Available online at www.sciencedirect.com
ScienceDirect
*Chinese Journal of Natural Medicines* 2021, **19**(9): 713-720
doi: 10.1016/S1875-5364(21)60087-0
Chinese Journal of Natural Medicines

•Research article•

# Comparison of *Murraya exotica* and *Murraya paniculata* by fingerprint analysis coupled with chemometrics and network pharmacology methods

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Available online 20 Sep., 2021

**[ABSTRACT]** There are two source plants for the traditional Chinese medicine Murrayae Folium et Cacumen (MFC) in *Chinese Pharmacopoeia*, i.e. *Murraya exotica* L. and *M. paniculata* (L.) Jack. Herein, a chemical comparison of *M. exotica* and *M. paniculata* by high performance liquid chromatography (HPLC) fingerprint analysis coupled with chemometrics and network pharmacology was performed. The main peaks in the fingerprints were identified by liquid chromatography coupled with ion trap/time-of-flight mass spectrometry (LC-IT-TOF-MS) and authenticated by references. The chemometrics results showed that the HPLC fingerprints of these two species were clearly divided into two categories using hierarchical cluster analysis (HCA) and principal component analysis (PCA), and a total of 13 significantly differentiated markers were screened out by orthogonal partial least squares-discriminant analysis (OPLS-DA). However, the following network pharmacology analysis showed that these discriminated markers were found to act via many common targets and metabolic pathways, indicating the possibly similar pharmacological effects and mechanisms for *M. exotica* and *M. paniculata*. The above results provide valuable evidence for the equivalent use of these two plants in clinical settings. Moreover, the chromatographic fingerprint analysis coupled with chemometrics and network pharmacology supplies an efficient approach for the comparative analysis of multi-source TCMs like MFC.

**[KEY WORDS]** *Murraya exotica*; *Murraya paniculata*; Multi-source TCM; HPLC fingerprint; Chemometrics; Network pharmacology

**[CLC Number]** R917 **[Document code]** A **[Article ID]** 2095-6975(2021)09-0713-08

## Introduction

Traditional Chinese Medicines (TCMs) have been widely used to treat various diseases in China for a long time. In recent years, quality control becomes one of the core issues of TCM research, attracting increasing attention for resarchers, especially for those working on herbal medicines with multiple sources [1-3]. These multi-source TCMs, originated from two or more plants, play a positive role for meeting the increasing demands of clinical use and pharmaceutical industry. However, the multi-source herbs may differ in their chemical constitution and quantity [4], which then influences the therapeutic effects and makes the quality control difficult. Therefore, it is necessary to evaluate the chemical and pharmacodynamic consistency of the TCMs from multiple sources, so as to ensure the efficacy, stability, and safety of clinical medication.

Murrayae Folium et Cacumen (MFC) is a representative TCM originated from two plants, *Murraya exotica* L. (ME) and *Murraya paniculata* (L.) Jack (MP), and recorded in *Chinese Pharmacopoeia* (2020 Edition) [5]. Its official medicinal parts are the fresh leaves and twigs of plants. MFC (*Jiulixiang* in Chinese) has been commonly used in South China for the treatment of various diseases, especially inflammatory lesions and the pain symptoms caused by traumatic injuries or rheumatic arthralgia and gastralgia [6]. Modern pharmacological studies show that MFC possesses anti-inflammatory, antinociceptive, antibacterial, antioxidant, and anti-diabetic activities [7-11]. This herb is also a main material in

**[Received on]** 08-Jun.-2021
**[Research funding]** This work was supported by the National Natural Sciences Foundation of China (Nos. 81773864, 81973199, and 81473106) and the National Key Research and Development Project (No. 2019YFC1711000).
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These authors have no conflict of interest to declare.
Dedicated to the 90th Anniversary of the Founding of Shenyang Pharmaceutical University

*Sanjiu Weitai* granule, a famous Chinese complex prescription for gastric diseases [12]. However, the names of *M. exotica* and *M. paniculata* were ever mix-used in the 1980s and 1990s [13-14], making the existing information for both confused. Meanwhile, to the best of our knowledge, there has been no comparison between the chemical constituents of these two medicinal plants to date [15]. Therefore, it is necessary to develop a specific method to comprehensively compare the chemical constituents between *M. exotica* and *M. paniculata*.

Chromatographic fingerprint analysis is an effective and comprehensive technique for quality assessment of herbal medicines [16-17]. As a statistical tool, chemometrics can overview the complex data from the chromatographic profiles. Therefore, the combination of fingerprints and chemometrics is an important tool for systemic quality assessment of TCMs [18-19]. Moreover, network pharmacology analysis has been applied to investigate the systematic action of TCMs and recognized as a powerful strategy for drug discovery in light of its ability to explore the complex relationships among components, diseases and targets [20-21]. Hence, in this study, a chromatographic fingerprint method was developed to compare the chemical constituents between *M. exotica* and *M. paniculata* by high performance liquid chromatography-diode array detector (HPLC-DAD), and the main peaks were identified by high-performance liquid chromatography coupled with ion trap-time-of-flight mass spectrometry (HPLC-IT-TOF-MS). Subsequently, the fingerprints from the two different source plants were differentiated by hierarchical cluster analysis (HCA), principal component analysis (PCA), and orthogonal partial least squares-discriminant analysis (OPLS-DA). Finally, network pharmacology study was performed to explore the effect of these differentiated markers on the pharmacodynamics and mechanisms of *M. exotica* and *M. paniculata*.

## Materials and Methods

### *Chemicals, reagents, and plant materials*

All the botanical samples studied were collected by China Resources SanJiu Medical & Pharmaceutical Co., Ltd. (Shenzhen, China), and authenticated by one of the authors, Professor TU Peng-Fei, as the dry leaves and twigs of *Murraya exotica* L. and *Murraya paniculata* (L.) Jack. The collection information of the 97 tested samples is summarized in Tables S1 and S2. The specimens were deposited in the Herbarium of the Peking University Modern Research Center for Traditional Chinese Medicine. All the samples were ground into powder, and passed through a 60-mesh sieve. Meanwhile, an equal amount of the powder from each sample was pooled within species to generate a homogenized quality control (QC) sample of *M. exotica* and *M. paniculata*, respectively. The ground powder was kept under cool and dry conditions before use.

Twenty-three standard references were isolated from the dry leaves and twigs of *M. exotica* and *M. paniculata* in our laboratory, and their structures were confirmed by various spectroscopic analyses [22-25]. All the solvents used for sample preparation were of analytical grade and the solvents used for HPLC were of HPLC grade. Deionized water was prepared on a Milli-Q water purification system (Millipore, Bedford, MA, USA). Methanol was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Acetonitrile used for HPLC was obtained from Tianjin Biaoshiqi Science & Technology Development Co., Ltd. (Tianjin, China).

### *Preparation of sample solutions*

Each sample powder (0.25 g) was accurately weighed and extracted with 25 mL of 80% aqueous methanol under ultrasonication (250 W, 40 kHz) at room temperature for 30 min. Subsequently, the extraction solution was filtered through 0.22 μm membrane and injected for HPLC. The standard solutions of the references were prepared in an appropriate concentration with 80% aqueous methanol and stored at 4 °C until use.

### *Instrumentation and analytical conditions*

HPLC fingerprint analysis was carried out on an Agilent 1100 series HPLC-DAD system (Agilent Technologies, Santa Clara, CA, USA) equipped with a G1322A vacuum degasser, a G1312A binary pump, a G1313A auto sampler, a G1316A column compartment, and a G1315A diode array detector. An Agilent Eclipse XDB $C_{18}$ (250 mm × 4.6 mm, 5 μm) column was used for separation. A binary gradient elution system was composed of water (A) and acetonitrile (B) with a gradient elution system: 0−30 min, 5%−35% B; 30−60 min, 35%−45% B; 60−70 min, 45%−65% B; 70−77 min, 65%−100% B; 77−80 min, 100% B; 80−80.01 min, 100%−5% B; 80.01-88 min, 5% B. The column temperature was 30 ºC; injection volume was 15 μL, and the flow rate was 1.0 mL·min$^{-1}$. The DAD detector was set at 320 nm for acquiring chromatograms.

LC-IT-TOF-MS analysis was performed on a Shimadzu LC-IT-TOF-MS platform (Shimadzu, Kyoto, Japan) equipped with a DGU-20A3 degasser, two LC-20AD pumps, a SIL-20AC auto sampler, a CTO-20A column oven, and a SPDM20AD detector. The chromatographic separation was the same as the fingerprint analysis. MS parameters were set as follows: alternative ion mode; electrospray voltage, +/−3.5 kV; detector voltage, 1.7 kV; the temperature of curved desolvation line (CDL) and heat block, 200 ºC; nebulizing gas ($N_2$), 1.5 L·min$^{-1}$; drying gas ($N_2$) pressure, 100 kPa; scan ranges, *m*/*z* 100–1500 for $MS^1$, *m*/*z* 100–1200 for $MS^2$, and *m*/*z* 50–800 for $MS^3$; collision energy, 50% for $MS^2$, and $MS^3$ with a region pressure of $1.4 \times 10^{-4}$ Pa; ion trap pressure, $1.8 \times 10^{-2}$ Pa; ion accumulation time, 30 ms. The mass axis was calibrated using the sodium trifluoroacetate. Ultra-high purity argon was used as the collision gas for the collision-induced dissociation (CID) experiments.

### *Method validation*

All the tests were carried out on the QC samples of *M. exotica* and *M. paniculata*. The extraction solutions were prepared following the same procedures mentioned above. The precision was determined by replicating HPLC injection of the same sample solution for six times per day. Six independent samples were extracted and analyzed in parallel for evaluation of the repeatability. The stability was assessed by meas-

uring a single sample solution stored at room temperature for 0, 12, 24, and 36 h, respectively.

*Data processing and analysis*

The raw data obtained from HPLC analysis were processed by ChemPattern (version 2017, Chemmind Technologies, Beijing, China) for peak alignment, picking, and normalization to produce a matrix containing peak area and retention time ($t_R$). The multivariate statistical analysis (MVA) for the fingerprint data was performed by SIMCA-P software (v 14.0, Umetric, Umeå, Sweden) and MetaboAnalyst (http://www.metaboanalyst.ca). The quality of all MVA models was controlled by evaluating the $R^2$ and $Q^2$ values. Unsupervised PCA and HCA were first employed to assess the clustering trends of different groups. Subsequently, the scatter plots of supervised OPLS-DA were used to discriminate the classes and the combination of *S*-plot and variable influence in projection (VIP) plot from the OPLS-DA model was used to identify the differentiated signals. The CV-ANOVA *P* value and permutation plots (200 permutations) were calculated to determine the reliability of the OPLS-DA model. The univariate significance of selected variables was subsequently confirmed by the area under the receiver operating characteristic curve (*AUC*) and Student's *t*-test by MetaboAnalyst.

*Network pharmacology analysis*

The procedures were listed as follows: (1) The potential targets of the above identified discriminated makers were derived from SwissTargetPrediction (http://swisstargetprediction.ch/) and PharmMapper Sever (http://www.lilab-ecust.cn/pharmmapper/). The nomenclature for the target proteins were standardized according to the Uniprot website (https://www.uniprot.org/). (2) The inflammation-related targets were screened out from GeneCards database (https://www.genecards.org/) and OMIM database (https://omim.org/) with duplicates removed. (3) The intersection of (1) and (2) was considered as the predicted target of selected markers against inflammation. (4) The protein-protein interaction (PPI) network was established by STRING (https://www.string-db.org/) and Cytoscape 3.7.2 (Cytoscape Consortium, CA, USA). (5) The KEGG signaling pathway analysis was derived from the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/).

## Results and Discussion

*Optimization of chromatographic conditions and method validation*

The extraction method for *M. exotica* and *M. paniculata* was optimized in our previous report [26]. Therefore, the ultrasonic extraction method was used in the present study. After comparison, 80% aqueous methanol was used as the solvent (Fig. S1). In order to achieve satisfactory separation among the chromatographic peaks of the test solutions, the column temperature (25, 30 and 35 °C), the detection wavelength, and the ratio of mobile phase were optimized. Varying the ratio of water and acetonitrile in the mobile phase provided significant improvement in separation, yielding narrow and better-resolved peaks. The change of temperature affected the separation effect in the chromatogram. In this experiment, as the temperature increased, the resolution of the two chromatographic peaks at 40 min became better, but the two peaks at 48 min became worse. After comparison, the column temperature was finally selected at 30 °C (Fig. S2). The DAD wavelengths were monitored and most chemical components showed the biggest absorbance at 320 nm. Therefore, the wavelength of 320 nm was selected as the optimum detection wavelength (Fig. S3).

To ensure the validity of this proposed method, the precision, repeatability and stability were evaluated by calculating the relative standard deviations (RSDs) of the relative retention time (RRT) and the relative peak area (RPA) of nine main peaks ($t_R$ 18.35, 24.28, 26.25, 30.13, 39.84, 42.89, 44.41, 46.99 and 65.49 min), while phebalosin ($t_R$ 48.15 min) was used as a reference peak. The precisions of RRT and RPA of all the determined peaks were below 0.27% for RRT and 3.83% for RPA. The repeatabilities of RRT and RPA were found to be less than 0.91% and 3.78%, respectively. The stability test was performed with the sample solutions for 36 h. The RSD values were less than 0.34% for RRT and 2.19% for RPA. All the results indicated that the established HPLC method for fingerprint analysis of *M. exotica* and *M. paniculata* is valid and applicable.

*Establishment of standard fingerprint and identification of chemical constituents*

The fingerprints of 25 batches of *M. exotica* and 72 batches of *M. paniculata* were obtained and analyzed by the software of ChemPattern, and their standard fingerprints were produced accordingly (Fig. 1).

LC-IT-TOF-MS was subsequently employed to identify the main peaks of the fingerprints of these two plants. The negative and positive ion modes were both adopted for MS analysis. The results indicated that the chemical constituents of *M. exotica* and *M. paniculata* showed more obvious responses in the positive mode than in the negative mode. Therefore, the positive ion mode was adopted. A total of 52 major constituents were identified by comparing $t_R$, $[M + H]^+$, and fragment ions with previous data and the reference substances, including 33 coumarins and 19 polymethoxylated flavonoids (Table 1). Many common peaks, such as peaks **1**–**4**, **6**, **16**, **24**–**25**, **27**, **37**, **41**, and **44**–**45** were observed in the chromatograms of both *M. exotica* and *M. paniculata* (Fig 2).

*Similarity evaluation of samples from different sources and habitats*

The similarity evaluation of the chromatographic fingerprints of samples from different sources and habitats was performed by comparing with the standard fingerprint of *M. exotica* or *M. paniculata*, respectively, and the similarity values are calculated and listed in Tables S1 and S2. For *M. exotica* samples (ME1–25), the similarity values of 22 batches were more than 0.84, indicating that their chemical constitutions are similar and the quality of the samples from different habitats are consistent and stable. On the other hand, for the 72 batches of *M. paniculata* (MP1–72), the similarity values of 41 batches were over 0.80, while the other 31 batches fell in a range of 0.46−0.80. These findings indicated that the chemical constitutions of *M. paniculata* from differ-

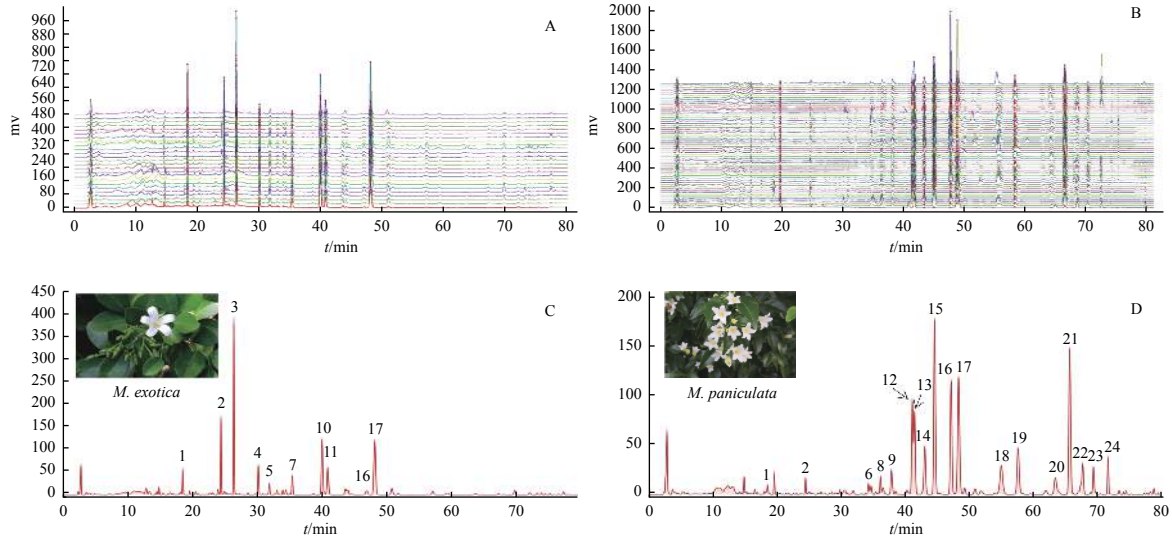


**Fig. 1 HPLC chromatographic fingerprints of 25 batches of *M. exotica* (A) and 72 batches of *M. paniculata* (B), and the reference fingerprints of *M. exotica* (C) and *M. paniculata* (D). 17(S): phebalosin**

ent habitats are not always similar, and some of them vary greatly. Among them, the chemical constitutents of *M. paniculata* from Vietnam ($n$ = 7) and Hechi ($n$ = 16) were the most stable, and most of their similarity values were between 0.80−0.96, while the similarity values of *M. paniculata* from Baise ($n$ = 4), Chongzuo ($n$ = 4), Guilin ($n$ = 3) and Guangdong Province ($n$ = 4) were mostly below 0.77. Besides, it is noticeable that the similarity values of 17 batches from Hezhou varied greatly, with a big range of 0.46−0.92. The great variation for the fingerprint similarities of *M. paniculata* from different habitats is probably associated with its wild resource and the variation of ecological environment. Therefore, it is necessary to notice the quality stability of *M. paniculata*, while artificial planting may be adopted as a solution.

*Chemometrics analysis*

The HCA dendrogram of the data matrix was initially employed to analyze the fingerprints of *M. exotica* and *M. paniculata* and the results are shown in Fig. S4A. According to the results, the samples from different plant sources were classified into two different categories. Moreover, the scatter score plot of PCA pattern analysis also showed a clear separation trend for the fingerprints of *M. exotica* and *M. paniculata* (Fig. S4B). All these unsupervised results indicatee that the established fingerprint method can distinguish the different sources of MFC.

Then, a supervised OPLS-DA model was employed to sharpen the established PCA model and identify the potentially discriminated markers between *M. exotica* and *M. paniculata*. As expected, a remarkable separation between these two plants was observed in the OPLS-DA score plot ($R^2X$ = 0.47, $R^2Y$ = 0.95, $Q^2$ = 0.923; Fig. 3A). In the permutation plots, all the blue $Q^2$-values were lower than all green $R^2$-values, suggesting non-overfitting for the OPLS-DA pattern model. The result of CV-ANOVA $P$ value (< 0.05) also suggested that the OPLS-DA model was highly significant and validated. The combination of $S$-plot (an absolute $P$ (corr) > 0.4 was used as the cutoff value) and VIP plot (VIP > 1.5) from the OPLS-DA model was performed to identify the potentially differentiated variables as the conspicuously characteristic markers with a high confidence level (Fig. 3B). Finally, a total of 13 chemical markers contributing to the separation of *M. exotica* and *M. paniculata* were selected and 12 markers were identified by comparing the retention time and $[M + H]^+$ ions with the standard references and previous data. All these selected markers were further confirmed by univariate ROC curve analysis ($AUC$ > 0.8) and Student's $t$-test ($P$ < 0.05). Among them, seven coumarins including minumicrolin ($t_R$ 18.31, peak **1**), murrangatin ($t_R$ 24.23, peak **2**), meranzin hydrate ($t_R$ 26.25, peak **3**), murpaniculol ($t_R$ 30.13, peak **4**), auraptenol ($t_R$ 35.22, peak **11**), hainanmurpanin ($t_R$ 39.84, peak **16**), and meranzin ($t_R$ 40.71, peak **17**) were more abundant in *M. exotica*, while five flavones, including 5,7,8,3′,4′-pentamethoxyflavone ($t_R$ 37.67, peak **14**), 5,7,8,3′,4′,5′-hexamethoxyflavone ($t_R$ 41.08, peak **19**), 5,7,3′,4′,5′-pentamethoxyflavone ($t_R$ 44.41, peak **23**), 5-hydroxyl-6,7,8,3′,4′-pentamethoxyflavone ($t_R$ 65.49, peak **40**), and 5-hydroxy-6,7,8,3′,4′,5′-hexamethoxyflavone ($t_R$ 69.11, peak **42**) existed in *M. paniculata* with high abundance (Fig. S5).

*Network pharmacology analysis*

Although there are certain differences in the chemical contituents between *M. exotica* and *M. paniculata*, it is not clear whether these differences can definitely affect the consistency of their efficacy. To explore the influence of these differentiated markers on the pharmacodynamics and mechanisms of *M. exotica* and *M. paniculata*, we conducted network pharmacology analysis. First, the potential targets of 12 accurately identified discriminated markers were predicted by combing PharmMapper Server with SwissTargetPrediction database, and 366 targets were obtained for seven coumarins of *M. exotica* and 237 targets for five flavones of *M. paniculata* after removing the repetitive ones. Among them, 154 common targets were obtained, suggesting that even if the chemical constituents are different, the similar pharmacological activities may be produced (Fig. S6). Our recent pharmacodynamics study showed that the ethanol extracts of *M.*

**Table 1 Identification of the major compounds in HPLC fingerprints of *M. exotica* and *M. paniculata* by LC/ESI-IT-TOF-MS$^n$**

| No. | $t_R$/min | Formula | $[M+H]^+$/$[M+NH_4]^+$ | | | $MS^2$/(*m/z*) | $MS^3$/(*m/z*) | Identification | Source |
|---|---|---|---|---|---|---|---|---|---|
| | | | Meas./Da | Pred./Da | Error/ppm | | | | |
| **1** * | 18.31 | $C_{15}H_{16}O_5$ | 277.1082 | 277.1031 | 4.17 | 259.0761, 231.0951 [a], 205.0471 | 189.0571 | Minumicrolin | ME, MP |
| **2** * | 24.23 | $C_{15}H_{16}O_5$ | 277.1082 | 277.1031 | 4.17 | 259.0904, 231.1105 [a], 189.0635 | 189.0587 | Murrangatin | ME, MP |
| **3** * | 26.25 | $C_{15}H_{18}O_5$ | 279.1243 | 279.1188 | 5.75 | 261.0915 [a] | 243.0956, 189.0486 | Meranzin hydrate | ME, MP |
| **4** * | 30.13 | $C_{15}H_{16}O_5$ | 277.1082 | 277.1031 | 4.17 | 259.0933, 231.0971 [a], 189.0755 | 189.0490, 131.0365 | Murpaniculol | ME, MP |
| **5** | 30.49 | $C_{16}H_{18}O_5$ | 291.1230 | 291.1188 | 1.03 | 273.0998, 219.0614 [a] | 189.0476, 161.0830 | Omphamurin | MP |
| **6** * | 31.59 | $C_{15}H_{14}O_4$ | 276.1215 | 259.0926 | 5.95 | 231.0738, 189.0484 [a] | 131.0545 | Murralongin | ME, MP |
| **7** | 33.19 | $C_{17}H_{18}O_6$ | 319.1150 | 319.1137 | 8.22 | 301.1083 [a], 259.1239, 231.0965, 205.0287 | 259.0609, 231.1043 | Murrangatin acetate | ME |
| **8** | 33.78 | $C_{15}H_{16}O_4$ | 278.1385 | 261.1082 | 0.71 | 243.0834 [a] | 201.0563 | Murraol | ME |
| **9** | 34.28 | $C_{14}H_{12}O_4$ | 245.0790 | 245.0769 | 7.52 | 212.0410, 203.0833 [a] | 188.0593 | Osthenon | ME |
| **10** * | 34.52 | $C_{18}H_{18}O_6$ | 329.1034 | 329.0980 | 4.37 | 314.0739 [a] | 286.0797, 271.0614 | 5-Hydroxy-7,3′,4′-trimethoxyflavone | MP |
| **11** * | 35.22 | $C_{15}H_{16}O_4$ | 278.1401 | 261.1082 | 5.44 | 243.1101, 189.0534 [a] | 131.0473 | Auraptenol | ME |
| **12** * | 35.81 | $C_{19}H_{18}O_7$ | 359.1112 | 359.1086 | 3.71 | 343.0756 [a], 298.0841 | 328.0509, 299.0528 | 5-Hydroxy-7,3′,4′,5′-tetramethoxyflavone | MP |
| **13** | 36.30 | $C_{20}H_{22}O_7$ | 375.1422 | 375.1399 | 4.36 | 211.0560 [a], 191.0857 | 196.0652, 178.0284, 150.0452 | 6,7,8,3′,4′-Pentamethoxyflavanone | MP |
| **14** * | 37.67 | $C_{20}H_{20}O_7$ | 373.1260 | 373.1243 | 5.86 | 343.0794 [a] | 327.0591, 315.0776 | 5,7,8,3′,4′-Pentamethoxyflavone | MP |
| **15** | 38.71 | $C_{18}H_{16}O_4$ | 297.1112 | 297.1082 | 3.16 | 279.0769 [a], 243.0660, 189.0411 | 243.1044, 189.0797 | Unknown | ME |
| **16** * | 39.84 | $C_{17}H_{18}O_6$ | 319.1157 | 319.1137 | 6.02 | 259.0978, 231.0978 [a], 189.0759 | 189.0744, 131.0581 | Hainanmurpanin | ME, MP |
| **17** * | 40.71 | $C_{15}H_{16}O_4$ | 278.1385 | 261.1082 | 0.71 | 243.1110, 189.0361 [a] | 131.0383 | Meranzin | ME |
| **18** * | 40.95 | $C_{19}H_{18}O_6$ | 343.1196 | 343.1137 | 5.80 | 327.0792 [a], 299.0841 | 312.0542, 299.0897, 283.0552 | 5,7,3′,4′-Tetramethoxyflavone | MP |
| **19** * | 41.08 | $C_{21}H_{22}O_8$ | 403.1359 | 403.1348 | 7.07 | 373.0874 [a] | 345.0951, 312.0634, 284.0684 | 5,7,8,3′,4′,5′-Hexamethoxyflavone | MP |
| **20** * | 42.89 | $C_{20}H_{20}O_7$ | 373.1275 | 373.1243 | 1.83 | 343.0812, 329.0968 [a], 312.0946 | 313.0699, 268.0691 | 5,6,7,3′,4′-Pentamethoxyflavone | MP |
| **21** | 43.68 | $C_{15}H_{16}O_4$ | 261.1139 | 261.1082 | 6.78 | 243.0908, 201.0160, 189.0652 [a] | 131.0355 | Isomeranzin | ME |
| **22** | 44.07 | $C_{22}H_{24}O_9$ | 433.1475 | 433.1454 | 4.19 | 403.1032 [a], 372.1231, 357.0947 | 373.0543, 357.0674, 345.0458 | 3,5,6,7,3′,4′,5′-Heptamethoxyflavone | ME |
| **23** * | 44.41 | $C_{20}H_{20}O_7$ | 373.1257 | 373.1243 | 6.66 | 312.0912 [a], 283.0879 | 283.0896, 268.0630 | 5,7,3′,4′,5′-Pentamethoxyflavone | MP |
| **24** * | 46.99 | $C_{21}H_{22}O_8$ | 403.1372 | 403.1348 | 3.84 | 342.1051 [a] | 312.0631, 281.0725, 253.0760; 239.0671 | 5,6,7,3′,4′,5′-Hexamethoxyflavone | ME, MP |
| **25** * | 48.15 | $C_{15}H_{14}O_4$ | 259.0974 | 259.0926 | 3.55 | 231.0864 [a], 189.0373 | 189.0457 | Phebalosin | ME, MP |
| **26** * | 49.08 | $C_{21}H_{22}O_8$ | 403.1394 | 403.1348 | 1.63 | 373.0907 [a] | 330.0670, 301.0556 | 5,6,7,8,3′,4′-Hexamethoxyflavone | MP |
| **27** | 50.82 | $C_{22}H_{24}O_9$ | 433.1467 | 433.1454 | 6.04 | 403.1024 [a] | 371.0761, 342.1152 | 3,5,6,7,8,3′,4′-Heptamethoxyflavone | ME, MP |
| **28** | 51.34 | $C_{16}H_{18}O_5$ | 291.1216 | 291.1188 | 3.79 | 273.1030, 219.0613 [a] | 189.0216, 161.0736 | 5,7-Dimethoxy-8-(3-methyl-2-keto-butyl)-coumarin | MP |
| **29** | 51.53 | $C_{17}H_{18}O_5$ | 303.1237 | 303.1188 | 3.31 | 243.1271 [a] | 189.0737, 187.0282 | Isomurralonginol Acetate | ME |
| **30** * | 54.68 | $C_{22}H_{24}O_9$ | 433.1464 | 433.1454 | 6.73 | 403.0984 [a], 357.0938 | 357.0888, 331.0761, 313.0586 | 5,6,7,8,3′,4′,5′-Heptamethoxyflavone | MP |
| **31** | 55.88 | $C_{20}H_{24}O_6$ | 361.1646 | 361.1606 | 0.10 | 343.1768 [a], 259.0905, 231.0803 | 259.0609, 231.0947, 189.0476 | Minumicrolin isovalerate | ME |
| **32** | 56.93 | $C_{20}H_{24}O_6$ | 361.1646 | 361.1606 | 0.10 | 343.1575 [a], 259.0914 | 231.1029 | Casegravol isovalerate | ME |

Continued

| No. | $t_R$/min | Formula | $[M+H]^+/[M+NH_4]^+$ | | | $MS^2$/(*m/z*) | $MS^3$/(*m/z*) | Identification | Source |
|---|---|---|---|---|---|---|---|---|---|
| | | | Meas./Da | Pred./Da | Error/ppm | | | | |
| **33** | 57.12 | $C_{20}H_{20}O_6$ | 357.1334 | 357.1293 | 0.38 | 275.0562, 203.0426 [a] | 177.0689 | Kimcuongin | ME |
| **34** * | 57.52 | $C_{19}H_{18}O_7$ | 359.1157 | 359.1086 | 8.85 | 326.0795 [a], 298.0819 | 298.0802, 270.1033 | 5-Hydroxy-6,7,3′,4′-tetramethoxyflavone | MP |
| **35** | 59.08 | $C_{23}H_{26}O_{10}$ | 463.1589 | 463.1560 | 2.11 | 433.1137 [a], 415.1063 | 403.0806, 371.0210 | 3,5,6,7,8,3′,4′,5′-Octamethoxyflavone | ME |
| **36** | 61.45 | $C_{20}H_{22}O_6$ | 359.1475 | 359.1450 | 3.95 | 275.0931 [a], 203.0431 | 203.0277, 177.0676 | Panitin D | MP |
| **37** | 62.79 | $C_{20}H_{22}O_6$ | 359.1497 | 359.1450 | 2.19 | 275.0912 [a], 203.0381 | 177.0455 | Exotimarin H | ME, MP |
| **38** * | 62.70 | $C_{20}H_{20}O_8$ | 389.1226 | 389.1192 | 1.27 | 356.0919, 328.0935 [a] | 295.0593, 267.0900 | 5-Hydroxy-6,7,3′,4′,5′-pentamethoxyflavone | MP |
| **39** | 63.56 | $C_{20}H_{22}O_6$ | 359.1513 | 359.1450 | 6.66 | 259.0821, 231.1008 [a], 189.0543 | 189.0460, 131.0424 | Murpaniculol senecioate | MP |
| **40** * | 65.49 | $C_{20}H_{20}O_8$ | 389.1227 | 389.1192 | 1.02 | 359.0741 [a], 341.0636 | 343.0373; 316.0493 | 5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone | MP |
| **41** | 67.41 | $C_{20}H_{24}O_6$ | 361.1651 | 361.1606 | 1.49 | 259.1219, 231.1018 [a], 189.0583 | 189.0281, 131.0527 | Murrangatin isovalerate | ME, MP |
| **42** * | 69.11 | $C_{21}H_{22}O_9$ | 419.1331 | 419.1297 | 1.34 | 389.0874 [a], 371.0748 | 346.0732, 328.0519, 300.0678 | 5-Hydroxy-6,7,8,3′,4′,5′-hexamethoxyflavone | MP |
| **43** | 69.18 | $C_{20}H_{22}O_6$ | 359.1505 | 359.1450 | 0.22 | 275.0939 [a], 257.0526 | 203.0487 | Isomurranganon senecioate | ME |
| **44** | 71.90 | $C_{20}H_{24}O_6$ | 361.1627 | 361.1606 | 5.18 | 259,0964 [a], 231.0956, 189.0554 | 231.1037, 189.0557, 161.0589 | Paniculonol isovalerate | ME, MP |
| **45** | 72.85 | $C_{20}H_{24}O_5$ | 345.1680 | 345.1657 | 4.79 | 243.0990 [a], 187.0176 | 187.0217 | Isomurralonginol isovalerate | ME, MP |
| **46** | 73.66 | $C_{30}H_{32}O_8$ | 521.2208 | 521.2131 | 7.31 | 301.1298, 261.1263, 243.0935 [a] | 189.0737, 187.0735 | Murradimerin A or isomer | ME |
| **47** | 74.02 | $C_{32}H_{34}O_{10}$ | 579.2232 | 579.2186 | 1.26 | 519.2020 [a], 261.1075, 231.1057, 189.0486 | 231.0954, 189.0598 | Murramarin A or isomer | ME |
| **48** | 74.14 | $C_{20}H_{22}O_7$ | 375.1431 | 375.1399 | 1.95 | 221.0702 [a], 181.0524 | 206.0557, 190.0497 | 6′-Hydroxy-3,4,5,2′,4′-pentamythoxychalcone | MP |
| **49** | 75.14 | $C_{30}H_{32}O_8$ | 521.2133 | 521.2131 | 7.01 | 261.1121 [a], 243.1247, 189.0542 | 189.0272 | Murradimerin A or isomer | ME |
| **50** | 76.19 | $C_{20}H_{26}O_6$ | 363.1801 | 363.1763 | 0.32 | 345.1667 [a], 261.1006, 243.1029 | 261.1078, 189.0737 | Muralatin M or isomer | ME |
| **51** | 76.78 | $C_{21}H_{26}O_6$ | 375.1750 | 375.1763 | −13.94 | 273.1056 [a] | 219.0751, 203.0487 | Omphamurin isovalerate | MP |
| **52** | 77.08 | $C_{26}H_{28}O_9$ | 485.1819 | 485.1806 | 2.67 | 377.1073, 309.1459, 283.1290, 203.0819, 189.0554 [a] | 131.0744 | Unknow | ME |

* Compound identified by comparing with reference standard; [a] Precursor-ion for next stage MS

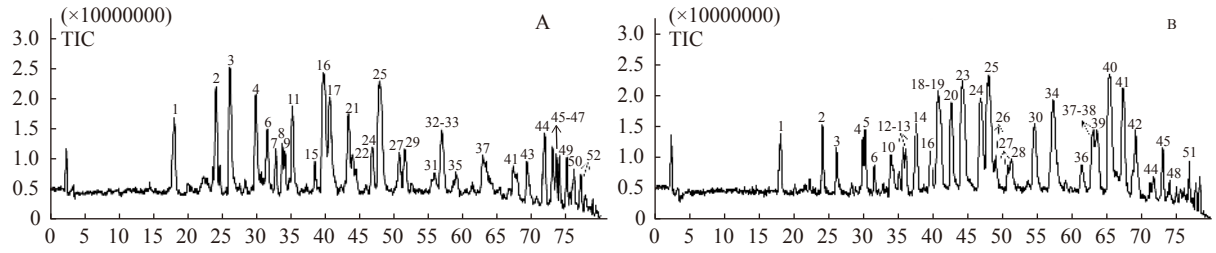


**Fig. 2 LC–IT-TOF-MS chromatograms for the homogenized samples of *M. exotica* (A) and *M. paniculata* (B)**

*exotica* and *M. paniculata* exhibited similar anti-inflammation and analgesia effects [27], which supports the above deduction.

With respect to the traditional use of MCF as an anti-inflammatory agent, we collected the inflammation-related targets from the OMIM and Genecards databases. After matching the component-related targets with the inflammation-related targets, 44 and 29 targets were identified respectively as the potential targets for differentiated components of *M. exotica* and *M. paniculata* to treat inflammation-related diseases. Among them, 22 common targets were found for both plants (Fig. S6). A PPI network for these common targets was then established and six targets (PTGS2, ALB, MMP9, MPO, MAPK14, and CASP3) exhibited high degree numbers (de-

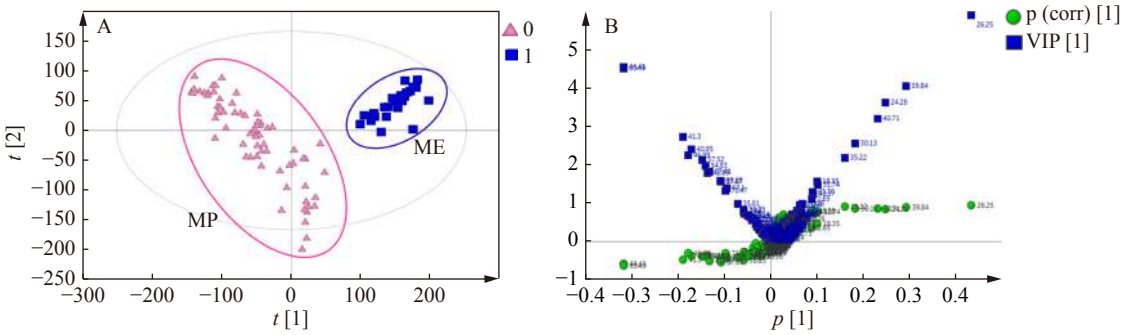


**Fig. 3 OPLS-DA score plot (A) and combination plot of *S*-plot and VIP values of *M. exotica* (ME) and *M. paniculata* (MP)**

gree > 12). According to the KEGG enrichment analysis, the common targets were mainly concentrated on the TNF signaling pathway, arachidonic acid metabolism, T cell receptor signaling pathway, Toll-like receptor signaling pathway, and neurotrophin signaling pathway. Among them, an important inflammation related TNF signaling pathway exhibited the highest *P* value for both plants. In addition, some of the predicted targets of *M. exotica* were shown to be specifically associated with the chemokine signaling pathway, B cell receptor signaling pathway, NF-*κ*B signaling pathway and other pathways, while those of *M. paniculata* were associated with the AMPK signaling pathway and Wnt signaling pathway. The pathways directly related to the anti-inflammatory activities of *M. exotica* and *M. paniculata* are shown in Fig. 4. Cytoscape was used to construct the network to visually reflect the relationship of component-target-pathway (Fig. 5). The above results indicated that the differentiated markers of *M. exotica* and *M. paniculata* might exert similar activities by acting on the same or different inflammation-related pathways.

## Conclusions

In the present study, the chemical constituents of *M. exotica* and *M. paniculata* were comparatively studied by

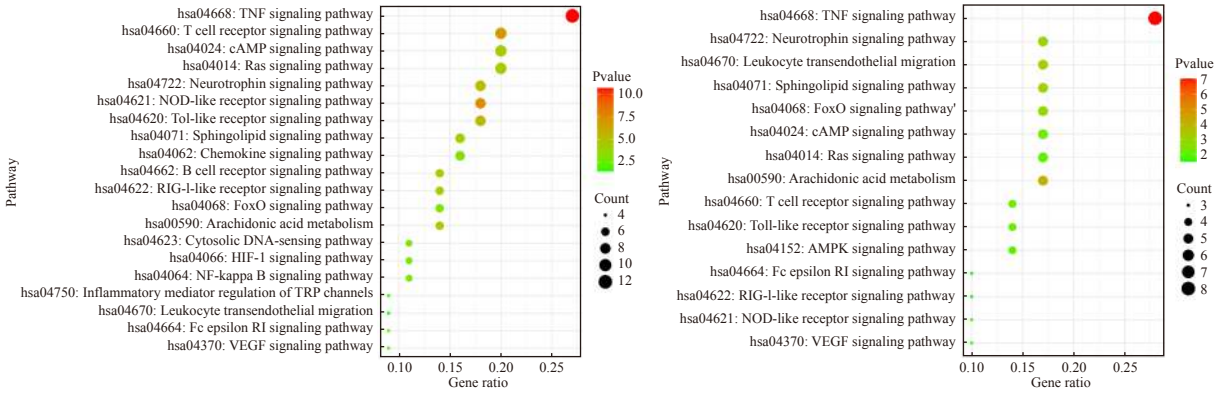


**Fig. 4 The bubble diagram of KEGG enrichment of differentiated components of *M. exotica* (left) and *M. paniculata* (right)**

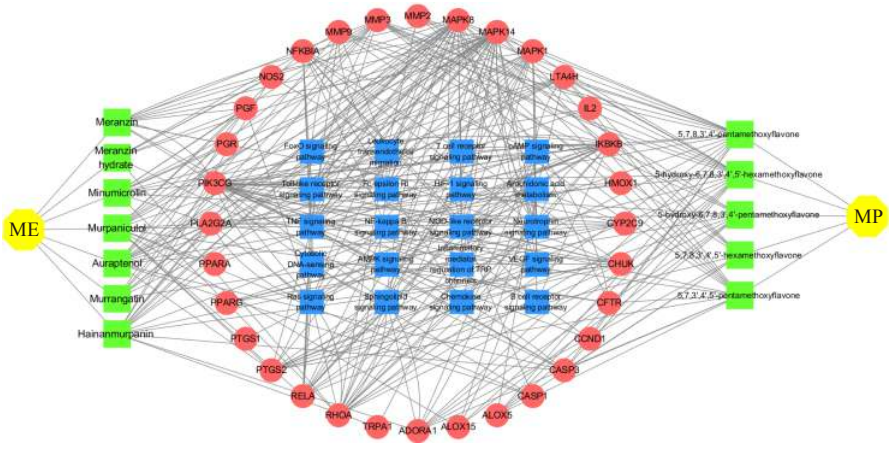


**Fig. 5 "Component-Target-Pathway" network diagram of differentiated components of *M. exotica* (ME) and *M. paniculata* (MP). The green squares represent the differentiated components, red circles represent the targets, and blue squares represent the pathways**

combination of HPLC and LC/MS fingerprint analysis with chemometrics and network pharmacology methods for the first time. A total of 52 compounds were identified from MFC by LC-IT-TOF-MS and comparison with the standard references. The fingerprint analysis and the HCA, PCA, and OPLS-DA results showed that there are certain differences in the chemical contituents between these two species. Thirteen differentiated chemical markers were subsequently screened out by OPLS-DA, among which, 7-methoxycoumarins were higher in *M. exotica*, while the polymethoxylated flavonoids were richer in *M. paniculata*. However, the following network pharmacology analysis demonstrated that these markers might possess the similar anti-inflammatory activity via some common targets and inflammation-related metabolic pathways. In sum, the above results supply theoretical foundation for the two-source collection of MFC in *Chinese Pharmacopoeia*, and provide valuable reference for the equivalent use of ME and MP in clinical settings. Moreover, our results proved that HPLC and LC/MS fingerprint coupled with chemometrics and network pharmacology analysis is an efficient approach for the evaluation of multi-source TCMs.

## Supporting information

Supporting information of this paper can be requested by sending E-mail to the corresponding author.

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**Cite this article as:** LIANG Hai-Zhen, DU Zhi-Yong, YUAN Shuo, LU Meng-Qiu, XING Jian-Yong, MA Qing, HAN Zheng-Zhou, TU Peng-Fei, JIANG Yong. Comparison of *Murraya exotica* and *Murraya paniculata* by fingerprint analysis coupled with chemometrics and network pharmacology methods [J]. *Chin J Nat Med*, 2021, **19**(9): 713-720.