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Advance in quality assessment of Chinese materia medica using microscopic and morphological methods

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[ABSTRACT] Quality evaluation plays a vital role in ensuring safety and effectiveness of Chinese materia medica (CMM). Microscopic and morphological technologies can be used to distinguish CMM's characteristics, such as shape, size, texture, section, and smell, for authenticity and quality control of CMM. The microscopic and morphological applications of novel micro-technology, colorimeter, and texture analyzer for CMM identification are summarized and the future prospect is discussed in this paper. Various styles and complex sources of CMM are systemically reviewed, including cormophyte medicinal materials, fruit and seeds, pollen grain, and spore materials.

[KEY WORDS] Chinese materia medica; Micro-technology; Colorimeter; Texture analyzer
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Introduction

Quality evaluation of Chinese materia medica (CMM) aims to ensure its safety and effectiveness. Microscopy and morphology are inheritance methods for identification of CMM, which is of great significance to guarantee the quality and safety of herbs [1]. By means of viewing and touching, microscopic and morphological identification can distinguish CMM's characteristics, such as shape, size, color, appearance, texture, section, and smell, for authenticity and quality control. There are many novel and efficient methods for microscopic and morphological identification of CMM, such as electronic eye, texture analyzer, electronic nose, and electronic tongue [2].

Recently, microscopic and morphological identification has been integrating continuously with the application of high-tech instruments, such as scanning electron microscope, fluorescence technology, and electronic technology, accelerating the automation process of identification and contributing to more rapid operation with more reliable results [3]. Therefore, it is of great importance to develop and apply the technology of microscopic and morphological identification. In this paper, the application of novel micro-technology, colorimeter and texture analyzer for microscopic and morphological identification of CMM will be summarized, and the future prospect will be discussed.

Microscopic Techniques

Microscopic identification has an integrated system to determine the quality of CMM, which can observe tissue structure. cell shapes, and inclusions. Micro-technology has been developed from the ordinary optical microscope to the scanning electron microscope, polarized microscopy, and fluorescence microscopy and its scope of application is also constantly expanded.

Optical microscope

Optical microscope has been widely used in the identification of CMM. Meanwhile, the optical microscope technology has been combined with a variety of techniques to estab-

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lish a systematic method, which can be applied to investigate the ingredient distribution in different tissues. Tan *et al*. have observed that saikoside is mainly distributed in the pericycle and secondary phloem of *Bupleurum chinense* DC., using a microscope coupled with vanillin-acetic acid and perchloric acid mixed reagent [4]. This technique can also be applied to evaluate the quality of herbs indirectly.

Qualitative microscopic identification

Qualitative microscopic identification of CMM is to observe tissue structure, cell shapes and inclusions, to identify the authenticity. Han *et al.* have studied the microscopic structure of above-ground portion of *Carthamus tinctorius* L. [5]. Some structural features are summarized as the main microscopic identification characteristics of the above-ground portion [5]. Ma *et al.* have presented the taxonomic identification of Coniferopsoda and Composotae pollens, which exist broadly in sedimentary strata [6]. Xu *et al.* have determined the microscopic identification standard of two leguminous herbs, semen *Astragalus complanatus* R. Br. and *Psoralea corylifolia* L., and provided reference for microscopic identification [7].

To identify *Agriophyllum squarrosum*, Lv *et al.* have observed the cross sections of the root and stem, and distinguished botanic tissue structures through microscopic methods [8]. The variation of age determination between two species of Astragali Radix is investigated [8]. The research has indicated that the microscopic characteristics, such as number of layers of phellem, continuing lignified xylem bundles within spring wood, and lignified parenchyma cells in the central part of the xylem, could be used to differentiate the root of *A. membranaceus* from the root of *A. membranaceus* var. *mongholicus* [9]. Fei *et al.* have performed an herbal study of *Rheum nobile radix* and Rhizome by microscopic and molecular identification, and obtained comprehensive identification information of *Rheum nobile* [10]. Microscopic and morphological characterization of *Begonia grandis*. is illuminated. The main characteristics appear to be prismatic crystals of calcium oxalate, non-glandular hairs, and vessel, providing identification information for *Begonia grandis* [11]. By means of microscopic technology, Fu *et al.* have identified the authenticity of Moschus, the microscopic characteristics of natural Moschus, domestic Moschus, and the counterfeit ones are summarized [12]. Liang *et al*. have described the morphological and microscopic characteristics of transverse section of *Psoralea corylifolia* L., and fluorescent speckles of test samples with the same color are found in the same place of psoralen and isopsoralen [13]. Wu *et al.* have compared microscopic characteristics of *Rauvolfia tetraphylla* and *Rauvolfia vomitoria*, and the obvious differences in their epidermal cells, phloem, xylem and medulla could be used to distinguish one from the other [14].

In addition to powder characteristics, there are ingredients that can be sublimated in CMM. The sublimate could be collected for the observation of its shape and color under a microscope. For instance, the sublimate of *Polygonum multi florum* Thunb. and its adulterants are observed under the microscope [15]. The genuine one shows crystal of yellow columnar and needle cluster, while the fake one is colorless or pale yellow. When added into lye, the genuine sublimate turns to red, while the fake one shows no change. These phenomena are directly correlated with chemical ingredients in the CMM. Therefore, the application of these physical phenomena can be unique for the authentication of herbal materials.

Furthermore, several new coupling technologies, such as microscopic method coupled with laser dissection (LMD) and ultra-high performance liquid chromatography quadrupole/time of flight-mass spectrometry (UHPLC-QTOF-MS), are used in qualitative evaluation of CMM. Liang *et al.* have combined the LMD with UHPLC-QTOF-MS to investigate the localization of ginsenosides in root and rhizome of *P. ginseng*, providing solid evidence for the accumulation of ginsenosides in cork, cortex, phloem, and xylem [16]. Additionally, Jaiswal *et al.* have determined the distribution of toxic alkaloids in tissues of aconite roots through LMD and UHPLC-QTOF-MS, contributing towards improved and effective management of therapeutically important toxic drugs, such as Aconite [17]. Meanwhile, they have compared tissue-specific metabolites and protodioscin content of *Asparagus cochinchinensis* (Lour.) Merr. And *Asparagus racemosus* Willd. used in China and India. Based on the analyses, it is indicated that Asparagus species from India and China can serve as substitute for each other in various therapeutic applications [18].

Quantitative microscopic identification

The quantitative microscopic identification is performed by the fixed constant of microscopic characteristics of CMM by volumetric analysis. This method not only presents a novel direction for determination of CMM, but provides a possible solution for the detection of the effective ingredients.

Li *et al.* have researched the microscopic characteristic constants of pollen grains in *Carthamus tinctorius* L.. They have detected the content of hydroxysafflor yellow A. and analyzed the correlation between the two sides [19]. Liu *et al.* have measured microscopic characteristic constants of cluster crystals of calcium oxalate in *Paeonia lactiflora* Pall.. The results suggest the correlation between microscopic characteristic constants and its chemical ingredients [20]. Feng *et al.* have collected *Sophora japonica* L. herbal materials from 11 different producing areas as well as 2 batches of products [21]. After studying the microscopic identification characteristics of herbal materials and its pills, they have found the results may contribute to the further improvement for microscopic identification standards [21]. Moreover, Li *et al.* have quantified pollen grains of *Sophora japonica* L., demonstrating a good correlation between the constant of pollen grains and the