

## Identification Tests of Aristolochic Acid in Crude Drugs by Reversed-Phase TLC/Scanning Densitometry

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We performed identification tests of aristolochic acid in Xixin, Mutong, Muxiang, and Fangji by reversed-phase TLC/scanning densitometry using aristolochic acid I as a marker compound. The developing solvents used were: I) acetonitrile/methanol/water mixture solution (3 : 1 : 1) and II) 2-butanone/methanol/sodium sulfate(1→20) mixture solution (2 : 1 : 1). The R<sub>f</sub> value of aristolochic acid I was 0.54 using the I system and 0.57 using the II system. The maximum absorption wavelengths of aristolochic acid I observed by scanning densitometry were 254 and 325 nm.

**Key words** — aristolochic acid, reversed-phase TLC, scanning densitometry

### INTRODUCTION

Aristolochic acid is a component of plants in Aristolochia and suspected to induce nephropathy.<sup>1–4)</sup> Use of crude drugs with derivations listed in the Pharmacopoeia of Japan raises no problems. However, the terms of crude drugs sometimes differ among countries, and products that are not in conformity with the Pharmacopoeia of Japan are in circulation in other countries. Therefore, when crude drugs/Chinese medicines are used, measures such as the identification of materials is necessary to prevent mixture of plants containing aristolochic acid. Xixin (Asiasarum Root, “Saishin” in Japanese), Mutong (Akebia Stem, “Mokutsu” in Japanese), Muxiang (Saussurea Root, “Mokko” in Japanese), and Fangji (Sinomenium Stem, “Boi” in Japanese) are considered to require attention because they may contain

aristolochic acid.<sup>5,6)</sup> For the analysis of aristolochic acid in these crude drugs, methods by HPLC or LC/MS have been reported.<sup>7–13)</sup> These methods are sensitive and accurate but costly because of the use of precise apparatuses and also time-consuming for adjustments and analysis. Xixin, Mutong, Muxiang, and Fangji are used for many purposes as combination materials of crude drug preparations. Therefore, for their efficient quality control, a method of simple rapid analysis of aristolochic acid is necessary.

Recently, we have used reversed-phase TLC/scanning densitometry for the identification of coal tar dyes in foods, cosmetics and crude drugs, and reported its usefulness.<sup>14–19)</sup> In this study, for simple rapid identification of aristolochic acid in crude drugs, analysis was performed by reversed-phase TLC/scanning densitometry.

### MATERIALS AND METHODS

**Samples** — A) Xixin 1 (Lot No. 032121), Xixin 2 (Lot No. 161104), Xixin 3 (Lot No. US262121); B) Mutong 1 (Lot No. 243053), Mutong 2 (Lot No. US262208), Mutong 3 (Lot No. 5A28M); C) Muxiang 1 (Lot No. 833023), Muxiang 2 (Lot No. US453228), Muxiang 3 (Lot No. 4K25M); D) Fangji 1 (Lot No. 163044), Fangji 2 (Lot No. US453112), Fangji 3 (Lot No. 5A28M); E) Guanmutong (*Aristolochia manshuriensis* Kom, “Kanmokutsu” in Japanese, Lot No. SB-1373); and F) Guangfangji (*Aristolochia fangchi* Wu, “Koboi” in Japanese, Lot No. SB-1374). A–D): crude drugs listed in the Pharmacopoeia of Japan; 3 products each (total, 12 samples). E) and F): 1 product each (total, 2 samples).

Xixin 1, Mutong 1, Muxiang 1, and Fangji 1 were purchased from Matsuura Yakuhin Co., Ltd. (Nagoya, Japan), Xixin 2 from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan), Xixin 3, Mutong 2,

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Muxiang 2, and Fangji 2 from Uchida Wakanyaku Co., Ltd. (Tokyo, Japan), Mutong 3, Muxiang 3, and Fangji 3 from Daiko Syoyaku Co., Ltd. (Nagoya, Japan), Guanmutong from China (Heilongjiang), and Guangfangji from China (Guangdong).

**Preparation of Sample Solutions** — Each powder sample (1.0 g) was mixed and shaken with 70% methanol (10 ml) for 15 min and filtrated to obtain sample solution.

**Preparation of Standard Solution** — Aristolochic acid I (1 mg) is dissolved in methanol (1 ml) to obtain standard solution.

Aristolochic acid I for crude drug purity tests (Matsuura Seiyaku Co., Ltd.; Nagoya, Japan) was used. All the other agents were of analytic grade (Wako Pure Chemical Industries, Ltd.; Osaka, Japan).

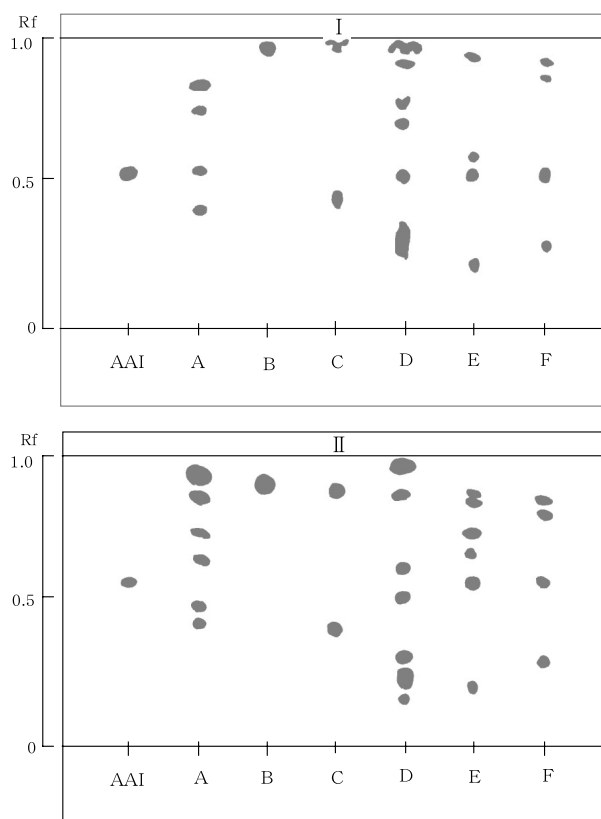
**Operating Conditions** —

**TLC Conditions:** The TLC plate was an RP-18F<sub>254</sub>S (Art. 15389, Lot No. OB257917, E. Merck, Darmstadt, Germany), and the solvent systems were I) acetonitrile/methanol/water mixture solution (3 : 1 : 1), and II) 2-butanone/methanol/sodium sulfate(1→20) mixture solution (2 : 1 : 1). Volume of spot; 10  $\mu$ l. Developing distance; about 10 cm. Method of detection; examine under UV light (wavelength: 254 nm) in the dark.

**Scanning Densitometric Conditions:** The scanning densitometer using dual wavelength flying spot scanning densitometer CS-9000 was obtained from Shimadzu (Tokyo, Japan). The wavelength scanning range was from 200 to 370 nm, slit size was 0.4  $\times$  0.4 mm and the UV absorption spectrum was measured by reflectance spectrophotometry.

## RESULTS AND DISCUSSION

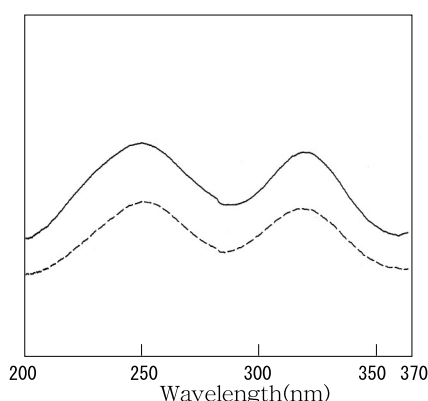
For the identification of aristolochic acid, standard solution was prepared using aristolochic acid I as a marker compound. Reversed-phase TLC was used. To separate aristolochic acid I from crude drugs, the solvent system was evaluated using water, acetonitrile, methanol, and 2-butanone. Using the following 2 developing solvents, components of each crude drug could be separated: I) acetonitrile/methanol/ water mixture solution (3 : 1 : 1) and II) 2-butanone/methanol/sodium sulfate(1→20) mixture solution (2 : 1 : 1). The R<sub>f</sub> value of the spot of separated aristolochic acid I standard solution was 0.54 using the I system and 0.57 using the II sys-



**Fig. 1.** Reversed-Phase TLC Chromatograms of Standard and Sample Solutions

I, II) AA I: standard solution of aristolochic acid I, A: sample solution of Xixin, B: sample solution of Mutong, C: sample solution of Muxiang, D: sample solution of Fangji, E: sample solution of Guanmutong, F: sample solution of Guangfangji. Plate: RP-18F254S (E. Merck, OB257917). Solvent systems: I) acetonitrile/methanol/water mixture solution (3 : 1 : 1), II) 2-butanone/methanol/sodium sulfate(1→20) mixture solution (2 : 1 : 1).

tem. When each crude drug was separated using the I system, a single spot was observed at the same R<sub>f</sub> value as that of the standard solution spot for Xixin, Fangji, Guanmutong, and Guangfangji. To confirm this spot to be aristolochic acid I, each crude drug was separated using the II system. For Xixin and Fangji, no spot was observed at the same R<sub>f</sub> value as that of the standard solution spot. For Guanmutong and Guangfangji, a spot was observed at the same R<sub>f</sub> value as that of the standard solution spot (Fig. 1). Therefore, to identify these spots by scanning densitometry, ultraviolet absorption spectra were measured for sample solution spots with the same R<sub>f</sub> value as that of the developed standard solution spot. As a result, the maximum absorption wavelengths of the standard solution spot of aristolochic acid I were 254 and 325 nm. The spectrum of the sample solution spot of Guanmutong was consistent with



**Fig. 2.** Ultraviolet Absorption Spectra of Standard and Sample Solutions Measured by Scanning Densitometry on the Developed Reversed-Phase TLC Plates

Maximum wavelengths of ultraviolet absorption spectra (nm): —: standard solution of aristolochic acid I (254, 325). - - - - -: sample solution of Guanmutong (254, 325).

the maximum absorption wavelengths and spectrum of the standard solution spot (Fig. 2). Similarly, the spectrum of the sample solution spot of Guangfangji was also consistent with that of the standard solution spot. However, the spectra of the sample solution spots of Xixin and Fangji, which showed the same Rf values as those of the standard solution spots using the I system, differed from the spectrum of the standard solution spot. These results suggest that Guanmutong and Guangfangji have a single spot at the same Rf value as that of the standard solution spot and the same spectrum as that of the standard solution spot, which confirmed the presence of aristolochic acid. The 3 products of Xixin, Mutong, Muxiang, and Fangji (Total, 12 samples) showed an Rf value and spectrum that differed from those of the standard solution spot, which confirmed the absence of aristolochic acid. The detection limit of this method was 100  $\mu\text{g}$  per 1 g sample. Quantification of these samples by HPLC showed 2.840 mg aristolochic acid I per 1 g Guanmutong and 0.379 mg aristolochic acid I per 1 g Guangfangji.

Aristolochic acid in crude drugs should be identified by at least 2 methods to prevent the misidentification of crude drug components and obtain more accurate information. This method is a simple rapid analysis method for the identification of aristolochic acid in crude drugs.

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