.

Preparation of sample solutions

The samples for HPLC were prepared using modifications of the methods by Fang et al. (2010). Powdered spica samples (0.10 g) were mixed with 20 mL of 75% methyl alcohol for 30 min, extracted through a 30 min ultrasonic treatment at 20°C and centrifuged at 10,000 rpm for 10 min. The upper solution was filtered through a 0.45 µm organic membrane before injection into the HPLC system.

Statistical analysis

All data were analyzed using SPSS 11.5 for Windows. The data were initially compared by analysis of variance (ANOVA), and the differences between means were detected using the Duncan test. p values ≤ 0.05 were considered significant.

Results and discussion

Changes in RA, UA, and OA in the spicas of *P. vulgaris* at different harvest times

The HPLC chromatograms fingerprint of the RA, UA and OA from *P. vulgaris* spicas and its reference standards are shown in Figures 2 and 3. The amounts of RA, UA, and OA in the spicas of *P. vulgaris* collected at five harvest times are presented in Table 2. The results indicate that the amounts of these major bioactive compounds varied with harvest times.

In dried spicas of *P. vulgaris*, the amount of RA decreased from 5th May to 25th June, and the UA and OA indices declined steadily during this period (Table 2). The sample harvested on 5th May had the highest amounts, including RA: 56.81 mg·g⁻¹, UA: 2.77 mg·g⁻¹, and OA: 0.91 mg·g⁻¹. The lowest amounts were obtained from the 25th June harvest (RA: 1.66 mg·g⁻¹, UA: 2.27 mg·g⁻¹, and OA: 0.43 mg·g⁻¹). Total average RA amount in samples harvested on 5th May (56.81 mg·g⁻¹) was significantly higher than that of 25th June (1.66 mg·g⁻¹). Moreover, the average amount of UA and OA in samples harvested on 5th May increased 22 and 111%, respectively, compared with the 25th June samples (Table 1). The maximum amounts of RA, UA, and OA occurred on 5th May during the spica formation period. Previous studies have reported that changes in the amount of the major bioactive compounds in spicas of *P. vulgaris* occur at different harvest times, such as mid-May (Board of Pharmacopoeia of P.R. China, 1977) and mid-June (Luo et al., 2005; Wang et al., 2011). Late June (Wang et al., 1993) was reported as the best harvest time. These differences might be affected by environmental stresses (e.g., light, heavy metal, and drought), genetics, climatic conditions, ecotype, cultural practices, or a combination of these factors (Guo et al., 2010; Wu et al., 2010; Chen et al., 2011; Liao et al., 2010); therefore, it is worthwhile to experimentally determine the optimal time for RA, UA, and OA production for each location and ecotype rather than rely on published data only. Our study reported that the preflowering stage (5th May) had higher RA, UA, and OA amounts compared to the fully developed mature stage (15th June). These results are in agreement with those of Zhang et al. (2007) and Shou et al. (2009) who observed a decline in RA, UA, and OA with ripening. The decline in RA, UA, and OA from preflowering to fully mature plants could be caused by a dilution effect because the total sample volume increased during this period (Jiang et al., 2009). Similar results have occurred in other species as well (Chen et al., 2003; Wu et al., 2004).

Determination of the most suitable harvest time for spicas of *P. vulgaris*

There is an increasing interest in the use of natural substances (Deans & Svoboda, 1993) because many herbal medicines are free from side effects and easy to obtain, considered “green” and healthy, and generate income. Chemical markers, however, are often used for quality control of medicinal products. According to the Chinese Pharmacopoeia 2010, RA is the standard component for quality control of Prunellae Spica, and its content should be more than 2.0 mg·g⁻¹. Many pharmacological investigations have shown that active components, such as RA, UA, and OA, have important pharmacological effects (Liu, 1995; Psoyeva et al., 2003) and different content requirements for the quality control of medicinal products. For example, as a marker for quality control of Xiakucao Oral solution, the content of RA per mL should be more than 1.00 mg (Jin et al., 2009). For Jiliukang Oral liquid, the content of UA...
should be more than 0.50 mg per 10 mL (Deng et al., 2000).

In summary, present study indicates that the content of bioactive components in the spicas of *P. vulgaris* is closely related to harvest time, which should be determined according to the dynamics of target compound accumulation. Our results suggest that the optimal harvest time for maximum RA, UA, and OA accumulation in the spicas of *P. vulgaris* is early May.

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**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**References**


