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Rapid discrimination of *Schisandra sphenanthera* and *Schisandra chinensis* using electronic tongue and ultra-performance liquid chromatography coupled with chemometrics

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ABSTRACT

Both *Schisandra sphenanthera* (*S. sphenanthera*) and *Schisandra chinensis* (*S. chinensis*) are used as traditional Chinese medicines, but they have different medicinal properties. Because *S. sphenanthera* is cheaper, it is often used as a counterfeit product for *S. chinensis*. In the present study, an electronic tongue (e-tongue) was used for discrimination of the two *Schisandraceae* species. In addition, the contents of schisandrin, schizandrol B, schisantherin A, deoxyschizandrin, and schisandrin B were determined simultaneously by ultra-performance liquid chromatography. Principal component analysis (PCA) and discriminant factor analysis (DFA) were used to establish the mathematical models for species identification, and the classification rates for both methods reached 100%. The e-tongue coupled with multivariate analysis exhibited the excellent performance and classification accuracy, and this was validated by the ultra-performance liquid chromatography results. This simple e-tongue technique could be useful for rapid and accurate identification of *S. sphenanthera* and *S. chinensis*.
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1. Introduction

Schisandra sphenanthera Rehd. et Wils. (*S. sphenanthera*) and *Schisandra chinensis* (Turcz.) Baill. (*S. chinensis*) belong to the *Schisandraceae* family. The former is abundant in South China, whereas the latter is primarily found in North China and adjacent regions, including Russia, Korea and Japan. For a long time, their dried fruits have been applied extensively to food and other application (e.g. medicine and pharmaceutical production). In China, *Schisandraceae* is widely used in the treatment of chronic coughs and asthma, enuresis, diabetes symptoms, diarrhea, spontaneous sweating, spermatorrhea, night sweats, palpitations, and insomnia. The 2000 edition of Chinese Pharmacopoeia list two different species, *S. sphenanthera* (Nanwuweizi) and *S. chinensis* (Beiwuweizi) [1]. Modern studies have shown that most of the pharmacological effects of berries from Chinese *Schisandraceae* can be attributed to their lignan contents, which make up approximately 1% of their chemical composition [2]. Recent research suggests that lignans, especially those with a dibenzocyclooctadiene skeleton such as schisandrin, schisandrol B, deoxyschizandrin, schisantherin A, and schisandrin B (Fig. 1), are major bioactive constituents in Chinese *Schisandraceae* [3]. There is evidence that these compounds have liver-protecting [4], anti-tumor [5], anti-HIV [6], anti oxidation activity [7], and regulate the central nervous system [8]. However, *S. sphenanthera* is enriched in schisantherin A and deoxyschisandrin, whereas *S. chinensis* mainly contains schisandrin and schisandrin B [9–10]. Currently, *S. chinensis* is more commonly used than *S. sphenanthera*. Because *S. sphenanthera* is the cheaper of the two, it has been used as a counterfeit of *S. chinensis* in herbal markets. Modern medical research has shown that they have different medicinal values. For example, *S. chinensis* inhibits the central nervous system and stimulates respiration. *S. sphenanthera* is primarily was useful in the prevention and treatment of hyperproliferation and inflammatory skin diseases [11]. Therefore, accurate and rapid discrimination of the two herbs is important to ensure clinical efficacy and authenticity of the product for commercial use.

Recently, several methods have been developed for identification of *S. sphenanthera* and *S. chinensis*, including random amplified polymorphic DNA (RARD) [12], inter sample sequence repeat (ISSR) [13], ITS2 sequences [14], high-performance liquid chromatography (HPLC) [15], ultra-performance liquid chromatography-mass spectrometry (UPLC-MS), and gas chromatography–mass spectrometry (GC–MS) [16]. However, there are some drawbacks with these methods, including complex sample preparation, long analytical time, large consume of organic reagents, and expensive cost. Thus, a fast and effective method for differentiation of Chinese *Schisandraceae* species is required.

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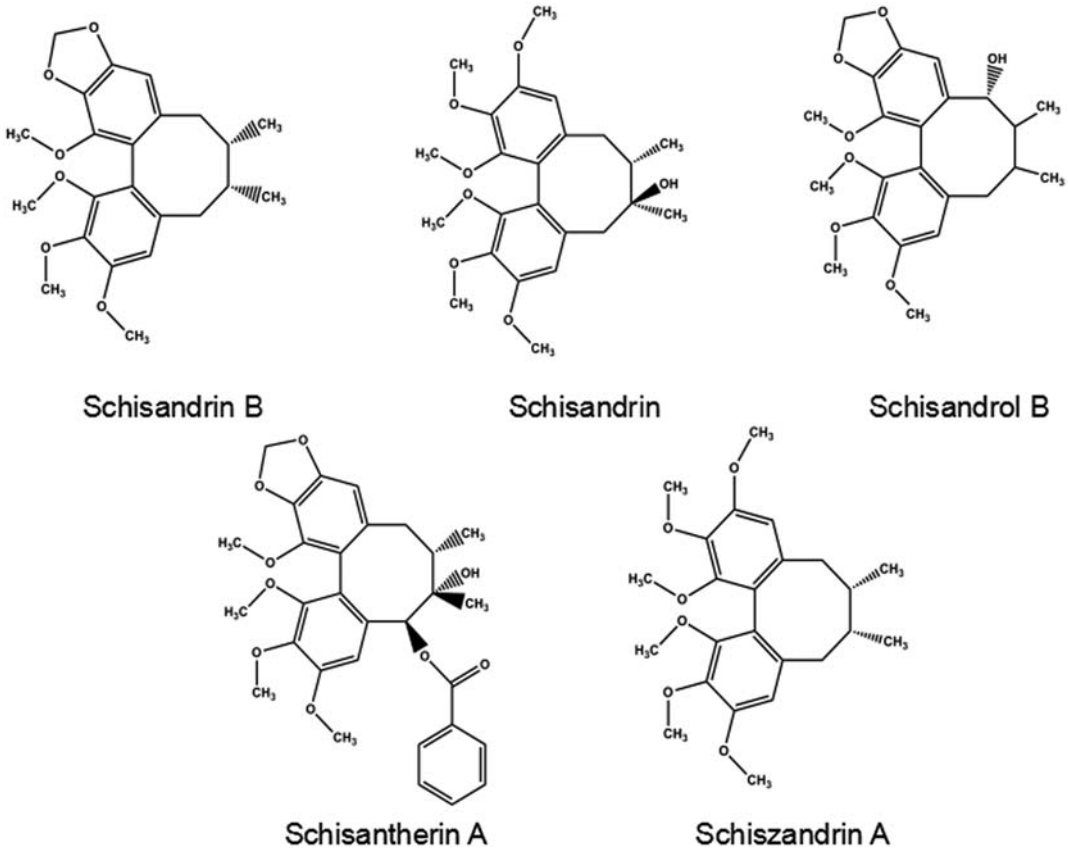

Fig. 1. Chemical structures of five lignans in *S. chinensis* and *S. sphenanthera*.

The electronic tongue (e-tongue) is an artificial taste recognition technology that is increasing in popularity for objective automated evaluation of samples. Analysis with the e-tongue is non-toxic, rapid, inexpensive, highly accurate and reliable [17–18]. To date, the method has been used for identifying various Chinese herbs, including the raw and processed of *Coptidis chinensis* [18], the geographical origins of *Scutellaria baicalensis* [19] and growth time of *Astragalu semembranaceus* var. *Mongholicus* [20]. Few studies identified *S. sphenanthera* and *S. chinensis* using the e-tongue.

In this study, the e-tongue was used for data acquisition, and ultra-performance liquid chromatography (UPLC) was applied for simultaneous quantitative determination of five bioactive lignans in *S. sphenanthera* and *S. chinensis*. Principal component analysis (PCA) and discriminant factor analysis (DFA) were used to establish identification models. UPLC data was used to validate the classification accuracy.

2. Materials and methods

2.1. Material

Twenty samples were randomly selected from a large number of *Schisandraceae* samlpes, which collected from different provinces of China. The details of the species, geographical origins of these samples are shown in Supplementary Table S1. As shown in Fig. 2, there is no significant difference in morphology of *S. sphenanthera* and *S. chinensis*. Each sample was identified by Professor Yuan Yuan of National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences. Each plant sample was oven dried at 50 °C for 48 h and ground into powder (finer than 40 mesh) using a pulverizer (FW135, Taisite Instrument Co., Ltd., Tianjing, China) and then stored at 20 °C in a moister buster cabinet (FU480, Hangzhou FRK Precise Electronics Co., Ltd., Hangzhou, China) before analysis.

Reference standards (purity ≥98.0%) of schisandrin (MUST-13061206), schizandrol B (MUST-14010312), schisantherin A (MUST-13110606), deoxyschizandrin (MUST-13080205) and schisandrin B (MUST-13091606) were purchased from Chengdu Mansite Biological Co.Ltd. (Chengdu, China); HPLC grade acetonitrile was purchased from Fishier Scientific USA (Fair Lawn, NJ, USA); Analytical grade methanol was obtained from Shanghai Chemical Corporation of China. Water was purified by Milli-Q water system (Millipore, USA) for UPLC and the e-tongue. Standard chemicals utilized for e-tongue start up were supplied by manufacturer (Alpha M.O.S., Toulouse, France).

2.2. E-tongue analysis

E-tongue analysis was based on previous method with few modifications [21]. Analyses were conducted with a portable α-ASTREE II e-tongue (Alpha M.O.S.). The device was equipped with a sensor array (ZZ, AB, GA, BB, CA, DA and JE) mounted around an Ag/AgCl reference electrode, which had a bi-layered polymer membrane that determined the device cross-selectivity to tastes [22]. In addition, the e-tongue system had a 48 position auto-sampler with 25 glass auto-sampler vials, an electronic unit for data acquisition, and a personal computer with a software package to record the difference between the reference electrode and the sensors.

Accurately weighed 2.0 g of sample powder were mixed and put into conical flask, refluxed with 200 mL of Milli-Q water for 1 h, and filtered through gauze. The filtrate was transferred to a 250 mL volumetric flask, diluted with Milli-Q water to the mark, then transferred to a 500 mL polypropylene centrifuge tube. The solution was centrifuged for 10 min at 8000 × *g* (Centrifuge 5424, Eppendorf, Hamburg, Germany). Aliquot (5 mL) of the supernatant was transferred and diluted to a total volume of 25 mL with Milli-Q water. Finally, the solution was filtered through a 0.45 μm polyvinylidene difluoride (PVDF) membrane, then transferred to 25 mL glass auto-sampler vial for e-tongue analysis.

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