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Short Review

# A Short Review Based on the Article Entitled "Establishment of Male and Female Eucommia Fingerprints by UPLC Combined with OPLS-DA Model and Its Application"

**Xinrui Wang, Jinling You, Huaqiang Li, Qi Wang, Yunjie Sheng, Luping Qin* and Xiongyu Meng***

*School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Binjiang, Hangzhou, PR China*

This study first combined the UPLC method with OPLS-DA chemometrics to establish a method for the identification of male and female fingerprints of *E. ulmoides*. In addition, the result showed that the OPLS-DA model could adequately distinguish male and female *E. ulmoides* and exhibit nearly complete goodness of fit and excellent predictive capability, thirteen and twelve major compounds were identified as chemical marker compounds, for the discrimination of male and female of Eucommiae Cortex (EC) and Eucommiae Folium (EF), respectively. While the UPLC the contents of the marker compounds varied among the samples, among which the contents of chlorogenic acid and protocatechuic acid were greater in males than that of females in all four origins Eucommiae Cortex and Folium. This also demonstrates that this quantitative method for the identification of male and female *E. ulmoides* can provide a reference for quality control and chemical composition studies of male and female *E. ulmoides*.

## Overview of Research Background and Purpose

*Eucommia ulmoides* Oliver. (*E. ulmoides*), a woody perennial dioecious deciduous tree of the monotypic genus Eucommia to China and has high economic value [1]. *E. ulmoides* contains a large number of active compounds that nourish the liver and kidneys as well as regulate blood pressure, meanwhile having anti-inflammatory, anti-bacterial, and antitumor [2,3]. Currently, *E. ulmoides* is widely cultivated for its extensive use in medicine and food [4,5]. The composition of the active ingredients of different species of *E. ulmoides* varies greatly, and Eucommia gum production shows significant sexual dimorphism between male and female leaves [6]. However, its dioecious nature prevents sex identification by traditional morphological observation early in development, thus hindering breeding and cash crop cultivation [7]. Therefore, the identification of males and females by phenotypic differences at the adult plant stage is not a reliable basis for early sex identification, and it is particularly important to study their reproductive organ development and sexual differentiation.

The physiological and molecular mechanisms of sex differentiation and determination in plants have been extensively studied for many years. The mechanisms of sex determination in plants consist of two main systems: genetic sex determination and environmental sex determination, corresponding to the study of the genome and transcriptome, respectively.

The study of the evolution of sex chromosomes in plant identification has great importance, three sex-determining systems have generally been identified in dioecious plants: XY, ZW, and XO [8,9]. With the development of gene sequencing technologies, sex-determining genes are being discovered. For example, *Actinidia* spp., where the Y-encoded sex-determinant candidate gene acts as the Suppressor of Feminization (SuF) [10,11]. In genomic studies of *E. ulmoides*, a desirable sex-linked locus MSL4 has been identified using RAD-seq/ddRAD-seq technology. It was found that MSL4 is highly conserved in all males, stable and reproducible, for the identification of genes that contribute to the understanding of sex chromosome phylogeny in higher plants [12].

Transcriptomes are investigated by deep sequencing technology (i.e., RNA-Seq), and analyzing transcriptomic differences in dioecious flower buds contributes to the screening of gender-related differentially expressed genes (DEGs) [13]. For instance, transcriptome studies of *Trachycarpus fortunei*, Shrub Willows (*Salix suchowensis*) and *Ginkgo biloba* have interpreted the functional elements of the genome, revealed the molecular components of cells and tissues, and understanding development and disease [14-16]. Transcriptomic studies on *E. ulmoides* have sequenced the transcriptomes of female and male buds using the Illumina platform and detected 67,447 and 58,236 single nucleotide polymorphisms in male and female buds of *E. ulmoides* respectively, which provides a valuable resource for conservation genetics and functional genomics studies [17]. However, metabolomics studies are also a way to evaluate the chemical composition of male and female herbal medicines [18]. Our team conducted a series of studies to develop a number of studies on the quality evaluation of herbal plants including the evaluation of flavonol glycosides content of male and female *Ginkgo biloba*, and the effects of geographical location and growth period on formation and accumulation of iridoid glycosides in *Morinda officinalis* [19,20]. Based on the

***Corresponding authors:** Luping Qin, School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Binjiang, Hangzhou, PR China, Tel: Tel: +86 057161768167; E-mail: lpqin@zcmu.edu.cn

Xiongyu Meng, Qin, School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Binjiang, Hangzhou, PR China, E-mail: mengxiongyu@zcmu.edu.cn

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metabolomics research methods such as UPLC combined with multivariate statistical methods for the overall assessment and variance analysis of the chemical components of Chinese herbal medicines, this study aimed to establish a simple and rapid UPLC method combined with OPLS-DA model to establish fingerprint profiles of male and female *E. ulmoides* from different origins and different medicinal parts (bark and leaves) of *E. ulmoides*.

## Research Summary and Outlook

In our study, we first examined the chromatographic performance, detector wavelength, column temperature and mobile phase composition to determine the optimal UPLC method, and method validation was performed. The chromatographic fingerprints showed thirty-six and thirty-seven peaks in the EC and EF chromatograms respectively, with good fingerprint similarity and small differences in the chemical composition species between the male and female fingerprints. The content was determined by the standard curve method, and it was found that the content of chlorogenic acid and protocatechuic acid was higher in males than in females in all four origins of EC and EF. In addition, the OPLS-DA model could adequately distinguish between male and female *E. ulmoides*. The model exhibit nearly complete goodness of fit and excellent predictive capability, thirteen and twelve major compounds were identified as chemical marker compounds. The above results suggest that the combination of UPLC fingerprinting and chemometric analysis may provide valuable insights into the application of UPLC fingerprinting in the identification of male and female *E. ulmoides*.

Nevertheless, there are some limitations to this study. Only seven chemical components were used to quantify male and female *E. ulmoides*. In addition to these, a large number of chemical components have not been studied qualitatively and quantitatively, and therefore an LC-MS method could optionally be used to identify and quantify all the peaks. It should also be noted that the composition and content of *E. ulmoides* compounds are influenced by a variety of factors, including site, origin, and growth age in addition to the sex of male and female, and therefore the differences in chemical composition between the male and female need to be analyzed in relation to environmental factors. Therefore, the transcriptomes of male and female *E. ulmoides* can be studied to make a more reliable and accurate assessment of the chemical composition and content of males and females by combining transcriptomic molecular information and metabolomic information. In the future, our team will use GC-MS, LC-MS metabolomics, and RNA-seq transcriptomics methods to further investigate the multifactorial effects on the chemical composition and content of male and female *E. ulmoides*.

Finally, dioecious plants are a large group in nature and there are few studies on the differences in the chemical composition of the metabolomics of dioecious plants, which could be further studied and applied to male and female plants in the future by applying various histological techniques from multiple perspectives.

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## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

Research data are not shared.

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