# Supplementary Materials

## Quantitative and Differential Analysis between *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd. Using High-Performance Liquid Chromatography-Mass Spectrometry and Gas Chromatography-Mass Spectrometry Coupled with Multivariate Statistical Analysis

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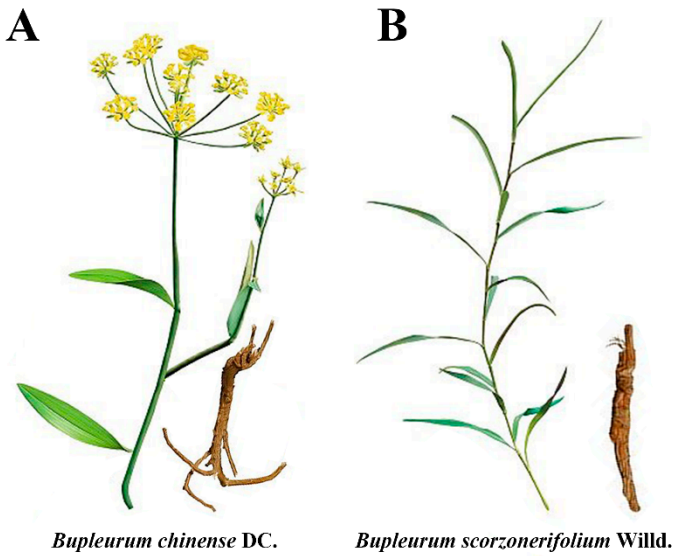


**Figure S1.** The pictures of *Bupleurum chinense* DC. (A) and *Bupleurum scorzonerifolium* Willd. (B).

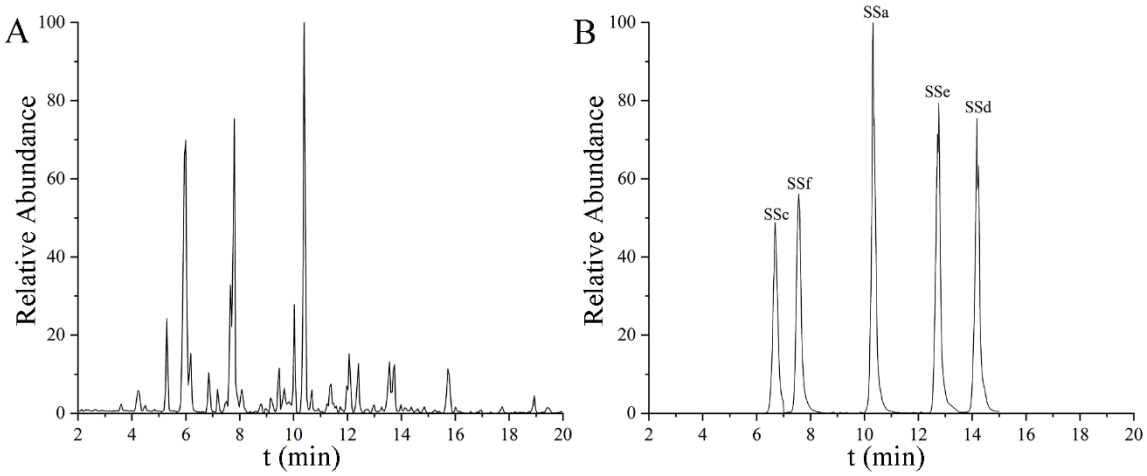


**Figure S2.** Total ion chromatograms of extracted saponins detected by HPLC-MS in full scan (A) and MRM mode (B).

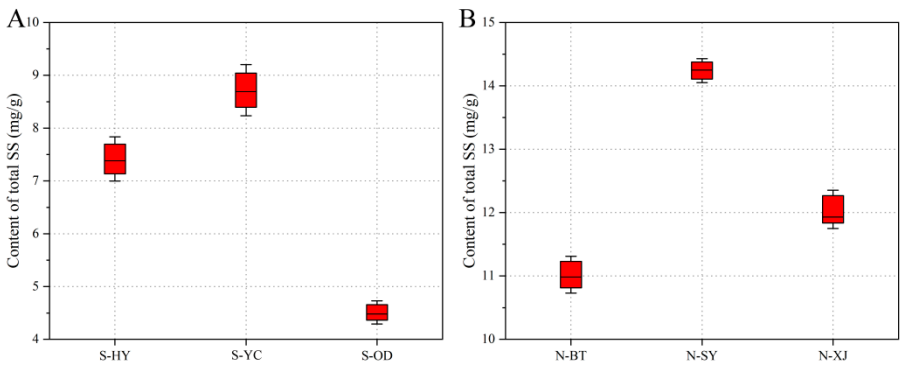


**Figure S3.** Boxplots of the total saikosaponin contents in the South (A) and North (B) RB samples from different growing regions.

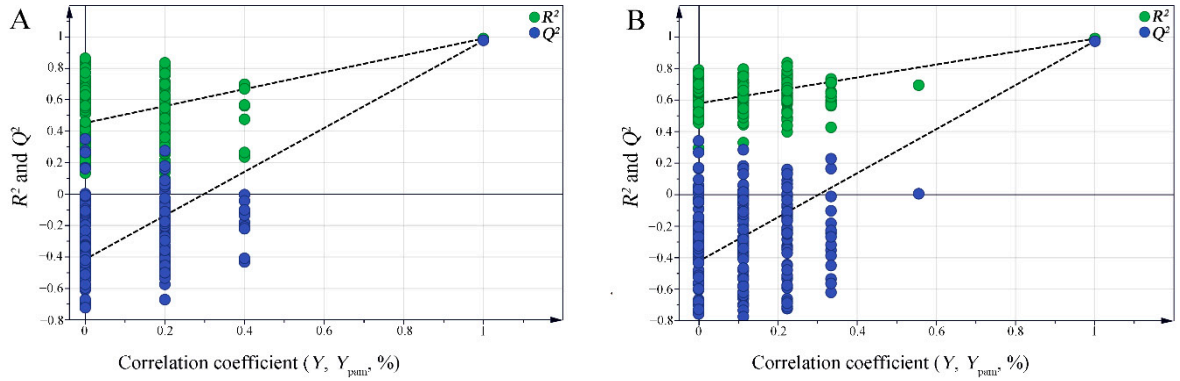


**Figure S4.** Permutation tests of PLS-DA models derived from the HPLC-MS data sets of saikosaponins (A) and the GC-MS data sets of volatile oils (B) in the Radix Bupleuri samples.

**Table S1.** The absolute contents of the five and total saikosaponins in the North and South RB samples.

| No. | Content of SS[1] (μg/mg) | | | | | |
|---|---|---|---|---|---|---|
| | SSa | SSc | SSd | SSe | SSf | Total SS |
| N-BT-1[2] | 3.523±0.008 | 0.820±0.006 | 5.853±0.007 | 0.048±0.004 | 0.334±0.011 | 10.578±0.047 |
| N-BT-2 | 3.572±0.023 | 0.829±0.002 | 6.070±0.029 | 0.036±0.013 | 0.398±0.009 | 10.904±0.050 |
| N-BT-3 | 3.632±0.012 | 0.834±0.010 | 6.233±0.009 | 0.055±0.007 | 0.308±0.006 | 11.063±0.016 |
| N-BT-4 | 3.568±0.009 | 0.821±0.031 | 5.877±0.004 | 0.057±0.017 | 0.328±0.007 | 10.652±0.023 |
| N-BT-5 | 3.710±0.012 | 0.815±0.043 | 6.314±0.023 | 0.033±0.008 | 0.349±0.009 | 11.221±0.011 |
| N-BT-6 | 3.714±0.012 | 0.845±0.022 | 6.730±0.004 | 0.042±0.002 | 0.378±0.012 | 11.710±0.021 |
| N-SY-1 | 4.564±0.051 | 0.935±0.066 | 8.076±0.046 | 0.095±0.003 | 0.619±0.007 | 14.290±0.056 |
| N-SY-2 | 4.639±0.064 | 0.962±0.005 | 7.873±0.034 | 0.107±0.009 | 0.628±0.006 | 14.209±0.087 |
| N-SY-3 | 4.378±0.039 | 0.955±0.005 | 8.470±0.035 | 0.094±0.011 | 0.638±0.009 | 14.535±0.077 |
| N-SY-4 | 4.589±0.054 | 0.983±0.009 | 8.164±0.067 | 0.141±0.002 | 0.651±0.023 | 14.528±0.022 |
| N-SY-5 | 4.219±0.006 | 0.968±0.015 | 7.971±0.008 | 0.111±0.013 | 0.613±0.029 | 13.882±0.052 |
| N-SY-6 | 4.182±0.051 | 0.951±0.013 | 8.097±0.005 | 0.105±0.002 | 0.664±0.019 | 13.998±0.067 |
| N-XJ-1 | 3.816±0.028 | 0.892±0.011 | 6.514±0.006 | 0.079±0.007 | 0.522±0.022 | 11.823±0.039 |
| N-XJ-2 | 3.670±0.039 | 0.891±0.009 | 6.888±0.041 | 0.055±0.009 | 0.503±0.002 | 12.037±0.022 |
| N-XJ-3 | 3.822±0.077 | 0.911±0.024 | 6.469±0.019 | 0.057±0.006 | 0.515±0.008 | 11.775±0.091 |
| N-XJ-4 | 3.956±0.008 | 0.922±0.003 | 7.452±0.014 | 0.062±0.008 | 0.469±0.009 | 12.866±0.068 |
| N-XJ-5 | 3.914±0.009 | 0.894±0.005 | 6.739±0.091 | 0.091±0.001 | 0.478±0.007 | 12.116±0.011 |
| N-XJ-6 | 3.855±0.042 | 0.860±0.029 | 6.418±0.006 | 0.066±0.007 | 0.494±0.004 | 11.694±0.041 |
| S-HY-1[3] | 2.499±0.049 | 0.611±0.016 | 3.851±0.009 | 0.024±0.002 | 0.146±0.014 | 7.118±0.087 |
| S-HY-2 | 2.533±0.023 | 0.614±0.007 | 3.993±0.047 | 0.020±0.002 | 0.174±0.007 | 7.327±0.059 |
| S-HY-3 | 2.576±0.025 | 0.611±0.003 | 4.101±0.079 | 0.019±0.003 | 0.135±0.004 | 7.438±0.073 |
| S-HY-4 | 2.530±0.024 | 0.605±0.005 | 3.867±0.038 | 0.024±0.001 | 0.143±0.002 | 7.169±0.079 |
| S-HY-5 | 2.632±0.028 | 0.623±0.007 | 4.154±0.047 | - | 0.152±0.001 | 7.573±0.091 |
| S-HY-6 | 2.634±0.025 | 0.631±0.005 | 4.428±0.068 | 0.019±0.003 | 0.165±0.001 | 7.873±0.089 |
| S-YC-1 | 2.888±0.042 | 0.705±0.007 | 4.573±0.072 | 0.027±0.002 | 0.182±0.003 | 8.375±0.075 |
| S-YC-2 | 2.928±0.027 | 0.715±0.014 | 4.742±0.074 | 0.024±0.004 | 0.216±0.002 | 8.625±0.086 |
| S-YC-3 | 2.977±0.072 | 0.721±0.026 | 4.870±0.086 | 0.019±0.001 | 0.168±0.003 | 8.754±0.087 |

| | | | | | | |
|---|---|---|---|---|---|---|
| S-YC-4 | 2.924±0.043 | 0.708±0.007 | 4.592±0.087 | 0.022±0.002 | 0.178±0.004 | 8.419±0.091 |
| S-YC-5 | 3.041±0.045 | 0.714±0.015 | 4.933±0.064 | 0.021±0.002 | 0.189±0.001 | 8.898±0.091 |
| S-YC-6 | 3.044±0.036 | 0.721±0.007 | 5.258±0.075 | - | 0.206±0.007 | 9.239±0.098 |
| S-OD-1 | 1.367±0.017 | 0.586±0.007 | 2.314±0.035 | 0.010±0.002 | 0.065±0.007 | 4.342±0.058 |
| S-OD-2 | 1.406±0.035 | 0.546±0.015 | 2.400±0.053 | - | 0.078±0.014 | 4.436±0.058 |
| S-OD-3 | 1.410±0.046 | 0.570±0.016 | 2.464±0.062 | 0.019±0.002 | 0.070±0.005 | 4.527±0.078 |
| S-OD-4 | 1.445±0.017 | 0.570±0.005 | 2.323±0.033 | 0.021±0.017 | 0.064±0.018 | 4.415±0.059 |
| S-OD-5 | 1.461±0.035 | 0.575±0.015 | 2.496±0.046 | 0.020±0.001 | 0.061±0.007 | 4.599±0.076 |
| S-OD-6 | 1.452±0.014 | 0.553±0.013 | 2.660±0.035 | 0.018±0.003 | 0.074±0.012 | 4.748±0.085 |

(1) SSa, SSc, SSd, SSe, SSf, and SS are the abbreviations of saikosaponin a, saikosaponin c, saikosaponin d, saikosaponin e, saikosaponin f, and saikosaponins, respectively.

(2) N-BT, N-SY, and N-XJ represent *Bupleurum chinense* DC. grown in Baotou city, Sanyuan county, and Xinjiang county, respectively.

(3) S-HY, S-YC, and S-OD represent *Bupleurum scorzonerifolium* Willd. grown in Heyang county, Yuncheng city, and Ordos city, respectively.

**Table S2.** ANOVA of the effects of variety and growing region on the contents of the five saikosaponins.

| Factor | SSa | SSc | SSd | SSe | SSf |
|---|---|---|---|---|---|
| Variety | * | * | * | * | * |
| Growing region | * | * | * | * | * |

* significant difference at the level of $p < 0.05$.

**Table S3.** Comparison of current and reported work

| | Present study | Reference 6* |
|---|---|---|
| Plants | 18 batches of two-year-old North RB and 18 batches of two-year-old South RB, collected from Shaanxi Province, Shanxi Province, and Inner Mongolia Autonomous Region, China. | 12 wild North RB and 12 wild South RB, at least one-year-old, collected from the suburban mountainous areas of Beijing, China, |
| Parts of the plants | Root | Root, stem, leaf, and flower |
| Extraction method | Sonication in 15% ammonia-methanol | Immersion in 70% aqueous methanol and |

| | | |
|---|---|---|
| | solution and steam distillation | HS-SPME |
| LC-MS conditions | HPLC-QqQ/MS in MRM and fullscan mode, C18 column, mobile phase: $H_2O$ with 0.1% formic acid (A) and acetonitrile (B) | HPLC-QqQ-LIT/MS in MRM and fullscan mode, C18 column, mobile phase: $H_2O$ (A) and acetonitrile with 0.1% acetic acid (B) |
| GC-MS conditions | GC-QqQ/MS in SIM and fullscan mode, EI at 70 eV, DB-5MS capillary column, helium at 1.2 mL/min in split mode | EI at 70 eV, DB-5MS capillary column, helium at 1.2 mL/min in splitless mode |
| Quantitative analysis | Absolute saikosaponin contents | Relative metabolite contents |
| Qualitative analysis | Tandem MS analysis, retrieval of references and NIST 14 database | Self-complied database |
| Statistical analysis | HCA, PCA, PLS-DA, ANOVA, T-test | HCA, PCA, OPLS-DA, Venn diagrams, pathway annotation and enrichment analysis |
| Results and conclusions | 1. The absolute content of 5 saikosaponins in Radix Bupleuri from different varieties and regions were obtained.<br>2. Twenty-one saponins and fifty-two volatile oils were identified as the differential compounds.<br>3. The content of saikosaponins was significantly higher in North RB than in South RB.<br>4. There were significant differences in saikosaponins and volatile oils between the two varieties, whether grown in the same region or not.<br>5. The identified differential compounds may be the main active components responsible for the differences in their clinical efficacy. | 1. There were significant differences in metabolites between different tissues.<br>2. 144 common differential metabolites were identified between different tissues.<br>3. The content of active components was higher in the root of North RB than in South RB.<br>4. Differential metabolites of the aboveground parts mainly concentrated in monoterpenoid biosynthesis, while those of the root mainly concentrated in sesquiterpenoid and triterpenoid biosynthesis.<br>5. The difference in metabolites and metabolic networks between the two varieties resulted in the difference in the clinical efficacy of Radix Bupleuri. |

* Qu, X.; Hu, S.; Li, T.; Zhang, J.; Wang, B.; Liu, C. Metabolomics Analysis Reveals the Differences Between *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd. *Front. Plant. Sci.* **2022**, *13*, 933849.