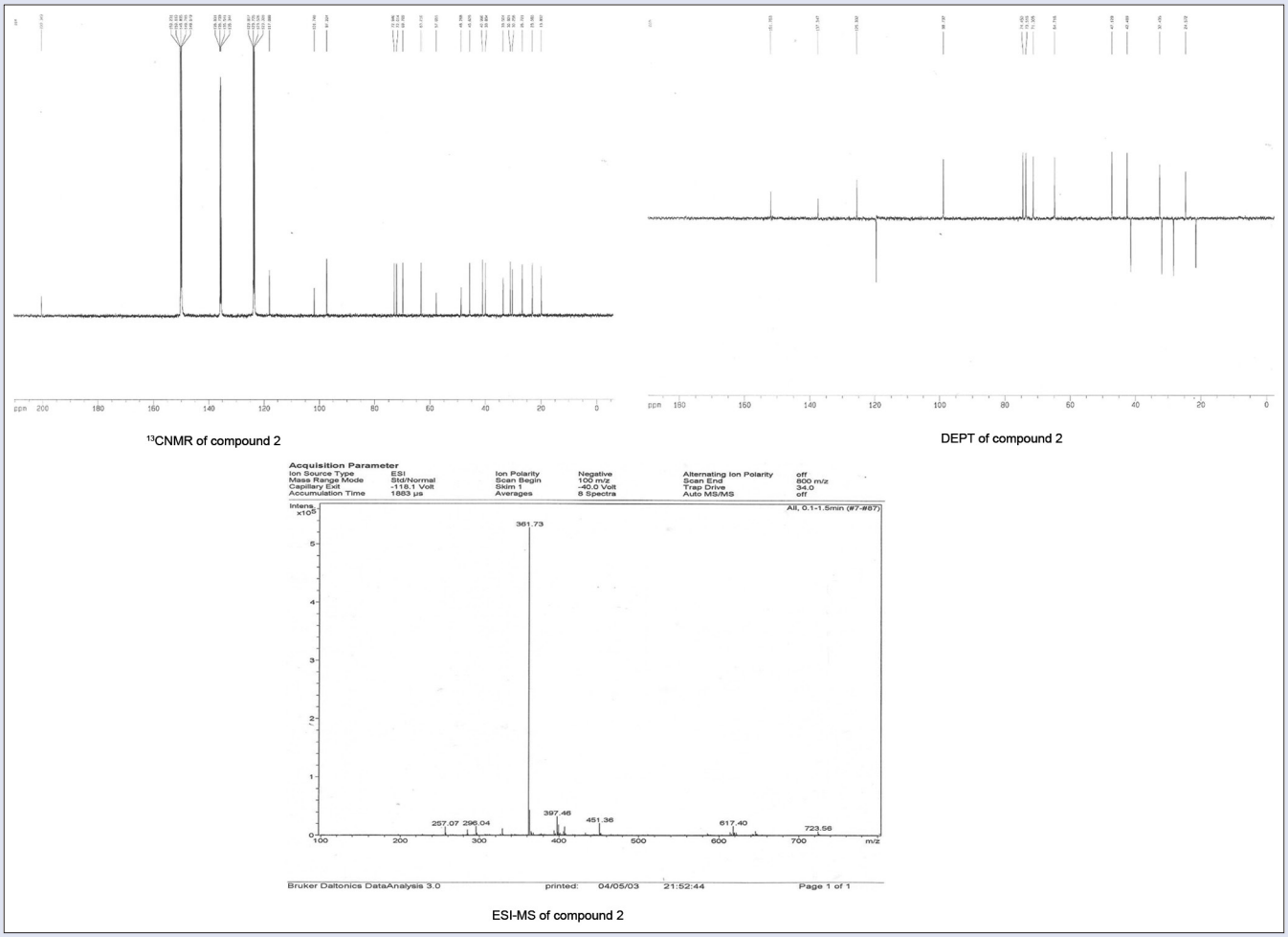

Figure 2: ^{13}CNMR spectrum and MS spectrum of compound 2

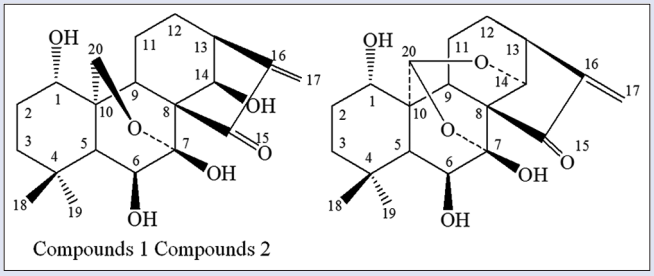

Figure 3: Structure of compound 1 (oridonin) and compound 2 (ponicidin)

content average of the oridonin and the ponicidin were 0.55% and 0.34%, respectively.

Stability test: Ten microliters of test sample solution was precisely measured and placed at room temperature. According to the above chromatographic conditions, the sample was injected and determined once every 2 h, and the determination was repeated five times for 10 h. The peak area values of the oridonin and the ponicidin were recorded, and the content determination results were calculated. The results showed that the RSD of the content average of the oridonin and the ponicidin in the tested sample solution were 0.85% and 0.30%, respectively. The results showed that the tested sample solution was stable within 10 hours.

Content determination of sample

About 2.0 g of *Rabdosia rubescens* powder collected from different months (July to October) was carefully weighed and prepared according to the above preparation method of test sample solution. Four test sample solutions were obtained. The contents of the oridonin and the ponicidin were calculated according to the above chromatographic conditions. The calculation results are shown in Table 2.

DISCUSSION

Determination of preparation method of test sample solution by HPLC method

In order to determine the preparation methods of test sample solution, different polar solvents, including methanol, ethanol, and acetone and different extraction methods, including ultrasonic extraction, soxhlet extraction, and maceration extraction were investigated, and the extraction time was optimized. The results found that continuous reflux extraction with methanol as a solvent for 8 h had the best effect. The extraction rate of the oridonin and the ponicidin were relatively the highest.

Selection of mobile phase in HPLC method

When selecting the mobile phase, methanol-water and methanol-phosphoric acid-aqueous solution systems were used.[18–20] It

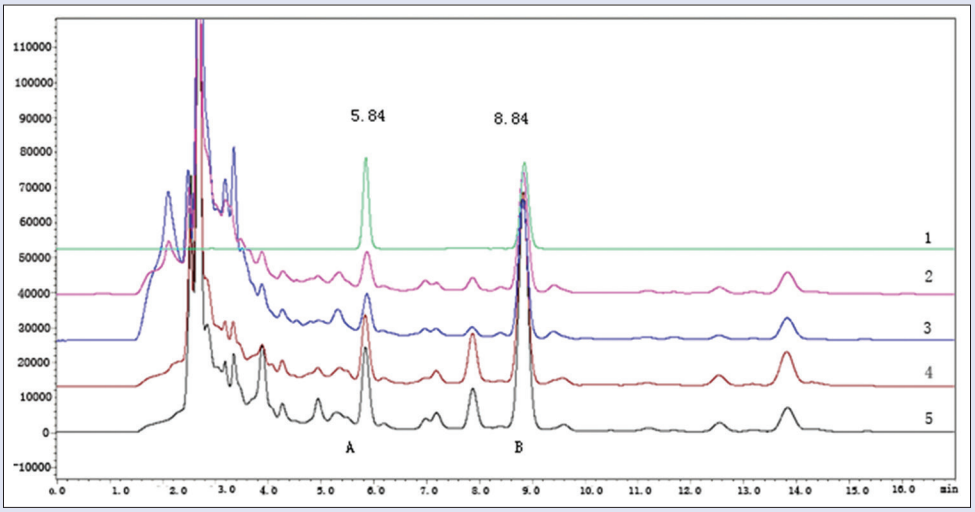

Figure 4: HPLC of reference substance and samples. (A: oridonin, the retention time is 5.84 min. B: ponicidin, the retention time is 8.84 min. 1: reference substance; 2: sample on July; 3: sample in October; 4: sample on August; 5: sample in September)

was found that the addition of acid had little effect on the separation degree and peak pattern. Therefore, methanol-water gradient elution was adopted. it can make the separation degree of the oridonin and the ponicidin reach more than 1.5, so methanol-water system was used in this experiment.

HPLC analysis of determination results

It has been reported[21] that the content of oridonin and the ponicidin in *R. rubescens* leaves is much higher than that in the whole *rubescens*, so the leaves of *R. rubescens* are used as medicinal parts in this study. It has been reported[22] that the best ripening and harvesting season of *R. rubescens* is from July to October every year, and the content of effective components is relatively highest. Therefore, in this study, leaves of *R. rubescens* at different periods from July to October were collected. The contents of the anti-cancer active ingredients oridonin and ponicidin were determined.

In this study, the chemical constituents of *R. rubescens* were extracted, isolated, and structure identified. and the oridonin and the ponicidin of the anti-tumor active components were used as reference substances to establish HPLC method. The content of *R. rubescens* collected from different periods was determined by HPLC method. The results showed that the best harvest time for *R. rubescens* was from August to September every year, and the content of the oridonin and the ponicidin was relatively highest. The specific harvest time was determined by the climate and precipitation of the year. The HPLC method established in this study was simple, rapid, accurate, reliable and reproducible, which provided a reference for drug application and resource utilization of *R. rubescens* and could be used for quantitative analysis of anti-cancer active ingredients in said herb.

Liu *et al.*[23,24] proved through experiments that the inhibition rate of cell growth of NB4 cells and leukemia HL-60 cell line cultured *in vitro* was treated with different concentrations of oridonin, and the results showed that oridonin could inhibit cell growth and induce cell apoptosis. Wang *et al.*[25] showed that *R. rubescens* extract could induce massive necrosis and apoptosis of ascites hepatoma H22 cells by intragastric administration in mice. Liu *et al.*[26] studied the mechanism of oridonin promoting phagocytosis of apoptotic bodies by macrophages differentiated from human lymphoma cells U937. Xiao *et al.*[27] found that oridonin had a significant inhibitory effect on the growth of human nasopharyngeal carcinoma cell line CNE cells. Liu *et al.*[28] observed the apoptosis of human colon cancer HCT8 cells induced by oridonin *in vitro*, and the apoptosis rate increased with the increase of concentration. Yang *et al.*[29] explored and found that oridonin has significant anti-DNA mutation effect. Guan *et al.*[30] showed that oridonin injection had a satisfactory effect on reducing the tumor size of liver cancer, relieving symptoms and improving the quality of life of patients. Oridonin has an obvious inhibitory killing effect on many cancer cell lines. Ponicidin had obvious cytotoxicity to ascites carcinoma cells cultured *in vitro*. Ponicidin has an inhibitory effect on a variety of transplanted tumors. It has an obvious anti-tumor effect on ascites cancer, S180 liver cancer, and L1 ascites cancer in mice, which significantly prolongs the survival time and makes some animals survive for a long time. It also has an obvious anti-tumor effect on reticulosarcoma and solid liver cancer.[31] Oridonin and ponicidin have good anti-cancer activity and low toxicity to normal cells, so they have good clinical application prospect. At present, there is no monomer preparation of oridonin and ponicidin in China except for the total extract preparation of *R. rubescens* in the early market. It is of great significance for the development and utilization of the abundant anti-tumor plant resources of *Rabdosia rubescens*.

Table 2: Contents of two anti-tumor active components in different harvesting time of *Rabdosia rubescens* (%, *n*=3)

Harvesting month	Planting area	Content of oridonin	Content of ponicidin
On July 18	Jiyuan city, Henan province	0.469%	0.124%
On August 20	Jiyuan city, Henan province	0.618%	0.203%
On September 20	Jiyuan city, Henan province	0.625%	0.216%
On October 18	Jiyuan city, Henan province	0.448%	0.127%

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Conflicts of interest

There are no conflicts of interest.

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