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Advances in Research of Identification of Cnidii Fructus and Its Adulterants

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Abstract In this paper, the traditional identification and modern identification technology of Cnidii Fructus and its adulterant were reviewed, and their advantages, disadvantages and practicability were summarized, to provide a reference for the rapid and accurate identification and quality evaluation of Cnidii Fructus.

Key words Cnidii Fructus, Adulterant, Traditional identification, Modern identification technique

1 Introduction

Cnidii Fructus is the dried ripe fruit of *Cnidium monnieri* (L.) Cuss. and has a long history of medicinal use. *Shennong's Classic of Materia Medica* took it as top grade drug[1]. It has the effect of dispelling wind and dampness, killing insects and itching, warming the kidney and aphrodisiac, and is used for pudendal itch and vaginal discharge, eczema itching, dampness impediment low back pain, kidney deficiency impotence, uterine cold infertility[2]. Modern studies have shown that its main active components are coumarin compounds, volatile oils and carbohydrates, which have pharmacological effects such as anti-tumor, anti-cancer, antipruritic, anti-allergic, anti-mutagenic, anti-arrhythmia, inhibiting heart, dilating blood vessels, anti-inflammatory, anti-osteoporosis and local anesthesia[3-9].

With the enhancement of the research, development and utilization of Cnidii Fructus, its market demand has increased, resulting in confusion and adulteration in the market, which has seriously affected the quality and clinical safety of Cnidii Fructus. At present, there are many adulterants of Cnidii Fructus in the market, mainly the same genus *Cnidium dauricum* (Jacquin) Fischer & C. A. Meyer, *Cnidium salinum* Turcz., *Cnidium japonicum* Miq., *Cnidium monnieri* (L.) Cuss. var. *formosana* (Yabe) Kitag, and plants of different genera in the same family *Daucus carota* L., *Torilis scabra* (Thunb.) DC., *Angelica biserrata* (Shan et Yuan) Yuan et Shan, *Foeniculum vulgare* Mill., *Anethum graveolens* L., *Apium graveolens* L., *Cicuta virosa* L., *Carum carvi* L., *Heracleum scabridum* Franch., *Saposhnikovia divaricata* (Turcz.) Schischk., *Cuminum cyminum* L., and dried fruit of *Lepidium apetalum* Willdenow[10-16]. Therefore, we summarized the identification of Cnidii Fructus and its adulterants, especially the research progress of modern identification technology, in order to provide new ideas and new methods for the rapid, non-destructive and accurate identification of Cnidii Fructus.

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2 Traditional identification methods of Cnidii Fructus and its adulterants

2.1 Character identification Cnidii Fructus is obviously different from its adulterant in plant morphology, fruit shape, size, color, longitudinal edge, oil tube, odor and other characteristics. Plants of the same genus *C. dauricum* (Jacquin) Fischer & C. A. Meyer, *C. japonicum* Miq., and *C. salinum* Turcz. have similar fruit shape and difficult to distinguish, so they can only be distinguished in plant morphology. The involucral bracts of *C. dauricum* (Jacquin) Fischer & C. A. Meyer are long ovate-obovate with very wide membranous margins, while the involucral bracts of *C. monnieri* are linear with only narrow membranous margins; involucral bracts of *C. japonicum* Miq. and *C. salinum* Turcz. are not margined with fine lashes[17]. However, Cnidii Fructus sold in the market is the fruit without the original plant, so it is impossible to accurately identify its origin. In nature and taste, Cnidii Fructus is different from *Carum carvi*, *Lepidium apetalum* Willd., *Illicium verum*, and *Apium graveolens* seeds. Cnidii Fructus tastes pungent and cool, and has a tingling sensation[10,18]. *Carum carvi* tastes spicy[14]. *L. apetalum* Willd. tastes slightly spicy[12]. *Illicium verum* tastes slightly sweet[12]. *Apium graveolens* seeds is slightly bitter in taste and cool and pungent in nature[12,19-20], light taste[21], and slightly numbs tongue[11]. Cnidii Fructus can be distinguished from Angelicae Pubescentis Radix in shape and size. The fruit of Cnidii Fructus is elliptic, 2 – 4 mm long and about 2 mm in diameter. The fruit of Angelicae Pubescentis Radix is obovate-oblong, 5 – 10 mm in length and 5 – 9 mm in diameter. *Daucus carota* and *Toriliss cabra* (Thunb.) DC. fruits have hair and Cnidii Fructus fruit is glabrous[10]. In summary Cnidii Fructus is a fruit and seed medicinal material, and it is difficult to distinguish it from adulterant in appearance and shape, especially when the fruit of the same genus is more similar in appearance and characteristics, and adulterant is added, it is even more difficult to distinguish it. Therefore, it is difficult to judge only by experience in the practical application process.

2.2 Microscopic identification Microscopic identification method is simple and objective, through observing the schizocarp transec-

tion of Cnidii Fructus and its adulterants *Carum carvi* and *Apium graveolens seeds*, *their distinctive features are significant. Cnidii Fructus is semicircular*, *acute and pentagonal in shape*, *with thick* mesocarp, abnormally prominent longitudinal ribs, a vascular bundle in the middle, bead-like endocarp cell wall[22], small endosperm, and fine calcium oxalate cluster crystals in each aleurone grain of endosperm cells[11,14,19,22]. Mesocarp cells of *Carum carvi* are mostly compressed and irregular in shape, and endocarp cells vary in length[14]. *Apium graveolens* seeds are semicircular and obtuse pentagonal[22], there are vascular bundles at the longitudinal edge of the mesocarp[11], the endosperm is large, and there are large calcium oxalate cluster crystals in the aleurone grains of the endosperm cells, which are obviously obtuse pentagonal[19,22].

2.3 Physical and chemical identification Zhou Lushan *et al.*[23] applied the water test method to identify Cnidii Fructus and *Apium graveolens* seeds. Cnidii Fructus floats on the water surface, while the *Apium graveolens* seeds sink. Ultraviolet spectroscopy was used to identify Cnidii Fructus and *Apium graveolens* seeds, which have different absorption wavelengths to achieve the purpose of identification[24-25]. Cnidii Fructus can be distinguished from *Apium graveolens* seeds by the detection of osthole by TLC[11,19,24]. Zhu Jinhua *et al.*[19,22] separately took 2 g of Cnidii Fructus and *Apium graveolens* seeds powder, added 20 mL of ethanol, heated and refluxed for 30 min, filtered, took several drops of the filtrate on a white porcelain plate, and observed under an ultraviolet lamp (365) nm. Cnidii Fructus showed blue fluorescent yellow light, and *Apium graveolens* seeds shows bright blue fluorescence; took another 2 mL of filtrate, add the same amount of 3% sodium oxide solution, heated for 5 min, cooled, and then added 1-2 drops of newly prepared diazo-p-nitroaniline test solution, Cnidii Fructus was cherry red, *Apium graveolens* seeds were light brown. Ding Shaochun *et al.*[15] used thin layer chromatography and ultraviolet spectroscopy to distinguish Cnidii Fructus, *Anethum graveolens*, and Cicuta virosa. If Cnidii Fructus is adulterated with *Anethum graveolens*, Cicuta virosa and *Apium graveolens* seeds, the distinguishing characteristics of Cnidii Fructus still exist by thin layer chromatography, and the three cannot be distinguished. Qin Luping *et al.*[26] used polyacrylamide gel electrophoresis to analyze Cnidii Fructus, *C. dauricum* (Jacquin) Fischer & C. A. Meyer, *Daucus carota* seeds, and *Toriliss cabra* (Thunb.) DC. seeds and found that the number and position of electrophoresis bands were significantly different, so they could be distinguished.

3 Modern identification techniques for Cnidii Fructus and its adulterants

Zhu Li *et al.*[16] used HS-SPME-GC-MS method to analyze the volatile components of Cnidii Fructus and its adulterant *Cuminum cyminum*, and found that there were obvious differences in the volatile components between them. Based on high-resolution melting curve (HRM) analysis method, Zhang Mengting *et al.*[27] achieved simple and rapid identification of four spices *Foeniculum vulgare* Mill., *Cuminum cyminum* L., *Carum carvi*, *Anethum graveolens* L. and their adulterants Cnidii Fructus and *Saposhnikovia divaricata*. Wu Yutian identified Salviae Miltiorrhizae Radix Et Rhizoma, Isatidis Radix and Cnidii Fructus by convolution transform and information visualization of near-infrared spectroscopy of traditional Chinese medicines, and the classification results were consistent with those of traditional plant taxonomy. Guo Liheng *et al.*[11] identified Cnidii Fructus and *Apium graveolens* seeds by HPLC.

4 Conclusions and prospects

At present, there are many adulterants of Cnidii Fructus in the market, and they are fruit medicinal materials with similar characters, so it is difficult to identify them, and the identification methods are mainly traditional empirical identification, which is subjective and objective. Zhong Yulan *et al.*[28] used near-infrared spectroscopy combined with discriminant analysis to rapidly identify the quality of *Panax notoginseng* powder and its adulterants. Wu Xiyu *et al.*[29] established a *Zanthoxylum bungeanum* Maxim. powder and its adulterants by applying near-infrared technology and using discriminant partial least squares (DPLS) and support vector machine (SVM) qualitative identification model. Gong Jianting *et al.*[30] achieved the rapid and accurate identification of Curcumae Radix, Curcumae Rhizoma, Curcumae Longae Rhizoma, and Wenyujin Rhizoma Concisum by collecting the odor fingerprints of four kinds of *Curcuma* L. by electronic nose combined with the discriminant model established by XGBoost. Zhang Danchun *et al.*[31] applied ITS2 and psbA-trnH sequences to identify *Pulsatilla chinensis* and adulterants (*Potentilla chinensis*, *Potentilla discolor*, *Polycarpaea corymbosa*) for DNA barcode molecules, confirmed the universality of bar code in *Pulsatilla chinensis* and adulterants, which provides a basis for their quality evaluation. Near-infrared technology has the advantages of rapid and non-destructive detection means, low detection cost, no need to treat samples and fast speed, and is widely used in the identification of origin, quality evaluation and identification of mixed products of traditional Chinese medicine[32-33]. Electronic nose is an electronic system that imitates biological nose. It has the characteristics of simple operation, fast detection and accurate identification. It has been widely used in food quality detection, environmental quality control, grain storage and identification, medicine and health and other fields. In recent years, it has also been used to evaluate the authenticity of traditional Chinese medicine, with great potential[34-36]. DNA barcode molecular identification technology has unique advantages in the identification of original species of medicinal plants. It has the advantages of accurate and rapid identification, good repeatability and stability, strong practicability and clear judgment criteria. It can realize the accuracy, digitization and standardization of the identification of Chinese medicinal materials, and has developed rapidly in the identification of Chinese medicinal materials[37-38]. Therefore, the above techniques can be used to establish a rapid and non-destructive identification method

and model for Cnidii Fructus and its adulterant, which can provide a reference for its quality evaluation and a supplement for its identification and detection means.

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