



ORIGINAL ARTICLE

Morphological and microscopic characterization of five commonly-used testacean traditional Chinese medicines



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Abstract Testacean traditional Chinese medicine (TTCM), derived from the outer shell of sea or freshwater mollusks, is a special and important category of Chinese medicinal materials. To ensure the effective use of TTCM, a comparative identification study was performed on five commonly-used testacean drugs, including *Haliotidis Concha*, *Arcae Concha*, *Meretricis Concha*, *Ostreae Concha* and *Margaritifera Concha* (*Shijueming*, *Walengzi*, *Geqiao*, *Muli* and *Zhenzhumu* in Chinese, respectively). Typical morphological photographs of the crude drugs were acquired, and the key microscopic characteristics of the derived powders under normal light microscope and polarized light microscope were summarized. The major results can be concluded as follows: (1) the original species involved in the five TTCMs could be distinguished by their respective interspecies morphological characteristics; (2) the key identification characteristics of the five powdered crude drugs were mainly crystal fragments, with the fragment features under both normal light and polarized light microscope providing powerful points for differentiating the five commonly-used testacean drugs. This study demonstrated that it is feasible to provide authentication for these five kinds of TTCMs by the combination of morphology with microscopy.

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1. Introduction

Testacean traditional Chinese medicine (TTCM) is mainly from the outer shell of sea or freshwater mollusks, with properties of both mineral and animal drugs. In China, use of this kind of medicine can be traced back to 200 BC in Shen Nong Ben Cao Jing, the first traditional Chinese medicine book. It is estimated that about 100 kinds of testacean drugs are documented in Chinese Marine Materia Medica, a contemporary monograph descriptive of marine drugs¹. Recently, abundant shell resources are attracting more and more scientists to investigate their medicinal value.

Haliotidis Concha, Arcae Concha, Meretricis Concha, Ostreae Concha and Margaritifera Concha are recorded in the Chinese Pharmacopoeia, with Chinese names of Shijueming, Walengzi, Geqiao, Muli and Zhenzumu, respectively. The five kinds of TTCMs are used extensively as effective medicines to induce sedation, calm the nerves, improve eyesight or to soften hard masses and eliminate nodulations²⁻⁶. However, commercial shell drugs are mostly applied in coarse powder, which is prone to give rise to species confusion due to their similarity in appearance and color. What is worth noting is that authentication work for the five kinds of TTCMs has not been established.

These testacean medicinal materials are composed mainly of calcium carbonate or calcium sulfate, in hard texture. For mollusk shells, previous research was mainly on their formation, structure and organic matrix⁷⁻⁹. Although some methods including X-ray diffraction, infrared and atomic absorption spectrometry have been applied to identification, expensive instruments are needed¹⁰⁻¹². It is well known that microscopic techniques are fast, accurate, economical as well as simple, and have been used widely for the authentication of herbal, animal and even mineral medicines¹³⁻¹⁵. A few microscopic studies^{1,16} on TTCMs have been reported, however, only cursory hand-drawing pictures on cross section were provided and none of them showed detailed color illustrations of the characteristics of powder. Herein we perform the identification of Haliotidis Concha, Arcae Concha, Meretricis Concha, Ostreae Concha and Margaritifera Concha by morphological and microscopic study. Morphological characteristics are depicted with color photographs and powder microscopic characteristics using normal light microscopy combined with polarized light microscopy.

2. Materials and methods

2.1. Materials

Twenty-five samples were collected from different main production areas. Among these samples, there were 7 batches of Haliotidis Concha, 5 batches of Arcae Concha, 4 batches of Meretricis Concha, 4 batches of Ostreae Concha and 5 batches of Margaritifera Concha. All these shells were obtained from coastal areas and used as crude experimental materials without calcination after rinsing, cracking and powdering. The details of each sample are presented in Table 1. These samples were authenticated by Prof. Huijun Li (China Pharmaceutical University, Nanjing, China) and deposited at the State Key Laboratory of Natural Medicines of China Pharmaceutical University.

2.2. Apparatus and software

A digital camera (Cannon EOS 500D, Japan) was used for taking morphological photographs of the typical shell samples. A TCM

grinder (TAISITE FW135, China) was used to prepare the powdered crude drugs. An optical microscope (Nikon E200, Japan) equipped with a digital camera (Nikon Smart V550D, Japan) was used to observe the powder features and Smart V550D Capture software to record the photographs.

2.3. Reagents

Dilute glycerin was prepared according to procedures described in Appendix XV B, Pharmacopoeia of the People's Republic of China, 2010¹⁷.

2.4. Methods

The exterior features of each authenticated sample were examined by observing, measuring and touching. The color digital photographs of typical samples were taken with a digital camera. Each sample of these crude drugs was powdered using a grinder and passed through a 300 µm sieve, sealed with dilute glycerin when observed under the microscope. The powder of each sample was observed for at least 10 slides. The distinctive representative characteristics under normal light and polarized light microscope were chosen and imaged.

3. Results

3.1. External morphology

The typical color photos and external morphological characteristics of the 5 kinds of TTCMs (15 species in total) are given in Fig. 1 and Table 2. *Haliotis diversicolor* Reeve, *Haliotis discus hannai* Ino, *Haliotis ruber* (Leach), *Haliotis asinina* Linnaeus and *Haliotis laevigata* (Donovan), as the original species of Haliotidis Concha; *Arca subcrenata* Lischke, *Arca inflata* Reeve, *Arca granosa* Linnaeus as the species of Arcae Concha; *Meretrix meretrix* Linnaeus, *Cyclina sinensis* Gmelin as the species of Meretricis Concha; *Ostrea talienwhanensis* Crosse, *Ostrea rivularis* Gould, as the species of Ostreae Concha; *Hyriopsis cumingii* (Lea), *Cristaria plicata* (Leach), *Pteria martensii* (Dunker), as the species of Margaritifera Concha, were studied in this paper.

3.2. Powder microscopy

The key microscopic characteristics of the five powdered crude drugs under normal and polarized light microscopy are given in Fig. 2 and Fig. 3. The detailed descriptions of each drug powder composed of their respective original species are described below. The transverse section view of prismatic layer fragments can be observed in the powder of Haliotidis Concha, Ostreae Concha and Margaritifera Concha, of which the comparison data are displayed in Table 3.

3.2.1. Microscopic characteristics of Haliotidis Concha

Nacreous layer fragments are visible everywhere, differing in size, mostly in polygonal or irregular shapes; surfaces are with slightly granular nature. These fragments are usually imbricate or in dune shape, some with a distinct wavy margin; under the polarized microscope, they show dark blue centrally and yellow at the margin. Polygonal dark brown or tan fragments can be observed, opaque under the polarized microscope. Prismatic layer fragments

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