

Title	New methods for identification of alismatis rhizoma by means of electrophoresis, paper partition chromatography, and thin-layer chromatography
Sub Title	
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Publisher	共立薬科大学
Publication year	1984
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.29 (1984.) ,p.24- 25
JaLC DOI	
Abstract	
Notes	抄録
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000029-0024

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**New Methods for Identification of *Alismatis Rhizoma* by Means
of Electrophoresis, Paper Partition Chromatography,
and Thin-Layer Chromatography***

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Alismatis Rhizoma, the rhizome of *Alisma orientale* Juzepczuk or related species (Alismataceae), has been used as an important component of many oriental pharmaceutical preparations. However, no effective method for its identification in decoctions and preparations is yet available.

In recent years, we have proposed new methods for identification of *Zizyphi Fructus* and *Angelicae Radix* by means of electrophoresis with color and precipitation reactions. In this paper, we report the development of several methods for identification of *Alismatis Rhizoma* by utilizing precipitation procedures, electrophoresis, paper partition chromatography (PPC), and thin-layer chromatography (TLC).

Precipitation Procedures The crude drug was sliced and extracted with water in a boiling water-bath. After filtration, 5% cetyltrimethyl ammonium bromide was added to the filtrate, then the mixture was centrifuged. The precipitate was dissolved in 0.5 M sodium chloride and the solution was centrifuged, if necessary. The resulting solution was added to four volumes of ethanol. After centrifugation, the precipitate was mixed well with 90% ethanol, then centrifuged again. This treatment with 80% ethanol was repeated three times. The final precipitate was dried *in vacuo*, then dissolved in water. This solution was used as the sample for electrophoresis, PPC, and TLC.

Electrophoresis Electrophoresis was carried out with Separax (Fuji Film Co., 6×21 cm long) using buffer A, 0.08 M pyridine-0.04 M acetic acid (pH 5.4), at 420 volts for 30 min. The inside of the apparatus was cooled with dry ice. The sample was applied in a line at a distance of 7 cm from the cathode, and was visualized with 0.5% toluidine blue in 3% acetic acid. After being dipped for five min, the membrane was washed with 1% acetic acid.

PPC The sample was subjected to PPC by the ascending method using Toyo-Roshi No. 51 and solvent A, methanol: formic acid: water (10: 2: 1). It was visualized with the toluidine blue reagent as described above, and with the Hanes-Isherwood reagent.

TLC This was carried out by the ascending method with Avicel SF cellulose (Funakoshi Co.) using the same solvent A as in PPC. The Hanes-Isherwood reagent was used for detection.

* 本報告は *Chem. Pharm. Bull.*, 32, 2845—2847 (1984) に発表.

Color Reaction of the Samples Obtained by the Precipitation Procedures Alismatis Rhizoma and thirty-seven other crude drugs used in related pharmaceutical preparations were treated by the precipitation procedures described above. When the final samples were subjected to cellulose acetate electrophoresis, detection with toluidine blue reagent showed that nine samples in addition to Alismatis Rhizoma were positive, while twenty-eight samples were negative. All of the positive samples gave reddish-violet spots.

Results of Electrophoresis The electrophoretic patterns of the color reaction-positive crude drugs are shown. Alismatis Rhizoma gave a single clear spot having a relative migration value of 3.50 with respect to standard phenol red.

Results of PPC and TLC Alismatis Rhizoma was the only sample which gave a single spot having R_f values of 0.46 in PPC and 0.56 in cellulose TLC. Any crude drug of the others tested gave no spot in PPC and TLC. For the purpose of detecting the spots in PPC, the toluidine blue reagent was superior to the Hanes—Isherwood reagent in sensitivity. However, the toluidine blue reagent was not suitable for TLC because of the required dipping process. The Hanes—Isherwood reagent resulted in sensitive detection of the spot from Alismatis Rhizoma in TLC.

A New Characteristic Color-reactive Substance in Alismatis Rhizoma Both the precipitation reaction with cetyltrimethyl ammonium bromide and the color reaction with toluidine blue usually depend on various acidic polysaccharides in crude drugs. However, we did not find any acidic polysaccharide in the water extract from Alismatis Rhizoma. Based on the result of reaction with the Hanes—Isherwood reagent, we concluded that the compound responsible for the characteristic reactions of Alismatis Rhizoma was a kind of phosphate. We isolated this substance from the crude drug, and identified it as a new natural product, lactose hexaphosphate. Details of the structural elucidation of this substance will be reported in a separate paper.