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# Research Article
# Anti-Colon Cancer Activity of *Patrinia villosa* Extract and Analysis of its Components based on UPLC-QTOF-MS/MS

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## Abstract

**Background and Objective:** *Patrinia villosa* was first recorded in *Shennong Bencao Jing* and enjoys a long medicinal history. Modern pharmacological studies show that it has definite anti-tumour pharmacological activity. The objective of this study was to determine its pharmacological activity against colon cancer metastasis and reveal its active components. **Materials and Methods:** In this study, the CCK-8 method, Transwell chamber experiment and wound healing assay were adopted to explore the inhibitory activity of *Patrinia villosa* extract on the proliferation, migration and invasion of human colon cancer SW620 cells. Furthermore, UPLC-QTOF-MS/MS technique was used to analyze the extract and the drug-containing plasma after oral administration of the extract in mice. **Results:** *Patrinia villosa* extract can inhibit the proliferation, migration and invasion of human colon cancer SW620 cells. Eighteen chemical components, including salicylic acid, daphnetin, oleuropein, isochlorogenic acid B and luteolin were identified in the extract. Among them, daphnetin, oleuropein and isochlorogenic acid B were first discovered in Patrinia. Besides, six chemical components, including salicylic acid and luteolin were introduced into the blood with prototypes. **Conclusion:** This study provides a basis for the clinical rational application of *Patrinia villosa* in the treatment of colon cancer metastasis and the disclosure of its pharmacodynamic material basis.

Key words: Anti-colon cancer metastasis, chemical components, components in plasma, *Patrinia villosa* (Thunb.) Juss., UPLC-QTOF-MS/MS

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

As first recorded in Sheng Nong's herbal classic, *Patrinia villosa* (Thunb.) Juss. has been used in China for thousands of years[1]. It uses dried whole herbs as medicine, with a pungent taste and cold nature and it is mainly produced in Northern, Eastern, Central, Southern and Southwest China. Besides, this medicine has the effects of clearing heat and detoxification, promoting dampness and expelling pus, facilitating blood circulation and removing blood stasis. The study on chemical constituents showed that *Patrinia villosa* (PV) contains saponins, flavonoids, sterols, iridoids and volatile oils[2,3]. Furthermore, traditional Chinese medicine believes that PV is the first choice for the treatment of intestinal carbuncle and abdominal pain and it is commonly used in the anti-tumour, anti-virus and anti-inflammatory treatment[2-5].

In this study, through the *in vitro* anti-tumour pharmacological efficacy test and literature investigation[6-8], the therapeutic efficacy sites of PV for colon cancer metastasis were determined. Targeting the scientific problem of unclear substances *in vivo* and *in vitro* of *Patrinia villosa* extract (PVE) in the treatment of colon cancer, UPLC-QTOF-MS/MS technology was adopted[9,10] to study the chemical constituents *in vivo* and *in vitro* of the extract and the drug-containing plasma of the extract after oral administration in mice.

Therefore, this study provided a scientific basis for revealing the pharmacodynamic substance basis and substance metabolism rule of PV in the treatment of colon cancer and also laid a foundation for the rational clinical application of PV in the treatment of colon cancer metastasis.

## MATERIALS AND METHODS

**Study area:** This study was carried out at the analysis and test centre, Liaoning University of Traditional Chinese Medicine, China from December, 2020 to May, 2021.

**Reagents and Materials:** The PV was purchased from Hebei Baicaokangshen Pharmaceutical Co., Ltd. and identified by Professor Xu Liang (Liaoning University of Traditional Chinese Medicine) as the *Patrinia villosa* (Thunb.) Juss. of the Valerianaceae, Patrinia genus. Besides, the human colon tumour SW620 cell line was bought from the cell bank of the Typical Culture Preservation Committee of the Chinese Academy of Sciences, while rutin, apigenin, caffeic acid, salicylic acid, scutellarin, chlorogenic acid, ferulic acid, isoorientin, isovitexin and luteolin (purities >98%) were obtained from Chengdu Pufeide Biotechnology Co., Ltd. (China). Apart from that, LC-MS-grade methanol and acetonitrile were from Merck (Germany), whereas, LC-MS-grade formic acid was acquired from Thermo Fisher Technology Co., Ltd. (USA).

**Preparation of PVE:** The PV powder were refluxed with 15 times 60% ethanol 3 times (1 hr each time). Then, vacuum spin steam at 40°C until the crude drug concentration is 0.1 g $mL^{-1}$. After that, PVE was purified with macroporous adsorption resin HPD-300. The extraction ratio was 3.01%. The mixed extract was dissolved in methanol to prepare a solution of 0.5 mg $mL^{-1}$ for UPLC-QTOF-MS/MS analysis.

**Preparation of plasma samples:** Sixteen Kunming mice, with a weight of about 20±2 g, were purchased from Liaoning Changsheng Biotechnology Co., Ltd. Besides, all experiments were performed following the approved animal protocols and guidelines established by the Medicine Ethics Review Committee for Animal Experiments of Liaoning University of Traditional Chinese Medicine. After one week of adaptive feeding, the mice were randomly classified into the blank group and the PVE group, with 8 mice in each group. Mice in the drug group were given PVE intragastric administration at a dose of 15.16 mg/20 g/day, while the mice in the blank group accepted an equal volume of purified water every day. After continuous gavage for one week, they fasted 12 hrs before the last gavage. Afterwards, the blood of the orbital venous plexus was collected and placed in a centrifuge tube containing 1% heparin sodium. After standing for 30 min, it was centrifuged at 3500 r $min^{-1}$ for 5 min and the supernatant was absorbed for later use. In addition, accurately absorb 200 µL plasma from each group into the 1.5 mL EP tube and add 1 mL of methanol solution precooled at 4°C, followed by vortex for 2 min and ultrasonic for 2 min. After that, it stood at -20°C for 10 min and was centrifuged at 8000 rpm $min^{-1}$ for 10 min at 4°C. Then, absorb the supernatant, dry it and add 50 µL corresponding deproteinization solvent to redissolve, followed by vortex for 2 min, ultrasonic for 2 min and being centrifuged at 4°C 8000 rpm $min^{-1}$ for 10 min. Afterwards, the supernatant was taken for determination.

**CCK-8 cell viability assay:** Human colon tumour cell line SW620 was cultured in the L-15 medium featuring 10% fetal bovine serum at 37, 5% $CO_2$ and saturated humidity. Cells in the logarithmic growth phase were plated in 96-well plates and treated with different concentrations of PVE (0.50, 0.75, 1.00, 1.25, 1.50, 1.75 mg $mL^{-1}$) for 24 hrs. After that, the optical density value (OD) was determined by the CCK-8 method[11], cell viability and $IC_{50}$ were confirmed according to the percentage of cell proliferation.

**Transwell chamber experiment:** Dilute Matrigel matrix glue diluent with the L-15 medium (dilution ratio = 1:8), was quickly added to Transwell upper chamber and placed in the 37 incubators for drying. Then, SW620 cells in the logarithmic growth stage were digested with trypsin and the 0.625 mg $mL^{-1}$ concentration of the drug serum-free medium was added to the administration group. Besides, the blank group was replaced with the serum-free medium, which was continuously cultured in the incubator for 24 hrs. As $1\times10^6$ cells/well were inoculated into the Transwell invasion chamber, each well was 200 µL and the lower chamber was added with the complete medium (containing 15% fetal bovine serum). Apart from that, the volume of each well was 600 µL. Continue to culture for 24 hrs, dye with crystal violet and dry it and then take photos under the microscope for counting (5 visual fields in each group at random)[12].

**Wound healing assay:** The SW620 cells in the logarithmic growth phase were inoculated into 6-well culture plates at a density of 1-$4\times10^5$ cells per well, being cultured for 24 hrs. Afterwards, draw a fine mark perpendicular to the wall of the cell culture plate with a gun head, wash the cell surface with PBS three times and add the culture medium containing 1% fetal bovine serum as the blank control, while the culture medium containing 1% fetal bovine serum with 0.625 mg $mL^{-1}$ concentration of drugs as the drug stimulation group (3 wells in each group). Next, the trace widths of different treatment groups were observed after 0, 12 and 24 hrs, followed by the blank group being taken as reference and the relative migration distance of the drug groups at different times being calculated[13].

**Identification of *in vitro* chemical constituents of PVE:** Chromatography was performed on the Agilent 1290 series HPLC system (Agilent Technologies, USA). Chromatographic separation was performed on an Agilent poroshell 120 SB-C18 chromatographic columns (100×4.6 mm, 2.7 µm). The mobile phase was 0.1% formic acid aqueous solution (A) and acetonitrile (B). Besides, the gradient elution procedure lasted for 0~25 min (5→70% B) while the flow rate was 0.4 mL $min^{-1}$ and the column temperature was maintained at 30°C. Moreover, the injection volume was 10 µL.

Mass spectrometry was conducted on the Agilent 6550 iFunnel Q-TOF equipped with an ESI source (Agilent Technologies, USA). The electrospray ion source was determined by the negative ion mode. The capillary voltage, the sheath gas temperature, the dry gas temperature, the sheath gas flow rate, the dry gas flow rate, the atomizer pressure, the fragmentation voltage, the mass range and the collision energy were 4000V, 350 degrees, 250 degrees, 11 and 13 L $min^{-1}$, 45 psig, 125V, 100~1500 m/z and 20 and 40 eV, respectively.

**Identification of *in vivo* chemical constituents of PVE:** The mobile phase was 0.1% formic acid aqueous solution (A) and acetonitrile: methanol (95:5) (B). The gradient elution procedure lasted for 0~45 min (5→100% B), while other chromatographic and mass spectrometry analysis conditions were the same as those of *in vitro* chemical constituent analysis.

**Data processing:** The SPSS19.0 software was employed to analyze the data and the quantitative data were in line with normal distribution, expressed as the Means±SEM as indicated. Apart from that, a comparison between the two groups was performed by the independent sample T-test, when $p<0.05$ indicates a significant difference, while, $p<0.01$ refers to a very significant difference.

## RESULTS

**Cell viability, migration and invasion assay:** The CCK-8 method was adopted to detect the effect of different concentrations of PVE on the cell viability of human colon tumour SW620 cells after 24 hrs and the $IC_{50}$ value of its half inhibitory concentration. The results showed that different concentrations of PVE (0.50, 0.75, 1.00, 1.25, 1.50 and 1.75 mg $mL^{-1}$) could significantly inhibit the proliferation of colon tumour cells, the cell viability was 68.89±3.62, 53.37±2.69, 27.10±2.52, 20.56±2.17, 9.76±1.09 and 7.97±1.11%, respectively and there was a significant difference compared with the blank control group ($p<0.01$) (Fig. 1a). Here, it should be mentioned that the $IC_{50}$ value is 0.625 mg $mL^{-1}$.

The results of the Transwell chamber showed that compared with the blank group (1.00±0.005), a significant difference appears in the PVE group (0.54±0.025) ($p<0.01$) (Fig. 1b), which demonstrated that PVE can inhibit the invasion and metastasis of the colon cancer cells to a great extent. Furthermore, wound healing assay results showed that when at 12 hrs the relative migration distance of the blank group is 0.82±0.03, while the administration group is 0.93±0.04 and at 24 hrs the relative migration distance of the blank group is 0.69±0.03, while the administration group is 0.86±0.02. After statistical analysis, it manifested that compared with the blank group, there were significant differences in the administration group at 12 and 24 hrs ($p<0.05$) and the longer the administration time, the more obvious the differences (Fig. 1c). The results also indicated that PVE could inhibit the migration of colon cancer cells.

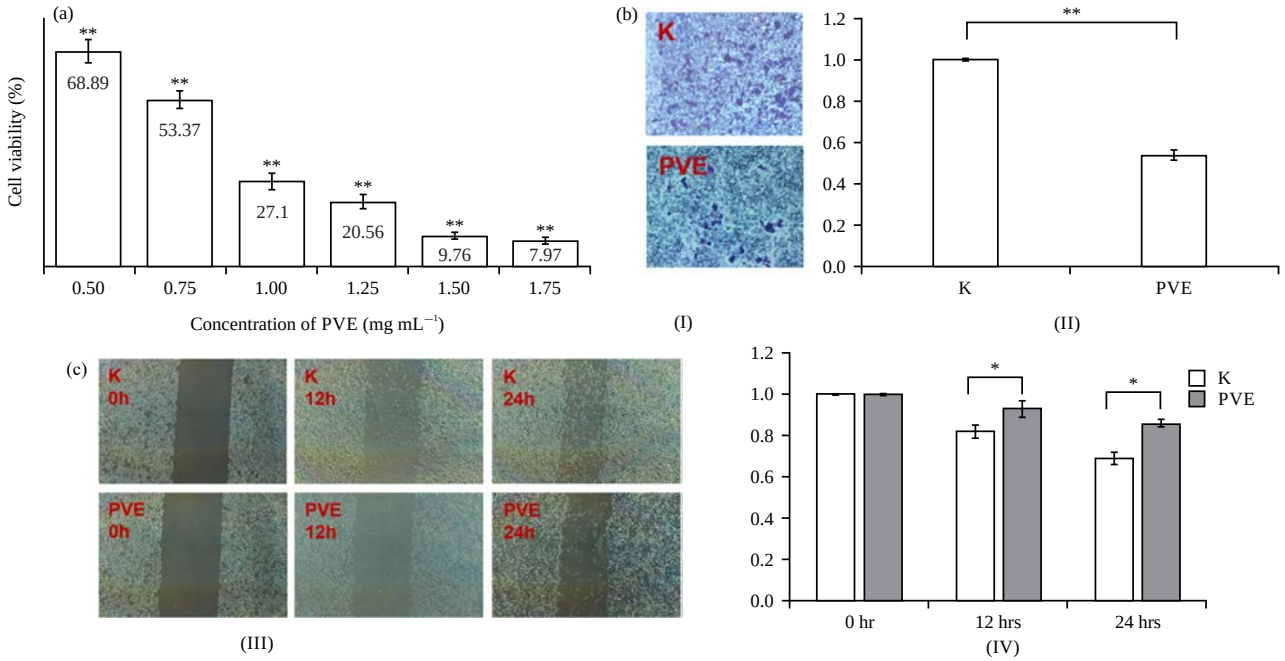



Fig. 1(a-c): Antitumor activity of PVE against colon cancer and its inhibitory effect on migration and invasion of SW620 cells, (a) Effects of PVE on the cell viability of SW620 cells, (b)(I) : Transwell chamber pictures of each group, (II): Relative number of cells in each group, (c) (III): Wound healing pictures of each group at different time points, (IV): Relative wound healing width of each group

*p<0.05 and **p<0.01

Table 1: Analytical results of *in vitro* chemical components of *Patrinia villosa* extract

| No. | Retention time (RT) (min) | Formula | Ion type | Theoretical mass (Da) | Calculated mass (Da) | Mass error (ppm) | Fragmentations | Identifications |
|---|---|---|---|---|---|---|---|---|
| 1* | 2.726 | $C_7H_6O_3$ | $[M-H]^-$ | 137.0244 | 137.0251 | -5.11 | 137,119 | Salicylic acid |
| 2* | 3.885 | $C_{16}H_{18}O_9$ | $[M-H]^-$ | 353.0878 | 353.0887 | -2.55 | 353,191 | Chlorogenic acid |
| 3 | 4.001 | $C_9H_6O_4$ | $[M-H]^-$ | 177.0193 | 177.02 | 3.95 | 177,75 | Daphnetin[14] |
| 4* | 4.133 | $C_9H_8O_4$ | $[M-H]^-$ | 179.0350 | 179.0352 | -1.12 | 179,146 | Caffeic acid |
| 5 | 4.713 | $C_{12}H_{14}O_6$ | $[M-H]^-$ | 253.0718 | 253.0722 | -1.58 | 253,194 | Dimethyl prephenate |
| 6 | 5.007 | $C_{27}H_{30}O_{15}$ | $[M-H]^-$ | 593.1512 | 593.1525 | -2.19 | 593,285 | Kaempferol-3-rutinoside[15] |
| 7* | 5.110 | $C_{27}H_{30}O_{16}$ | $[M-H]^-$ | 609.1461 | 609.1463 | -0.33 | 609,300 | Rutin |
| 8 | 5.243 | $C_{33}H_{40}O_{20}$ | $[M-H]^-$ | 755.2040 | 755.2057 | -2.25 | 755,384 | Camelliaside A[16] |
| 9 | 5.756 | $C_{25}H_{32}O_{13}$ | $[M-H]^-$ | 539.1770 | 539.1759 | 2.04 | 539,377,149 | Oleuropein[17] |
| 10* | 6.568 | $C_{21}H_{20}O_{11}$ | $[M-H]^-$ | 447.0933 | 447.0954 | -4.70 | 447,357 | Isoorientin |
| 11* | 7.644 | $C_{21}H_{20}O_{10}$ | $[M-H]^-$ | 431.0984 | 431.0998 | -3.25 | 431,413,311 | Isovitexin |
| 12 | 7.793 | $C_{27}H_{30}O_{15}$ | $[M-H]^-$ | 593.1512 | 593.1532 | -3.37 | 593,285,147 | Kaempferol-3-O-β-D-galactolipin (6→1)α-L-rhamnoside[18] |
| 13* | 7.992 | $C_{21}H_{18}O_{12}$ | $[M-H]^-$ | 461.0725 | 461.0746 | -4.55 | 461,285 | Scutellarin |
| 14* | 8.539 | $C_{10}H_{10}O_4$ | $[M-H]^-$ | 193.0506 | 193.0516 | -5.18 | 193,179 | Ferulic acid |
| 15* | 9.284 | $C_{15}H_{10}O_5$ | $[M-H]^-$ | 269.0455 | 269.0465 | -3.72 | 269,225,151 | Apigenin |
| 16 | 9.632 | $C_{25}H_{24}O_{12}$ | $[M-H]^-$ | 515.1195 | 515.1210 | -2.91 | 515,329 | Isochlorogenic acid B[19] |
| 17 | 9.913 | $C_{29}H_{36}O_{11}$ | $[M-H]^-$ | 559.2185 | 559.2209 | -4.29 | 559,285 | Phragmalin[20] |
| 18* | 11.669 | $C_{15}H_{10}O_6$ | $[M-H]^-$ | 285.0405 | 285.0419 | -4.91 | 151,133 | Luteolin |

*identified with standard

**Analysis of *in vitro* and *in vivo* chemical constituents of PVE:** In this study, 18 compounds *in vitro* were identified by the methods of reference substance comparison and database query, combined with the accurate molecular weight, fragment ion weight, retention time and molecular weight deviation (ppm) of the compounds (Table 1, Fig. 2a). To be specific, they were salicylic acid, chlorogenic acid, daphnetin, caffeic acid, dimethyl prephenate, kaempferol-3-rutinoside, rutin, camelliaside A, oleuropein, isoorientin, isovitexin, kaempferol-3-O-β-D-galactolipin(6→1)α-L-rhamnoside, scutellarin, ferulic acid, apigenin, isochlorogenic acid B, phragmalin and luteolin, most of which were flavonoids and

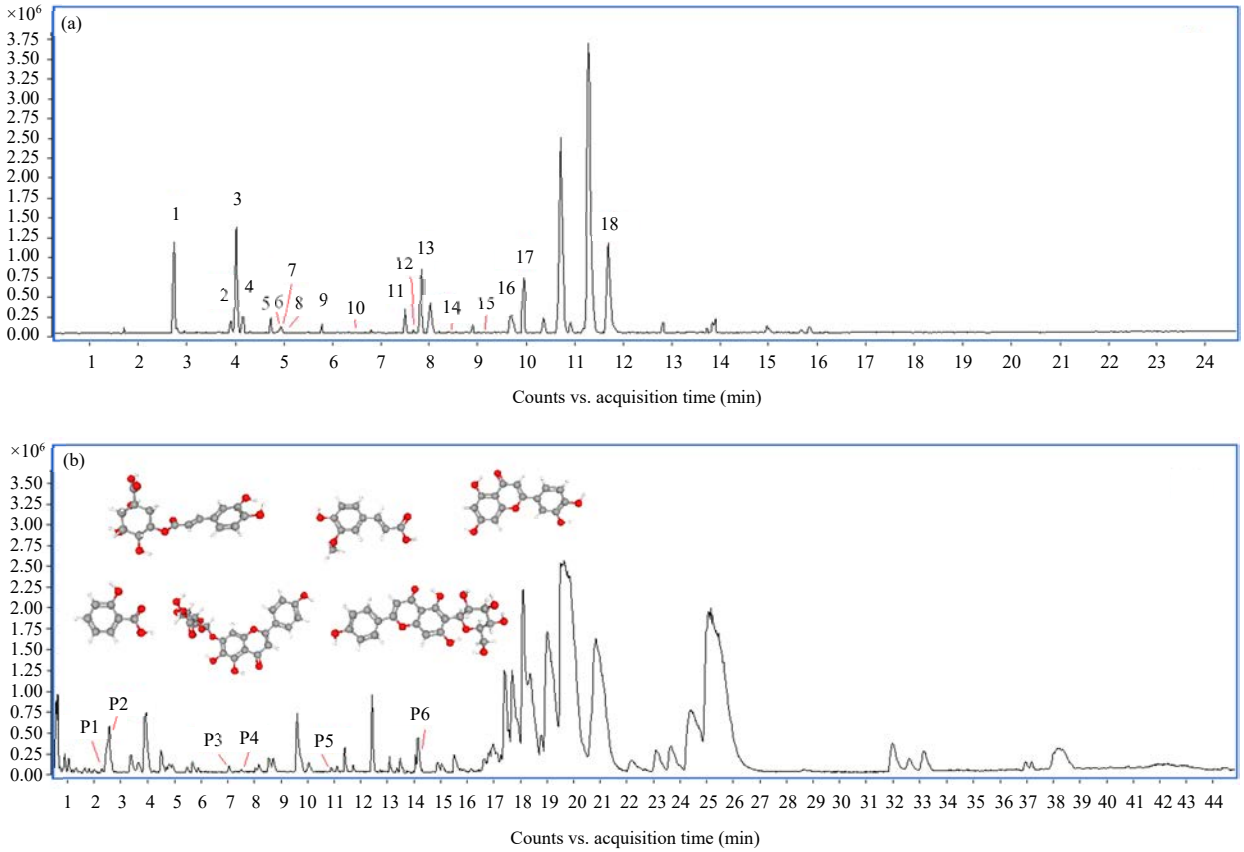



Fig. 2(a-b): Base peak chromatogram (BPC) of *in vitro* and *vivo* components of PVE in negative mode, (a) BPC of *in vitro* chemical components of PVE, and (b) BPC of *in vivo* blood components of PVE

Table 2: Analytical results of blood components from *Patrinia villosa* extract

| No. | Retention time (RT) (min) | Formula | Ion type | Theoretical mass (Da) | Calculated mass (Da) | Mass error (ppm) | Fragmentations | Identifications |
|---|---|---|---|---|---|---|---|---|
| P1* | 2.350 | $C_7H_6O_3$ | $[M-H]^-$ | 137.0244 | 137.0249 | -3.65 | 137,119 | Salicylic acid |
| P2* | 2.811 | $C_{16}H_{18}O_9$ | $[M-H]^-$ | 353.0878 | 353.0864 | 3.97 | 353,191 | Chlorogenic acid |
| P3* | 6.937 | $C_{21}H_{18}O_{12}$ | $[M-H]^-$ | 461.0745 | 461.0784 | -8.46 | 461,285 | Scutellarin |
| P4* | 7.517 | $C_{10}H_{10}O_4$ | $[M-H]^-$ | 193.0536 | 193.0548 | -6.22 | 193,179 | Ferulic acid |
| P5* | 10.752 | $C_{21}H_{20}O_{10}$ | $[M-H]^-$ | 431.0984 | 431.0967 | 3.94 | 431,413,311 | Isovitexin |
| P6* | 14.384 | $C_{15}H_{10}O_6$ | $[M-H]^-$ | 285.0405 | 285.0409 | -1.40 | 151,133 | Luteolin |

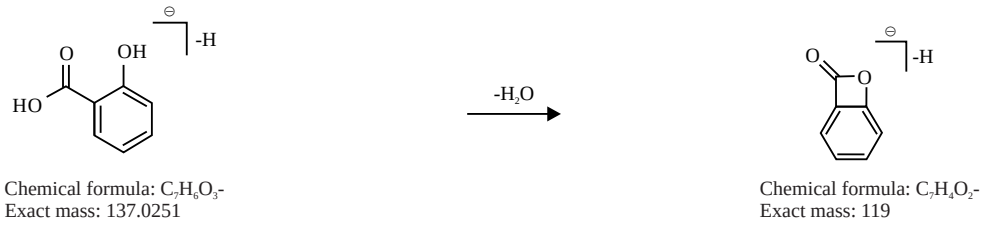
*Identified with standard

organic acids. Other than that, there were 9 flavonoids and 5 organic acids. Among these compounds, daphnetin, oleuropein and isochlorogenic acid B were found in Patrinia for the first time.

Besides, with the method of serum pharmacochemistry, 6 prototype blood components including salicylic acid, chlorogenic acid, scutellarin, ferulic acid, isovitexin and luteolin were identified in mice plasma by comparison of reference substances according to their retention time, MS and MS/MS data information (Table 2, Fig. 2b). Among the 6 compounds, there are 3 phenolic acids and 3 flavonoids.

Through the analysis of *in vitro* and *in vivo* chemical components of PVE, it was found that the chemical components contained in the extract and the blood components were mostly carboxyl or hydroxyl compounds and these compounds had a good response in the negative ion mode. Thus, in this study, the negative ion mode was adopted for QTOF-MS analysis. And taking the six prototype blood components as an example, the cracking law and identification process of these components is analyzed as follows.

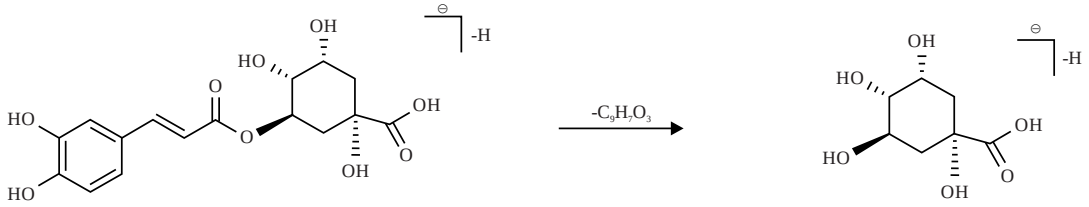
Peak 1 yielded the base peak $[M-H]^-$ at m/z 137.0251. Under the condition of 20V, one molecule $H_2O$ was removed to generate m/z 119 $[M-H-H_2O]^-$. Compared with the salicylic acid reference substance, the fragment ion information is consistent with it and it is determined to be salicylic acid (Table 2, Fig. 3). Peak 2 showed molecular ions at m/z 353.0878 $[M-H]^-$ and exhibited m/z 191 $[M-H-C_9H_7O_3]^-$ ions in the $MS^2$




Chemical formula: $C_7H_6O_3$-
Exact mass: 137.0251

Chemical formula: $C_7H_4O_2$-
Exact mass: 119

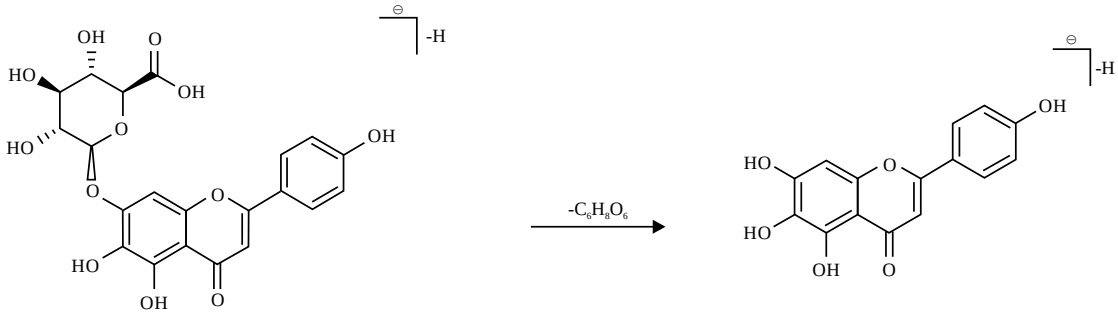
Fig. 3: Proposed fragmentation pathway of salicylic acid




Chemical formula: $C_{16}H_{18}O_9$-
Exact mass: 353.0887

Chemical formula: $C_7H_{12}O_6$-
Exact mass: 191

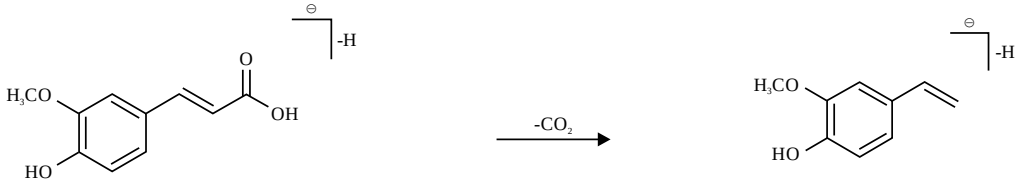
Fig. 4: Proposed fragmentation pathway of chlorogenic acid




Chemical formula: $C_{21}H_{18}O_{12}$-
Exact mass: 461.0746

Chemical formula: $C_{15}H_{10}O_6$-
Exact mass: 285

Fig. 5: Proposed fragmentation pathway of scutellarin




Chemical formula: $C_{10}H_{10}O_4$-
Exact mass: 193.0516

Chemical formula: $C_9H_{10}O_2$-
Exact mass: 149

Fig. 6: Proposed fragmentation pathway of ferulic acid

spectra, which arose from the loss of $C_9H_7O_3$. By being compared with the retention time and the fragmentation pattern, peak 2 was identified as chlorogenic acid (Table 2, Fig. 4). Moreover, in terms of peak 3, the base peak appeared at m/z 461.0745 $[M-H]^-$ and the main fragment ion was m/z 285 $[M-H-C_6H_8O_6]^-$. Beyond that, the loss of glucuronide (176 Da) was presented and it was the characteristics of flavonoid-O-glucuronide. Moreover, compared with the reference substance, it is identified to be scutellarin (Table 2, Fig. 5). Apart from that, the molecular ion of peak 4 is m/z 193.0548 $[M-H]^-$ and the fragment ion exists at m/z 179 $[M-H-CO_2]^-$ due to the loss of the neutral ion of $CO_2$. Besides, by comparison with the authentic reference standard, it is identified as ferulic acid (Table 2, Fig. 6). As for

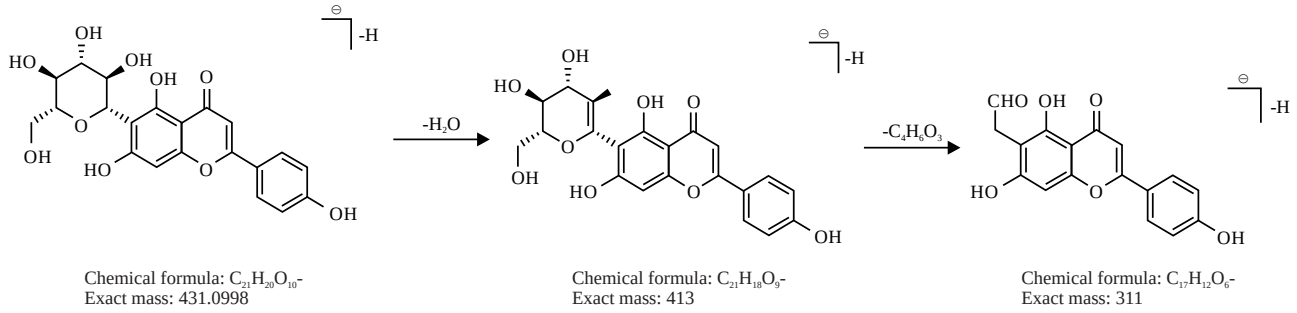



Fig. 7: Proposed fragmentation pathway of isovitexin

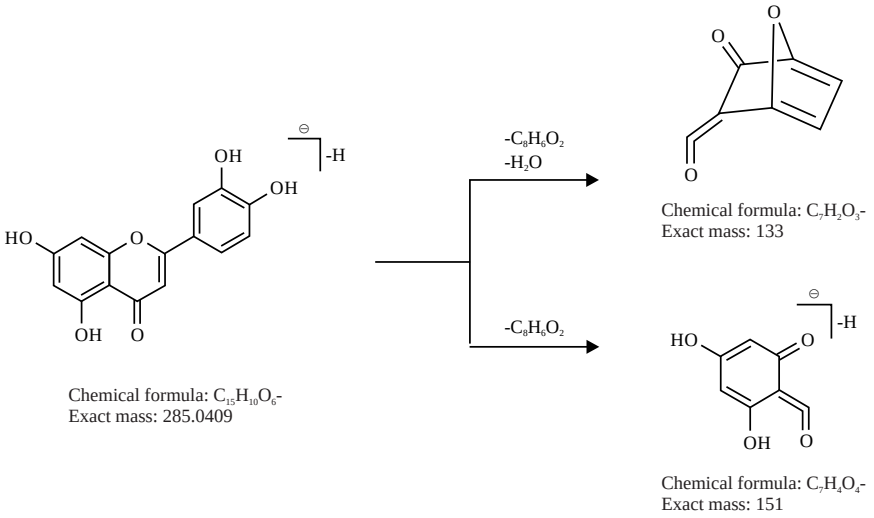



Fig. 8: Proposed fragmentation pathway of luteolin

peak 5, it showed a $[M-H]^-$ ion at m/z 431.0998 in the MS scan and produced the fragment ions at m/z 413 that represent the loss of $H_2O$ and $C_4H_6O_3$ was further removed to generate the m/z 311 $[M-H-H_2O-C_4H_6O_3]^-$ unit, which is the characteristic fragmentation of C-glycoside moiety. In addition, by comparison with the retention time and the fragmentation pattern, peak 5 was identified as isovitexin (Table 2, Fig. 7). Furthermore, the excimer ion peak of peak 6 is m/z 285.0409 $[M-H]^-$, while RDA cleavage occurs at 40V to obtain fragment ions m/z 151 $[M-H-C_8H_6O_2]^-$ and 133 $[M-H-C_8H_6O_2-H_2O]^-$. Considering that the secondary spectrum is consistent with that of the reference substance, it is inferred that peak 6 is luteolin (Table 2, Fig. 8).

## DISCUSSION

Previous literature reports on the treatment of colon cancer with PV mostly focused on its inhibition of tumour cell proliferation[7]. It was found that PV extracts with different polarities all had proliferation inhibitory effects on Caco-2 colon cancer cells and PVE presents best, with an inhibition rate of 84.38%[21]. But few people paid attention to its inhibition of colon cancer metastasis. Through this experiment, it is found that PVE has a clear pharmacological and pharmacodynamic effect of inhibiting the proliferation, invasion and migration of colon cancer cells. It can jointly inhibit cancer cells through a variety of mechanisms to achieve the purpose of treating colon cancer. The above research results provide a scientific theory for the drug formation of PVE and also present a scientific basis for verifying that PV is an ancient herbal medicine for the traditional treatment of intestinal carbuncle and abdominal pain.

Based on finding that PVE has significant inhibitory activity against colonic tumour metastasis, this study further revealed the chemical components and prototype absorbed components of PVE by using UPLC-QTOF-MS/MS technology combined with tandem mass spectrometry fragment ion information and reference substance comparison method. Most of the components absorbed in the blood are considered

potential active ingredients for further study[21,22]. The six blood inflow components found in this study, including salicylic acid, chlorogenic acid, scutellarin, ferulic acid, isovitexin and luteolin have been reported to have different degrees of antitumor activity. Salicylic acid has observationally been shown to decrease colorectal cancer (CRC) risk[23]. Chlorogenic acid is considered to be an effective chemical protective agent for cancer and possesses a significant inhibitory effect on colorectal cancer and other cancers[24]. Scutellarin can inhibit migration and increased the apoptosis of colorectal cancer cell lines[25]. Ferulic acid can inhibit colorectal cancer cell proliferation through down-regulating epidermal growth factor receptor (EGFR)[26]. At the same time, its metabolites produced under the action of intestinal flora *in vivo* have a significant inhibitory effect on the proliferation of chemotherapy-resistant cells[27]. Isovitexin is a chemopreventive compound with activity against various cancers through proapoptotic processes and/or autophagy[28]. It can regulate apoptosis and epithelial-mesenchymal transformation through the PI3K/Akt/mTOR signalling pathway, to reduce the tumour growth of human colon cancer cells[29]. Luteolin can regulate the expression of iNOS and COX-2 and inhibit the expression of MMP-2 and MMP-9, to play a role in the treatment of colon cancer[30].

The above findings provide some theoretical support for proving that they are the pharmacodynamic material basis for PVE to exert an anti-tumour effect. It also provides some confidence for the development of PVE as a drug for the treatment of colon cancer. In the future, the research group will reveal this scientific connotation through further research on the mechanism of action.

## CONCLUSION

In this study, it was discovered that PVE had definite pharmacological effects on inhibiting the proliferation, invasion and migration of colon cancer cells. A total of 18 compounds were identified *in vitro* when they were mainly flavonoids and organic acids. Among them, 6 were introduced into the blood as prototypes. Therefore, this study provided a scientific basis for revealing the pharmacodynamic substance basis and substance metabolism rule of PV in the treatment of colon cancer.

## SIGNIFICANCE STATEMENT

Previous literature reports on the treatment of colon cancer with PV mostly focused on its inhibition of tumour cell proliferation, but few people paid attention to its inhibition of colon cancer metastasis. Based on proving that PV has clear pharmacological activity against colon cancer metastasis, this study revealed its possible active components and their transformation law *in vivo* and found three new compounds in Patrinia for the first time, which laid a good foundation for the clinical rational application of PV in the treatment of colon cancer metastasis and the disclosure of the material basis of its efficacy.

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