Supplementary Material

Study on the Source and Characteristics of *Evodia rutaecarpa* Based on Chemical Pattern Recognition

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**Abstract**

In this study, the appearance and texture of *E. rutaecarpa* were linked with the chemical constituents to explore methods of classification of *E. rutaecarpa*. The Chemometrics such as Hierarchical cluster analysis (HCA), principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) models were used for analysis. According to the models, samples of *E. rutaecarpa* were divided into three categories based on their source: Evodia, Stone Tiger and Sparse Evodia. The Evodia category could be subdivided into two categories, one representing large fruits with a greater degree of cracking and the other representing large fruits with little or no cracking. The method provided by this study combines chemometrics with HPLC fingerprints, which can provide a basis and reference for the identification of *E. rutaecarpa* and enables establishment of a grade standard.
**KEYWORDS**: Evodia rutaecarpa, Chemometrics, source, characteristics

**Experimental**

**Materials and reagents**

The 31 batches of *E. rutaecarpa* samples were collected from eight different provinces (Zhejiang, Jiangxi, Guangxi, Guizhou, Sichuan, Hunan, Hubei, and Shanxi) of China. They were identified as the dried unripe fruit of *E. rutaecarpa* (Juss.) Benth., *E. rutaecarpa* (Juss.) Benth. var. officinalis (Dode) Huang and *E. rutaecarpa* (Juss.) Benth. var. Bodinier (Dode) Huang by Professor Li Tianxiang of the Tianjin University of traditional Chinese medicine. The sample information is shown in Table S1 and the physical charcters of Evodia were differentiated by chemometrics is shown in Table S2. Images of *E. rutaecarpa* samples from different sources are shown in Figure S1.

High performance liquid chromatography (HPLC)-grade methanol was purchased from Sigma–Aldrich (St. Louis, MO, USA) and HPLC-grade formic acid was obtained from Tianjin Kemiu Chemical Reagent Co., Ltd. (Tianjin, China). Water used as a chromatographic mobile phase was purified by a Milli-Q system (Millipore, Bedford, MA, USA). Dehydroevodiamine, evodiamine and rutacarpine were obtained from Shanghai Harmony Medical Technology Co., Ltd (Shanghai, China).

**Sample preparation**

Samples of *E. rutaecarpa* from various batches were collected, finely powdered, and passed through a 50-mesh sieve. Accurately weighed samples (0.2 g) of each powder were added to 80% methanol (10 mL), soaked for 1 h, and then sonicated for 40 min.

A mixed standard solution was obtained by dissolving accurately weighed samples of
dehydroevodiamine, evodiamine and rutacarpine in methanol.

**HPLC analysis**

A Shimadzu HPLC system (SHIMADZU-LC-20-AT, Japan) was used to determine the chemical composition. The chromatographic separation was performed on an HPLC Symmetry® C18 column (4.6×150 mm, 5.0 μm particle size; Waters), operated at 30°C. The mobile phase was composed of acetonitrile (solvent A) and water containing 0.1% formic acid (solvent B). A gradient elution program was employed as follows: 0–5 min (5%–11% A), 5–11 min (11%–13% A), 11–25 min (13%–20% A), 25–31 min (20%–40% A), 31–36 min (40%–45% A), 36–40 min (45%–75% A), 40–51 min (75%–90%, A) with a mobile phase flow rate of 1 mL/min. The detection wavelength was set at 254 nm with an injection volume of 5 μL. A photodiode array detector was used to collect chromatographic information from 200–400 nm. All solutions were filtered through a 0.22 μm nylon membrane prior to injection into the HPLC. The HPLC chromatograms of the sample and reference solution are shown in Figure S2.

**HPLC methodological evaluation**

Six successive injections of the same *E. rutaecarpa* sample solution were subjected to chromatography as described in section 1.3. The relative retention time and relative peak area of each common peak were calculated by using evodiamine (peak number 10) as the reference peak. The RSD for the relative peak retention time was < 2.0% and the RSD for the relative peak area was < 3.0%, indicating good precision.

Six samples of *E. rutaecarpa* were prepared in parallel from the same group of *E. rutaecarpa* samples according to the method in section 1.2. The relative retention time and relative peak area of each
common peak were calculated by using evodiamine (peak number 10) as the reference peak. The RSD for the relative peak retention time was < 1.6% and the RSD for the relative peak area was < 2.67%, indicating good repeatability.

Samples of the same Evodia solution, prepared according to section 2.2, were analyzed at 0, 2, 4, 8, 12, and 24 h. The relative retention time and relative peak area of each common peak were calculated by using evodiamine (peak number 10) as the reference peak. The RSD for the relative peak retention time was < 2.11% and the RSD for the relative peak area was < 2.98%, indicating that the sample was stable for 24 h.

**Software requirements**

LabSolutions/LCsolution workstation data management software (SHIMADZU, Japan) was used to collect chromatographic data on samples of *E. rutaecarpa*, including peak area, retention time and other related information. SOP of Similarity evaluation system for chromatographic fingerprint of TCM 2004A software (Chinese Pharmacopoeia Commission, China) was employed for evaluating fingerprint similarity. SPSS 19.0 (IBM, USA) was used to build a HCA unsupervised pattern recognition model. SIMCA-P11.5 demo version (Sartorius Scientific Instrument Co., Ltd., Germany) was used to establish a PCA unsupervised pattern recognition model and a PLS-DA supervised pattern recognition model.

**Tables**

| Table S1. Description of *E. rutaecarpa* samples. |
|-----------------------------------------------|
| Sample name      | Place of Origin | Origin code | Source                  |
| ZJ11-ZJ12-ZJ13-ZJ21-ZJ22-ZJ23 | ZheJiang       | ZJ         | Sparse Evodia            |
| ZJ31-ZJ32-ZJ33-ZJ41-ZJ42-ZJ43 |                |            |                         |
| Sample name | Place of Origin | Origin code | Source | Physical charcters* |
|-------------|-----------------|-------------|--------|--------------------|
| JX11-JX12-JX13-JX21-JX22-JX23 | JiangXi | JX | Stone Tiger |
| JX31-JX32-JX33-JX61-JX62-JX63 | JiangXi | JX | Sparse Evodia |
| JX41-JX42-JX43 | JiangXi | JX | Evodia |
| SX11-SX12-SX13-SX21-SX22-SX23 | ShanXi | SX | Sparse Evodia |
| SX31-SX32-SX33 | | | |
| HN11-HN12-HN13-HN21-HN22-HN23 | HuNan | HN | Evodia |
| HN31-HN32-HN33 | | | |
| HB11-HB12-HB13-HB21-HB22-HB23 | HuBei | HB | Evodia |
| HB31-HB32-HB33 | | | |
| GX11-GX12-GX13 | GuangXi | GX | Stone Tiger |
| GX21-GX22-GX23-GX31-GX32-GX33 | GuangXi | GX | Evodia |
| SC11-SC12-SC13-SC31-SC32-SC33 | SiChuan | SC | Evodia |
| SC41-SC42-SC43-SC51-SC52-SC53 | | | |
| SC21-SC22-SC23-SC61-SC62-SC63 | SiChuan | SC | Stone Tiger |
| GZ11-GZ12-GZ13 | GuiZhou | GZ | Stone Tiger |
| GZ21-GZ22-GZ23 | GuiZhou | GZ | Evodia |
| GZ31-GZ32-GZ33 | GuiZhou | GZ | Sparse Evodia |

Table S2. Physical charcters of Evodia differentiated via chemometrics.
*S3: Fruits with a greater degree of cracking; S4: Fruits with little or no cracking.

**Table S3.** Similarity evaluation of 31 batches of *E. rutaecarpa*

| Batches | Similarity | Batches | Similarity | Batches | Similarity | Batches | Similarity |
|---------|------------|---------|------------|---------|------------|---------|------------|
| GX1     | 0.928      | HB3     | 0.987      | JX5     | 0.884      | SC4     | 0.930      |
| GX2     | 0.949      | HN1     | 0.814      | JX6     | 0.996      | SC6     | 0.944      |
| GX3     | 0.820      | HN2     | 0.896      | SX1     | 0.789      | ZJ1     | 0.959      |
| GZ1     | 0.878      | HN3     | 0.904      | SX2     | 0.806      | ZJ2     | 0.868      |
| GZ2     | 0.874      | JX1     | 0.909      | SX3     | 0.848      | ZJ3     | 0.856      |
| GZ3     | 0.863      | JX2     | 0.952      | SC1     | 0.841      | ZJ4     | 0.855      |
| HB1     | 0.917      | JX3     | 0.972      | SC2     | 0.986      | SC5     | 0.937      |
| HB2     | 0.840      | JX4     | 0.893      | SC3     | 0.951      |

**Figures**

**Figure S1.** Images of *E. rutaecarpa* herbs from different sources: (a) Sparse Evodia, (b) Stone Tiger, (c) Evodia (not cracked), (d) Evodia (cracked)
Figure S2. HPLC chromatographic fingerprints of A. *E. rutaecarpa* sample; B. Mixed reference sample (7. dehydroevodiamine; 10. evodiamine; 11. rutacarpine)

Figure S3. Hierarchical cluster analysis of *E. rutaecarpa* with different provenance.
Figure S4. 2D PCA score plot (PC1 versus PC2) of all chromatographic fingerprints of *E. rutaecarpa* samples as listed in Table 1.

Figure S5. 3D PLS-DA score plot (PC1 versus PC2) of all chromatographic fingerprints of *E. rutaecarpa* samples as listed in Table 1. VIP (variable importance for the project) plot.

Figure S6. VIP (variable importance for the project) plot of PLS-DA.