中文摘要

不同基原淫羊藿化学成分差异及谱效关系研究

背景和目的：

淫羊藿是我国传统补益类药材，分布广泛，用药历史悠久，具有补肾阳，强筋骨和祛风除湿等功效，临床上常用于治疗骨质疏松、风湿病、高血压和心脏病等。中药化学成分是中药发挥药效的基础，而基原的遗传信息和环境因素会影响药材的生物合成和代谢途径，从而导致药材中的化学成分不同，进而影响临床药效。淫羊藿的主要特征成分是黄酮类化合物，具有很强的抗氧化作用，能够清除体内自由基，减少氧化应激的伤害，从而减少疾病的产生。中药质量评价与控制一直以来是中药研究的重点和难点。目前 2020 版药典中淫羊藿的质量评价方法是采用色谱法测定总黄酮醇苷的含量，所测定的成分单一且没有与淫羊藿药效相关联，无法全面评价淫羊藿质量，因此筛选与淫羊藿药效密切相关的潜在活性成分，对淫羊藿质量评价具有重要意义。本研究以不同基原淫羊藿为研究对象，结合 UPLC-Q-TOF/MS 技术，揭示不同基原淫羊藿化学成分差异，并基于谱效关系，探讨淫羊藿化学成分与抗氧化和保护氧化应激环境下的软骨细胞作用的相关性，为淫羊藿进一步开发和利用提供了药效物质基础。

方法：

1. 不同基原淫羊藿药材化学成分组学研究：以不同基原淫羊藿为研究对象，运用 UPLC-Q-TOF/MS 技术对淫羊藿的化学成分进行全面分析，并采用主成分分析（principal components analysis，PCA）、正交偏最小二乘法判别分析（Orthogonal partial Least Squares Discrimination Analysis，OPLS-DA）等分析方法，筛选不同基原淫羊藿药材的潜在差异化学成分，为不同基原淫羊藿药材的物质基础特异性提供科学依据。

2. 不同基原淫羊藿药材抗氧化作用研究：采用 DPPH、ABTS 和羟自由基三种检测方法评价淫羊藿药材抗氧化作用，并采用抗氧化活性综合指数（antioxidant potency composite，APC）比较不同基原淫羊藿药材的抗氧化活性。

3. 不同基原淫羊藿药材保护软骨细胞氧化应激损伤作用研究：利用过氧化

氢（Hydrogen peroxide，H_2O_2）诱导 C28/I2 细胞构建氧化应激损伤模型，以细胞活力作为指标，评价不同基原淫羊藿对氧化应激损伤的软骨细胞的保护作用。

4. 淫羊藿抗氧化谱效关系模型的建立：以淫羊藿化学成分的质谱数据作为自变量，APC 综合指数和软骨细胞存活率作为因变量，采用灰色关联度分析法（Grey Relational Analysis，GRA）与偏最小二乘分析法（Partial least square，PLS）进行谱效关联，建立淫羊藿化学成分与抗氧化、保护氧化应激损伤软骨细胞作用的谱效关系模型，筛选淫羊藿中与抗氧化、保护氧化应激损伤软骨细胞作用相关的活性成分。

5. 抗氧化关联成分药效验证：对与抗氧化、保护氧化应激损伤软骨细胞作用高度关联的化学成分进行体外实验验证研究。采用 DPPH、ABTS 和羟自由基三种检测方法验证其抗氧化能力，采用 CCK-8 法检测其对软骨细胞的保护作用，验证上述模型所筛选的结果的可靠性。

结果：

1. 不同基原淫羊藿药材化学成分组学研究：从淫羊藿药材中发现 3935 个共同化学成分特征离子，其中通过数据库和文献比对共鉴定了 53 个化学成分。通过 PCA 及 OPLS-DA 分析证明不同基原淫羊藿化学成分存在显著差异。初步鉴定 M1697、M1723、M1875、M2324、M2404、M2524、M2657、M3411、M3749、M3809、M3842、M3846、M3875、M3932 为不同基原淫羊藿的潜在差异成分。本部分为不同基原淫羊藿药材的物质基础研究提供了数据支撑。

2. 不同基原淫羊藿药材抗氧化作用研究：不同基原淫羊藿均具有一定清除 DPPH、ABTS 和羟基自由基的能力，APC 综合指数在 38.31~80.60%范围内，说明不同基原淫羊藿抗氧化能力不同。

3. 不同基原淫羊藿药材保护软骨细胞氧化应激损伤作用研究：通过甲苯胺蓝染色鉴定的 C28/I2 细胞系为软骨细胞。CCK-8 法实验确定构建 C28/I2 细胞氧化应激模型的 H_2O_2 浓度为 1.1 mM/L，作用时间为 24 h。100 μg/mL 的淫羊藿给药组与模型组相比，除 S3、S4、S7、S10、S14、S16、S17、S18、S20、S25、S31、S36 对软骨细胞无明显的保护作用外，其余 24 批次淫羊藿均对软骨细胞有明显的保护作用，具有统计学意义（P<0.05）。其中 S30（朝鲜淫羊藿）对软骨细胞保护作用最强，证明不同基原淫羊藿对氧化应激损伤软骨细胞的保护作用可能存在

差异。

4. 淫羊藿抗氧化谱效关系模型的建立：综合 GRA 和 PLS 模型分析结果，预测影响不同基原淫羊藿抗氧化活性差异的主要成分可能是 M3809、M1750、M3701、M3097、M1921；预测影响不同基原淫羊藿对保护氧化应激损伤软骨细胞活性差异的主要成分可能是 M3809、M3844、M3846、M736、M3412、M366、M1232；其中 M3809（朝藿定 B）是淫羊藿中与抗氧化和保护氧化应激损伤软骨细胞药效均相关的主要活性成分。因此，选取与抗氧化、保护软骨细胞氧化应激损伤作用均相关的朝藿定 B 进行后续药效研究。

5. 抗氧化关联成分药效验证：朝藿定 B 具有清除 DPPH、ABTS 和羟自由基的能力，并存在剂量依赖性。15 和 20 μM 的朝藿定 B 作用于氧化应激损伤软骨细胞，均可以明显提高细胞活力（$P<0.05$），说明其对软骨细胞具有保护作用，验证了上述模型所筛选的与抗氧化、保护氧化应激损伤软骨细胞活性相关的化学成分结果具有一定的可靠性。

结论：

本研究利用非靶向代谢组学技术，对不同基原淫羊藿的化学成分进行全面分析和鉴定，并揭示了不同基原淫羊藿的差异性成分。同时基于谱效关系，将淫羊藿化学成分与药效相结合，探讨了与抗氧化、保护氧化应激环境下的软骨细胞活性相关的化学成分。本研究为鉴别不同基原淫羊藿提供了数据参考，同时为淫羊藿质量评价提供了方法学借鉴。

关键词：

淫羊藿，代谢组学，抗氧化，软骨细胞，谱效关系

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Abstract

Study on the Difference in Chemical Composition and Spectrum-effect Relationship of Epimedium from Different Origins

Background and Objective:

Epimedium is a traditional tonic herb with a wide distribution and a long history of use in China. It is effective in tonifying kidney yang, strengthening tendons and bones, and dispelling wind and dampness, and is commonly used clinically to treat osteoporosis, rheumatism, hypertension and heart disease. The chemical composition of herbal medicines is the basis for their efficacy, while the genetic information and environmental factors of the primitive can affect the biosynthetic pathways and metabolic pathways of the herbs, thus leading to different chemical compositions in the herbs, which in turn affect the clinical efficacy. The main characteristic components of *Epimedium* are flavonoids, which have strong antioxidant effects and can scavenge free radicals in the body, reducing the damage of oxidative stress and reducing the development of diseases. Quality evaluation and control of Chinese medicine has been the focus and difficulty of Chinese medicine research. The current quality evaluation method of *Epimedium* in the 2020 version of pharmacopoeia is to determine the content of total flavonol glycosides by chromatography, the measured components are single and not associated with the efficacy of *Epimedium*, so it is not possible to comprehensively evaluate the quality of *Epimedium*. Therefore, it is important to screen the potential active components closely related to the efficacy of *Epimedium* for the quality evaluation of *Epimedium*. In this study, we investigated the differences in chemical composition of *Epimedium* from different basal sources, combined with UPLC-Q-TOF/MS technique to reveal the differences in chemical composition of *Epimedium* from different basal sources, and explored the correlation between the chemical composition of *Epimedium* and antioxidant and protective effects on chondrocytes under oxidative stress environment based on the spectrum-effect relationship, which provided the pharmacodynamic material basis for the further

development and utilization of *Epimedium*.

Methods:

1. A chemical composition histological study of different basal *Epimedium* herbs: The chemical components of *Epimedium* were comprehensively analyzed by UPLC-Q-TOF/MS technique, and principal components analysis (PCA), orthogonal partial Least Squares-Discrimination Analysis (OPLS-DA) were used to screen the potential differential metabolites of *Epimedium*. Squares Discrimination Analysis (OPLS-DA) were used to screen the potential differential chemical components of different basal herbs of *Epimedium* and provide scientific basis for the specificity of the material basis of different basal herbs of *Epimedium*.

2. Study on the antioxidant effect of *Epimedium* officinale from different origins: The antioxidant effects were evaluated by three assays, DPPH, ABTS and hydroxyl radicals, and the antioxidant potency composite (APC) was used to comprehensively evaluate the antioxidant activities of different basal sources of *Epimedium*.

3. Study on the protective effect of different basidiogenic *Epimedium* herbs against oxidative stress damage in chondrocytes: Hydrogen peroxide (H_2O_2) was used to induce oxidative stress injury model in C28/I2 cells, and cell viability was used as an indicator to evaluate the protective effect of different basidiogenic *Epimedium* on chondrocytes damaged by oxidative stress

4. Modeling of antioxidant spectrum-effect relationship of *Epimedium*: The mass spectral data of chemical components of *Epimedium* were used as independent variables, APC composite index and chondrocyte survival rate were used as dependent variables, and Grey Relational Analysis (GRA) and Partial least square (PLS) were used to establish the spectro-effective relationship model between chemical components of *Epimedium* and antioxidant and protection of chondrocytes damaged by oxidative stress, and to screen the active components of *Epimedium* related to antioxidant and protection of chondrocytes damaged by oxidative stress.

5. Validation of the efficacy of antioxidant-related ingredients: In vitro experimental validation studies were conducted on chemical components highly associated with antioxidant and chondrocyte-protective effects. Three assays, DPPH,

ABTS and hydroxyl radical, were used to verify their antioxidant capacity and CCK-8 method was used to detect their protective effects on chondrocytes to verify the reliability of the results screened by the above model.

Results:

1. Chemical composition study of *Epimedium* herbs from different base origins: 3935 common chemical composition characteristic ions were identified from *Epimedium* herbs, among which a total of 53 chemical components were identified by database and literature comparison. PCA and OPLS-DA analyses proved that there were significant differences in the chemical composition of *Epimedium* from different basal origins. Initially, M1697, M1723, M1875, M2324, M2404, M2524, M2657, M3411, M3749, M3809, M3842, M3846, M3875, M3932 were identified as potentially different components of different basal *epimediums*. This section provides data support for the study of the material basis of different basal *epimedium* herbs.

2. Study on the antioxidant effect of different basal herbs of *Epimedium*: different basal herbs of *Epimedium* have certain ability to scavenge DPPH, ABTS and hydroxyl radicals, and the APC composite index ranged from 38.31 to 80.60%, indicating that different basal herbs of *Epimedium* have different antioxidant ability.

3. Study on the protective effect of different basidiogenic *Epimedium* herbs against oxidative stress damage in chondrocytes: the C28/I2 cell line identified by toluidine blue staining was chondrocytes. the H_2O_2 concentration of 1.1 mM/L was determined by CCK-8 method to construct the oxidative stress model of C28/I2 cells, and the duration of action was 24 h. The administration group of 100 μg/mL of *Epimedium* had a significant protective effect on chondrocytes compared with the model group, except for S3, S4, S7, S10, S14, S16, S17, S18, S20, S25, S31, S36, S10, S14, S16, S17, S18, S20, S25, S31, and S36 had no significant protective effect on chondrocytes, except for S3, S4, S7, S10, S14, S16, S17, S18, S20, S25, S31, and S36, the remaining 24 batches of Icariin had significant protective effect on chondrocytes with statistical significance ($P<0.05$). Among them, S30 (*Korean Epimedium*) had the strongest protective effect on chondrocytes, which proved that there may be differences in the protective effect of different basal *Epimedium* on chondrocytes damaged by oxidative stress.

4. Modeling of the spectrum-effect relationship of *Epimedium*: Combining the results of GRA and PLS models, it was predicted that the main components influencing the difference in antioxidant activity of different basal sources of *Epimedium* might be M3809, M1750, M3701, M3097, M1921; The main components predicted to influence the difference in the activity of different basidiogenic *Epimedium* on the protection of chondrocytes damaged by oxidative stress may be M3809, M3844, M3846, M736, M3412, M366, and M1232; among them, M3809 (*Epimedin B*) was the main active ingredient in *Epimedium* that was associated with both antioxidant and protecting oxidative stress-injured chondrocytes. Therefore, patchouli B, which is related to both antioxidant and chondroprotective effects, was selected for the subsequent efficacy study.

5. Validation of the efficacy of antioxidant-associated ingredients: patchouli B has the ability to scavenge DPPH, ABTS and hydroxyl radicals in a dose-dependent manner. 15 and 20 μM of *Epimedin B* acting on oxidative stress-injured chondrocytes can significantly increase cell viability ($P<0.05$), indicating its protective effect on chondrocytes, which verifies that the above-mentioned models screened with antioxidant and protection of oxidative stress-injured chondrocytes are reliable, The results of the chemical components related to antioxidant and oxidative stress-injured chondrocytes protective activities screened by the above model were reliable.

Conclusions:

In this study, the chemical composition of *Epimedium* was comprehensively analyzed and characterized using non-targeted metabolomics techniques to reveal the differential composition of *Epimedium* from different base sources. Meanwhile, the chemical constituents of *Epimedium* were combined with pharmacological effects based on the spectral effect relationship to explore the chemical constituents associated with antioxidant and oxidative stress chondroprotection activities. This study provides a data reference for the identification of *Epimedium* from different base sources and also provides a methodological reference for the quality evaluation of *Epimedium*.

KeyWords:

Epimedium, Metabolomics, Antioxidant, Chondrocyte, Spectrum-effect relationship

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