Optimization of Identification Condition of Thin Layer Chromatography for Alpinia Katsumadai Hayata

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Abstract. Objective: To optimize the TLC identification conditions of Alpinia katsumadai Hayata. Methods: Qualitative identification of Alpinia katsumadai Hayata was carried out by TLC, toluene: ethyl acetate: methanol (15: 4: 1) and toluene: ethyl acetate: methanol (14.5: 4.5: 1) were used as developing solvent, then observed under 365nm ultraviolet lamp. Results: The optimized conditions were better than those of TLC in 2020 edition of Chinese Pharmacopoeia. The optimized spots were more clear, the separating capacity was better, and the stability of the conditions was better. After optimization, there were 9 spots in TLC. Conclusion: The method is simple, specific and reproducible, which can provide reference for the quality control of Alpinia katsumadai Hayata.

1. Introduction

Alpinia katsumadai Hayata is the dry and nearly mature seed of Alpinia katsumadai Hayata, and is a traditional Chinese medicine with the same origin as food and medicine. It has the effects of dissipating dampness, promoting the circulation of qi, warm interior and stop vomit. And it commonly used in the treatment of cold dampness closed-resistance, abdominal distension, belching and retching counterflow, no appetite and so on [1]. Its chemical constituents mainly include volatile oil [2], diphenylheptane [3], flavonoids [4], glycosides [5], nitrogen compounds, lactones, trace elements, etc [6]. It has many pharmacological effects such as anti-gastric ulcer, promoting gastrointestinal motility, anti-inflammation, anti-tumor, anti-oxidation, neuroprotection, etc [7]. At present, studies on the quality control of Alpinia katsumadai Hayata mainly include gas chromatography [8-10], high performance liquid chromatography [11] and infrared spectroscopy [12], but it has the disadvantages of high cost, long time and complex operation. TLC is a simple, rapid and qualitative identification method, it has the characteristics of fast and effective, low price and strong applicability, and is often used for qualitative identification of traditional Chinese medicine [13]. The 2020 edition of Chinese Pharmacopoeia already has the TLC identification method of Alpinia katsumadai Hayata, but through the experimental test, the method of Chinese Pharmacopoeia has less information and poor resolution [14, 15]. Therefore, the TLC conditions of Alpinia katsumadai Hayata were optimized in this paper, to provide references for the quality control and clinical application of Alpinia katsumadai Hayata.
2. Instruments and Materials

2.1. Instruments and reagents
High efficiency silica gel G (Qingdao Ocean Chemical Plant); Shenli Glass Spotting Capillary (Shanghai Shenli Glass Instrument Sales Co., Ltd.); Thermostat water bath (Changzhou Yuexin Instrument Manufacturing Co., Ltd.); Ultraviolet analyzer (Shanghai Zhiyan Scientific Instrument Co., Ltd.). Toluene, ethyl acetate and methanol are analytical purities.

2.2. Materials
The sources of the above different batches of *Alpinia katsumadai* Hayata are shown in Table 1.

| NO | Sample name          | Batch (source)         |
|----|----------------------|------------------------|
| S1 | *Alpinia katsumadai* Hayata | J2018090103-01         |
| S2 | *Alpinia katsumadai* Hayata | J2018090202-01         |
| S3 | *Alpinia katsumadai* Hayata | J2018090105-01         |
| S4 | *Alpinia katsumadai* Hayata | J2018090201-02         |
| S5 | *Alpinia katsumadai* Hayata | J2018090101-01         |
| S6 | *Alpinia katsumadai* Hayata | J2018090202-02         |
| S7 | *Alpinia katsumadai* Hayata | J2018090303-01         |
| S8 | *Alpinia katsumadai* Hayata | J2018090201-01         |
| S9 | *Alpinia katsumadai* Hayata | J2018090102-01         |
| S10| *Alpinia katsumadai* Hayata | J2018090203-01         |

3. Experimental methods

3.1. Preparation of sample solutions

3.1.1. Preparation method of Pharmacopoeia. Accurately weigh about 1g of *Alpinia katsumadai* Hayata powder, adding methanol 5mL, then heat it in a water bath and shake for 5 minutes, filtering, take filtrate as test solution.

3.1.2. Optimized sample preparation method for TLC identification. Accurately weigh about 1g of *Alpinia katsumadai* Hayata powder, adding methanol 10mL, then heat it in a water bath and shake for 5 minutes, filtering, take filtrate as test solution.

3.2. Determination method of TLC

3.2.1. Identification methods of Chinese Pharmacopoeia. According to thin layer chromatography (general rule 0502), take 5μL of the test solution and put them on the same silica gel G thin layer plate, respectively. Used toluene: ethyl acetate: methanol (15: 4: 1) as developing solvent, the mixture was developing, taken out and dried. Heat at 100°C until the spots are clear, and view under UV lamp (365nm).

3.2.2. Optimized identification method. Take 1μL of each sample solution and dot them on the same silica gel G thin layer plate. Use toluene: ethyl acetate: methanol (14.5: 4.5: 1) as developing agent, the mixture was developed, taken out and dried. Heat at 100°C until the spots are clear, and view under UV lamp (365nm).
4. Results

4.1. Pharmacopoeia condition results
Samples under UV light could be observed that there are 4 fluorescence spots in the thin layer of cardamom. Among them, the fluorescence spots of No.2, No.3 and No.4 are stronger than those of No.1. However, the information content of the four fluorescent spots is small and the resolution is poor. The color, number and Rf values of spots in different batches of *Alpinia katsumadai* Hayata are shown in Fig. 1 and table 1.

4.2. The optimized identification results
There were 9 spots in the TLC of *Alpinia katsumadai* Hayata. Among them, the big difference is that No.5 spot of S5, S6, S7, S8 and S10 batches is not obvious, and S2, S3 and S4 batches had good separation effect and clear spots, the spots of S9 and S10 batches were smaller and lighter in color. The spots 7, 8 and 9 of S1 were affected by edge effect. The color, number and RF values of spots in different batches of *Alpinia katsumadai* Hayata are shown in Fig. 2 and table 2.

![Figure 1. Thin layer chromatogram of Alpinia katsumadai Hayata in Pharmacopoeia.](image1)

![Figure 2. Optimized thin layer chromatogram of Alpinia katsumadai Hayata.](image2)
Table 2. RF values of TLC in Chinese Pharmacopoeia.

| NO | Rf value   |
|----|------------|
|    | 1   | 2   | 3   | 4   |
| S1 | 0.137 | 0.315 | 0.452 | 0.726 |
| S2 | 0.151 | 0.329 | 0.438 | 0.726 |
| S3 | 0.137 | 0.342 | 0.438 | 0.726 |
| S4 | 0.162 | 0.338 | 0.446 | 0.730 |
| S5 | 0.162 | 0.351 | 0.446 | 0.730 |
| S6 | 0.162 | 0.351 | 0.446 | 0.730 |
| S7 | 0.149 | 0.365 | 0.459 | 0.743 |
| S8 | 0.173 | 0.360 | 0.480 | 0.747 |

Table 3. Optimized RF values of TLC.

| NO | Rf value   |
|----|------------|
|    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
| S1 | 0.20 | 0.35 | 0.40 | 0.49 | 0.57 | 0.79 | 0.86 | 0.94 | 0.99 |
| S2 | 0.21 | 0.34 | 0.38 | 0.48 | 0.54 | 0.79 | 0.84 | 0.91 | 0.98 |
| S3 | 0.20 | 0.32 | 0.37 | 0.47 | 0.54 | 0.77 | 0.82 | 0.90 | 0.97 |
| S4 | 0.20 | 0.31 | 0.35 | 0.45 | 0.53 | 0.75 | 0.83 | 0.90 | 0.97 |
| S5 | 0.20 | 0.30 | 0.35 | 0.46 | 0.52 | 0.76 | 0.82 | 0.90 | 0.98 |
| S6 | 0.18 | 0.28 | 0.33 | 0.46 | 0.51 | 0.74 | 0.80 | 0.88 | 0.96 |
| S7 | 0.18 | 0.28 | 0.31 | 0.43 | 0.51 | -   | 0.78 | 0.88 | 0.95 |
| S8 | 0.18 | 0.28 | 0.33 | 0.42 | 0.59 | -   | 0.79 | 0.88 | 0.94 |
| S9 | 0.18 | 0.29 | 0.34 | 0.42 | 0.51 | 0.77 | 0.82 | 0.90 | 0.95 |
| S10| 0.17 | 0.36 | 0.39 | 0.48 | 0.55 | -   | 0.84 | 0.91 | 0.97 |

5. Discussion

Under the conditions of Chinese Pharmacopoeia (2020 Edition), the TLC identification of *Alpinia katsumadai* Hayata was carried out with toluene: ethyl acetate: methanol (15: 4: 1) as the developing solvent, then heated at 100℃ until the spots are clear, and was observed under 365nm UV light. The results showed that the separating capacity of the sample was poor, the spots were not round and the information was little. Therefore, the TLC conditions of *Alpinia katsumadai* Hayata were optimized in this paper. Toluene: ethyl acetate: methanol (14.5: 4.5: 1) was used as the developing solvent, the mixture was developed. The results showed that the best unfolding effect was observed under 365nm UV light, the chromatogram was clear, the information was more, the characteristic spots were well separated. And the size, color depth, location, quantity and Rf value of the spots in different batches were slightly different, indicating that the quality of different batches was slightly different. It may be affected by different processing process, storage conditions and so on [16]. The method is feasible and specific, it can provide a reference for the quality control and quality standard evaluation method of *Alpinia katsumadai* Hayata.

Acknowledgments

This work was financially supported by "Thousand level" innovative talents training project in Guizhou Province (Guizhou traditional Chinese medicine [ZQ2017005]).

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