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Quality evaluation of the artemisinin-producing plant *Artemisia annua* L. based on simultaneous quantification of artemisinin and six synergistic components and hierarchical cluster analysis

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ABSTRACT

Artemisia annua L. (*A. annua*) is one of the most widely used traditional Chinese Medicine, and is also an exclusive natural origin of artemisinin for the industrial production. Several sesquiterpenes, flavonoids and coumarin contained in *A. annua* could enhance the pharmacological effects of artemisinin. However, the contents of these components vary significantly in different places of origin and different parts of *A. annua*. To evaluate and differentiate its quality, an accurate and rapid high-performance liquid chromatography tandem mass spectrometric (HPLC–MS/MS) assay has been developed and validated for the simultaneous determination of artemisinin and six synergistic components in *A. annua*. The run time of analyzing one sample is only 6.0 min. The established method could serve as an accurate and rapid assay for the quality evaluation of *A. annua* from different places of origin and different parts of the plant. The hierarchical cluster analysis results indicated that the content of scopoletin and/or all seven components (artemisinin and six synergistic components) might be utilized to differentiate different parts and different plucking times of *A. annua*.

1. Introduction

Artemisinin (Fig. 1a) belongs to the sesquiterpene lactone family containing the specific endoperoxide bridge, which has been successfully discovered as an anti-malarial drug by research team led by Youyou Tu in 1970s. Till now, artemisinin and its semisynthetic derivatives are still the most important anti-malarial drugs available and ART-based combination therapy (ACT) has been recommended worldwide as first-line treatment for falciparum malaria since 2001 (WHO Antimalarial drug combination therapy, 2001; Gbotosho et al., 2011; WHO World Malaria Report, 2016).

It is just because of artemisinin, people all over the world have known *Artemisia annua* L. (*A. annua*, Qing-Hao in Chinese), which is an exclusive natural origin of artemisinin for the industrial production (Li and Luo, 2004; Tang and Zhang, 1957). In recent years, the comprehensive utilization of *A. annua* in the industry, but not only for the extraction of artemisinin, becomes a new research focus.

Besides artemisinin, *A. annua* also contains many other chemical components, including other sesquiterpene, volatile oil, terpenes, flavonoids and coumarins (Yang et al., 2003; Chen et al., 2008; Ćavar et al., 2012; Larson et al., 2013). Previous literatures mainly focused on artemisinin, since it is the anti-malarial active fraction, while little attention was paid to other components. Recently, other components have gained more and more attention because of their significant synergistic effects on artemisinin. In our previous studies (Zhang et al., 2016; Ji et al., 2008), arteannuin B, arteannuic acid and scopoletin was found to show significant synergistic effects on the anti-malarial effects of artemisinin, while these three components didn't have obvious anti-malarial effects. Arteannuin B was further confirmed to be one of main contributors in *A. annua* leading to enhanced antiplasmodial potency of artemisinin via regulation of its metabolism (Cai et al., 2017). Several flavonoids from *A. annua*, such as casticin, chrysosplenol D, chrysosplenetin and artemetin may contribute to the activity of artemisinin against *P. falciparum* (Ferreira et al., 2010; Li et al., 2015; Elford et al.,

Abbreviations: PLE, pressurized liquid extraction; HPLC–MS/MS, high-performance liquid chromatography tandem mass spectrometry; ESI, electrospray ionization; MRM, multiple reaction monitoring; ACT, ART-based combination therapy; UV, ultraviolet spectrophotometry; TLC, thin layer chromatography; GC, gas chromatography; HPLC-UV-ELSD, high performance liquid chromatography coupled with ultraviolet spectrophotometry and evaporation light scattering detection; ELISA, enzyme-linked immuno sorbent assay; LC–MS, liquid chromatograph-mass spectrometry; DMSO, dimethyl sulfoxide; CE, collision energy; DP, declustering potential; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; RE, relative error; HCA, hierarchical cluster analysis

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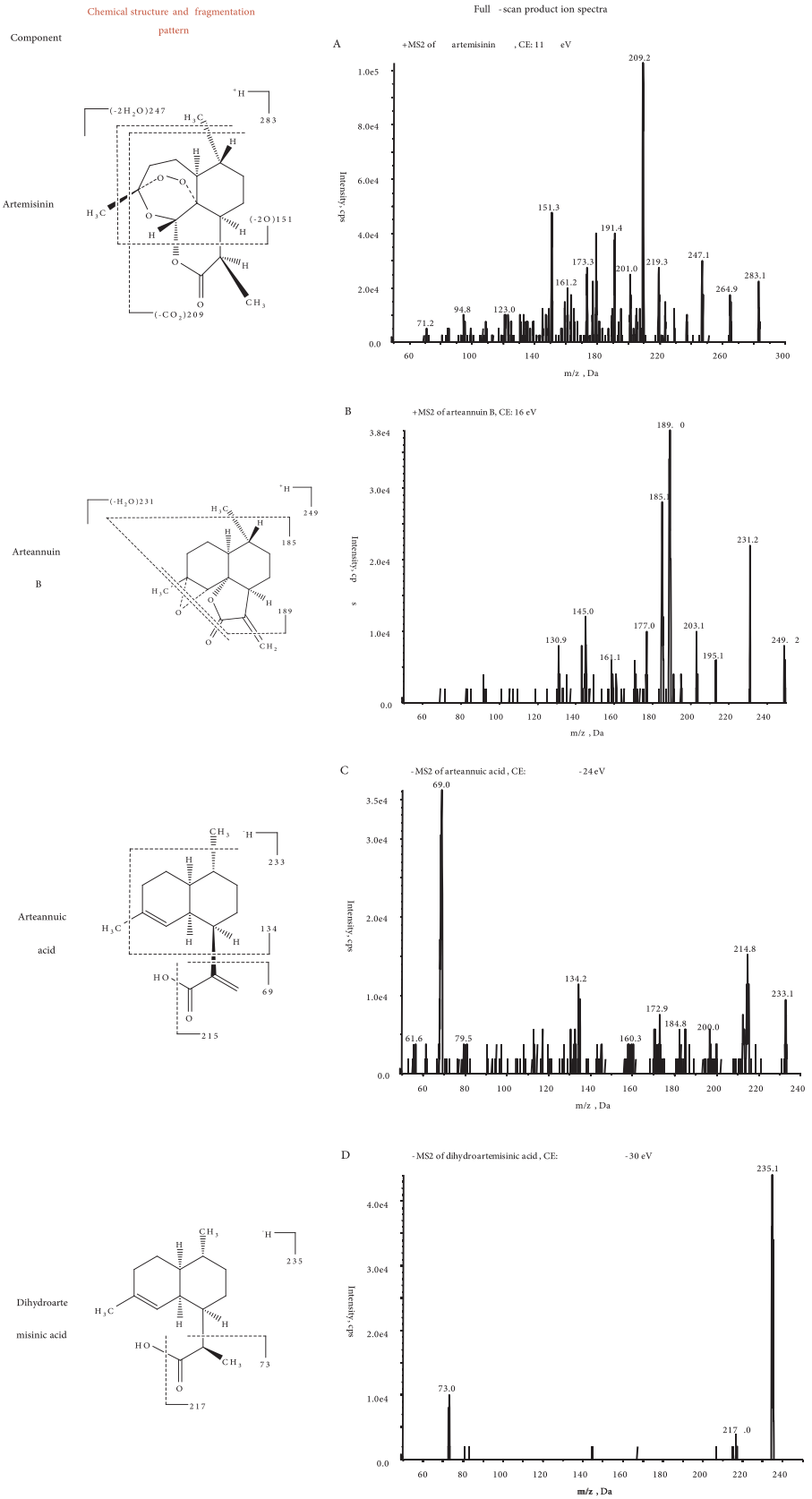

Fig 1. Chemical structures, fragmentation patterns and full-scan product ion spectra of $[M+H]^+$ or $[M-H]^-$ ions of artemisinin and six synergistic components in *A. annua* and positive internal standards (buspirone) and negative internal standards (chloroamphenicol).