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Identification and characterization of curcuminoids in turmeric using ultra-high performance liquid chromatography-quadrupole time of flight tandem mass spectrometry

Shuailong Jia[a], Zhifeng Du[a], Chengwu Song[b], Shuna Jin[c], Yang Zhang[d], Yulin Feng[e], Chaomei Xiong[a], Hongliang Jiang[a,*]

[a] Tongji School of Pharmacy, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan, Hubei, China
[b] College of Pharmacy, Hubei University of Chinese Medicine, Wuhan, China
[c] Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, State Key Laboratory of Environmental Health (incubation), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China
[d] Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China
[e] Jiangxi University of Traditional Chinese Medicine, Nanchang, China

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ABSTRACT

A three-step strategy was developed for systematic characterization of curcuminoids in turmeric. Based on UHPLC-QTOF-MS/MS analysis, 89 curcuminoids including 16 novel ones were identified in the turmeric samples using this approach. During the identification process, false positive results were excluded by combining the positive and negative ESI-MS/MS analyses. Moreover, the characterization of the keto and enol forms of type A, B and C curcuminoids was first discussed and they were clearly distinguished using negative ESI-MS/MS method with UV spectra analyses. The structures of detected curcuminoids were identified and rationalized in both ion modes. Additionally, the fragmentation behaviors of the 15 types of curcuminoids were clearly illustrated in this work, which will be helpful for detection and identification of corresponding trace curcuminoids in complex turmeric samples using UHPLC-QTOF-MS/MS methods.

1. Introduction

Turmeric, belonging to the Zingiberaceae famliy, is extensively distributed in East Asia and Southeast Asia such as China, Indonesia, Australia and India. The chemical constituents of turmeric mainly comprise curcuminoids, phenylpropene derivatives, terpenoids, flavonoids, steroids and alkaloids [1]. Among these components, curcuminoids have been extensively investigated because of their various pharmacological activities [2,3]. Curcumin, demethoxycurcumin and bisdemethoxycurcumin are the three major curcuminoids in turmeric extracts [4]. Various chemical studies indicated that curcumin exhibits keto-enol tautomerism due to the presence of the β-diketone system [5,6], as shown in Fig. 1. Additionally, it was reported that the keto and enol forms of curcumin derivatives showed obviously different bioactivities, which was related to corresponding diseases [7,8]. Thus, it is important to distinguish the keto and enol forms of curcuminoids. In addition to the three major curcuminoids, there are various minor curcuminoids in the turmeric, which attracted the attention of researchers due to their significant bioactivities [9–11]. Therefore, it is critical for us to explore the minor curcuminoid constituents in turmeric.

Various techniques have been applied for the analyses of curcuminoids in turmeric samples, such as HPLC, MS, GC-MS and LC-MS [12–15]. However, most researchers mainly focused on the identification and quantification of the three major curcuminoids. On the contrary, few studies were carried out for the systematically qualitative analyses of curcuminoids in turmeric. UHPLC-QTOF-MS/MS technology has been extensively used in qualitative and quantitative analyses for complex samples because of its high separation efficiency, accurate mass accuracy and excellent sensitivity [16–18]. In our previous studies, the three major curcuminoids along with other minor curcuminoids were characterized using various LC-MS methods [19,20]. Although limited curcuminoids

* Corresponding author.
E-mail address: jianghongliang668@163.com (H. Jiang).

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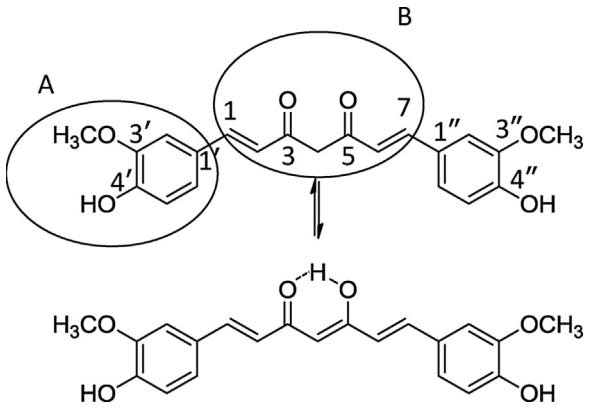

Fig. 1. The keto-enol tautomerism of curcumin.

were identified due to the use of different qualitative strategies, it laid the groundwork for our present work.

In the present study, a three-step strategy was developed for detecting and identifying the curcuminoids in turmeric samples by using a single UHPLC-QTOF-MS/MS platform. Firstly, potential curcuminoids were predicted through the combination of various skeletons and aryl groups. Secondly, the predicted compounds were verified by extracting the corresponding precursor ions in positive and negative modes to make sure whether they exist in the sample or not. Subsequently, detected compounds were characterized by their specific product ions and retention times based on our previous studies about the fragmentation patterns of known curcuminoids [19,20]. During the analyses of MS/MS spectra of potential curcuminoids, false positive results were excluded based on their product ions and respective retention time. Meanwhile, several unexpected compounds were detected, which were confirmed not to be the known types of curcuminoids. Therefore, the third step was to characterize and rationalize the structures of the newly observed curcuminoids based on their precursor ions, characteristic product ions and corresponding retention times. Finally, 101 chromatographic peaks (12 curcuminoids exhibited keto-enol tautomerism), 89 curcuminoids including 16 novel ones were detected and identified in turmeric extracts (Fig. 2).

2. Materials and methods

2.1. Chemicals and reagents

HPLC grade acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was produced by a Milli-Q water system (Millipore, Bedford, MA, USA). Formic acid (≥98%) of analytical grade and ammonium formate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The 10 reference standards were in-house isolated from the dried rhizome of turmeric. Their chemical structures were elucidated utilizing comprehensive spectral analysis of HR-ESI–MS, ^{1}H NMR and ^{13}C NMR data, and by comparison with the data previously published. The purity of these standards were above 98% according to HPLC-UV analysis as described [17].

2.2. Plant materials and sample preparation

Dried rhizomes of turmeric from different regions including Sichuan, Yunan, Guizhou, Fujian, Jiangsu, Guangdong and Guangxi Province of China and Myanmar were purchased from huge-hunter agriculture and products Co., LTD (Gaoyao city, Guangdong, China). They were identified and authenticated by Professor Keli Chen at the Hubei University of Chinese Medicine. The voucher specimens were deposited at the herbarium of Huazhong University of Science and Technology.

The 14 dried rhizomes of turmeric from different regions were ground into powder (24 mesh), the pulverized sample (1.0 g) was extracted with 5 mL of 80% aqueous methanol in an ultrasonic bath for 25 min at room temperature, before and after the ultrasound bath, the sample would be vigorously vortexed for 30 s. The extraction process was repeated twice with the solvents of 5 mL 50% aqueous methanol and 5 mL 100% methanol, respectively, then the three separate extracts were combined. The supernatants of these combined samples were centrifuged at 8000 rpm for 10 min followed by filtration. Another turmeric sample was pooled from the equivalent filtrate of the 14 individual samples. Totally, 15 samples were obtained for systematic identification and comparative analysis of curcuminoids.

2.3. LC–MS/MS conditions

The LC analysis was conducted on a Shimadzu UHPLC system (Kyoto, Japan) comprising a LC-30AD solvent delivery system, a SIL-30AC autosampler, a CTO-30A column oven, a DGU-20A3 degasser and a CBM-20A controller, with a PDA Detector (SPD-M20A). Full spectral scanning was performed from 190 to 800 nm, with range step at 1 nm. A Welch Ultimate UHPLC C18 column (100 mm × 2.1 mm, 1.8 μm) was used. The column oven temperature was set at 40 °C. The mobile phases used were as follows: (A) 0.1% formic acid in positive mode, 0.1% formic acid and 5 mM ammonium formate in negative mode and (B) acetonitrile. The flow rate was set at 0.3 mL/min. The elution program was as follows: 0.01 min, 20% B; 10 min, 35% B; 30 min, 55% B; 40 min, 95% B; 43 min, 95% B; 43.1 min, 20% B; 46 min, 20% B.

Mass spectrometric analyses were performed on a Triple TOF™ 5600 system with a Duo Spray source (AB SCIEX, Foster City, CA, USA) in positive and negative electrospray ionization (ESI) modes. The MS conditions were as follows: ion spray voltage, 4.5 KV/−4.5 KV; the ion source temperature, 600 °C; curtain gas, 30 psi; nebulizer gas (GS 1), 50 psi; heater gas (GS 2), 50 psi; declustering potential (DP), 80 V. The mass ranges were set at *m/z* 100–600 for TOF MS scan, 50–600 for TOF MS/MS experiments. In the TOF MS/MS experiments, the most intensive 8 ions from each full MS scan were selected for MS/MS fragmentation. Dynamic background subtraction was used to match the information dependent acquisition (IDA) criteria. The collision energy (CE) was set at 30 eV/−40 eV and the collision energy spread was 10 eV for MS/MS experiments. The data was analyzed using PeakView Software™ 1.2 (AB SCIEX, Foster City, CA, USA).

3. Results and discussion

3.1. UHPLC-QTOF-MS/MS conditions optimization

UHPLC system with a small-particle-size column (Welch Ultimate UHPLC C18 column, 100 mm × 2.1 mm, 1.8 μm) was chosen for separating the total extract of turmeric because of its great separation efficiency in the analyses of complex samples. 0.1% formic acid along with 5 mM ammonium formate (negative mode) and 0.1% formic acid (positive mode) aqueous solution (A), acetonitrile (B) were ultimately selected as mobile phases, considering its lower back pressure and better elution power compared with methanol-water system (aqueous solution).

The MS conditions were optimized using reference standards of 10 curcuminoids obtained from the turmeric. Both the positive and negative ion modes were evaluated to achieve optimal MS sensitivity for detection and to obtain abundant fragment ions for structural elucidation. Consistent with our previous study [20], better sensi-