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 Kemiou 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 ZJ31-ZJ32-ZJ33-ZJ41-ZJ42-ZJ43	ZheJiang	ZJ	Sparse Evodia

JX11-JX12-JX13-JX21-JX22-JX23 JX31-JX32-JX33-JX61-JX62-JX63	JiangXi	JX	Stone Tiger
JX41-JX42-JX43	JiangXi	JX	Sparse Evodia
JX51-JX52-JX53	JiangXi	JX	Evodia
SX11-SX12-SX13-SX21-SX22-SX23 SX31-SX32-SX33	ShanXi	SX	Sparse Evodia
HN11-HN12-HN13-HN21-HN22-HN23 HN31-HN32-HN33	HuNan	HN	Evodia
HB11-HB12-HB13-HB21-HB22-HB23 HB31-HB32-HB33	HuBei	HB	Evodia
GX11-GX12-GX13	GuangXi	GX	Stone Tiger
GX21-GX22-GX23-GX31-GX32-GX33	GuangXi	GX	Evodia
SC11-SC12-SC13-SC31-SC32-SC33 SC41-SC42-SC43-SC51-SC52-SC53	SiChuan	SC	Evodia
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.

Sample name	Place of Origin	Origin code	Source	Physical charcters*
JX51-JX52-JX53	JiangXi	JX	Evodia	S3
HN11-HN12-HN13-HN21-HN22-HN23	HuNan	HN	Evodia	S4
HN31-HN32-HN33	HuNan	HN	Evodia	S3
HB11-HB12-HB13-HB21-HB22-HB23 HB31-HB32-HB33	HuBei	HB	Evodia	S3
GX21-GX22-GX23	GuangXi	GX	Evodia	S3
GX31-GX32-GX33	GuangXi	GX	Evodia	S4
SC11-SC12-SC13-SC31-SC32-SC33	SiChuan	SC	Evodia	S4

SC41-SC42-SC43				
SC51-SC52-SC53	SiChuan	SC	Evodia	S3
GZ21-GZ22-GZ23	GuiZhou	GZ	Evodia	S3

*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

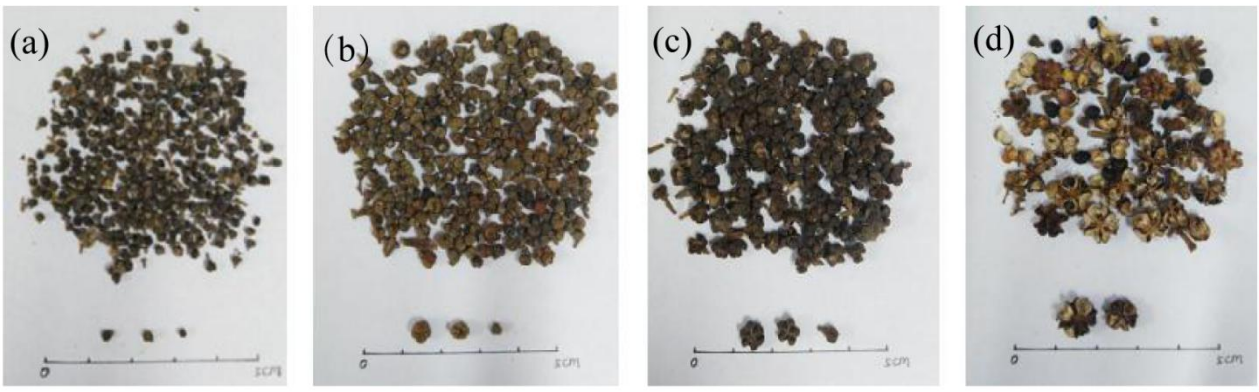

Figure S1. Images of *E. rutaecarpa* herbs from different sources: (a) Sparse Evodia, (b) Stone Tiger, (c) Evodia (not cracked), (d) Evodia (cracked)

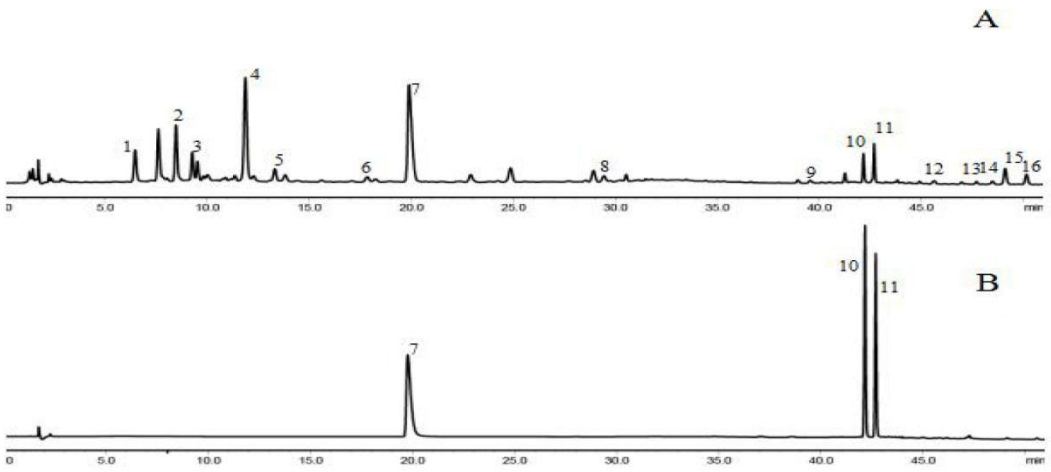

Figure S2. HPLC chromatographic fingerprints of A. *E. rutaecarpa* sample; B. Mixed reference sample (7. dehydroevodiamine; 10. evodiamine; 11. rutacarpine)

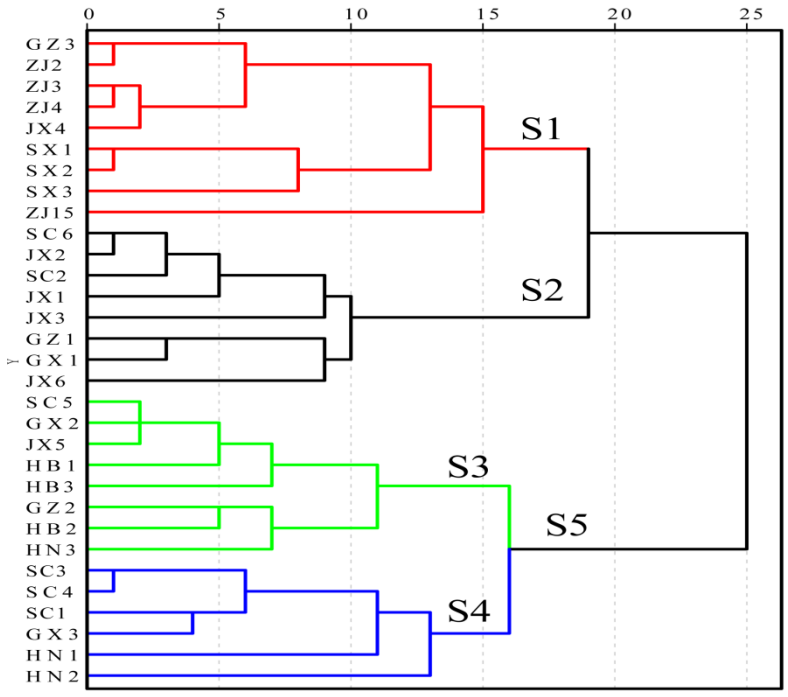

Figure S3. Hierarchical cluster analysis of *E. rutaecarpa* with different provenance.

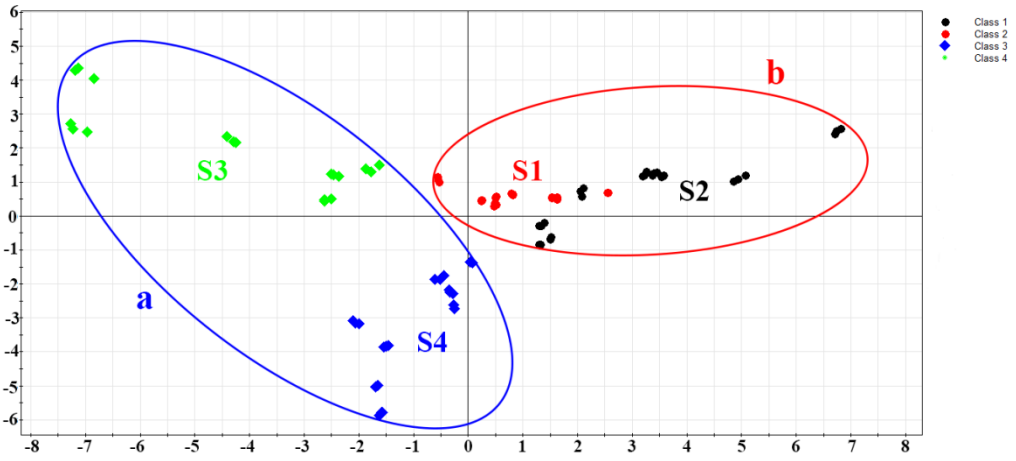

Figure S4. 2D PCA score plot (PC1 versus PC2) of all chromatographic fingerprints of *E. rutaecarpa* samples as listed in Table 1.

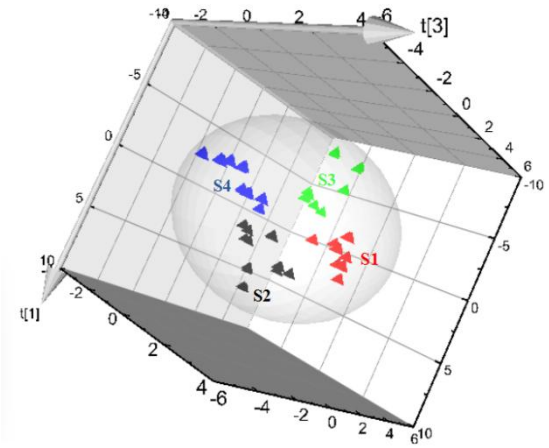

Figure S5. 3D PLS-DA score plot (PC1 versus PC2) of all chromatographic fingerprints of *E. rutaecarpa* samples as listed in Table 1. VIP (variable importance for the project) plot).

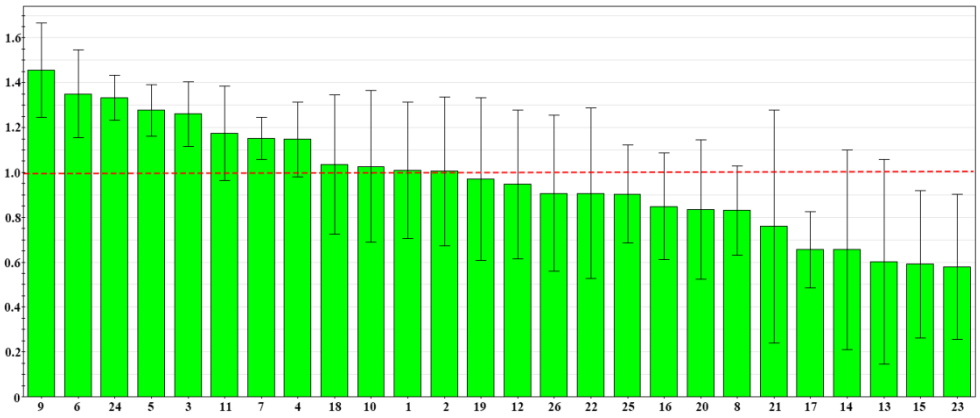

Figure S6. VIP (variable importance for the project) plot of PLS-DA.