A NEW METHOD FOR THE QUANTITATIVE ANALYSIS OF THE AQUEOUS EXTRACT OF PINELLIA TUBER CONTAINED IN SEVERAL CHINESE TRADITIONAL MEDICINES

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Antiserum specific to Pinellia tuber was elicited in a rabbit. The extract of the tuber was converted to solid-phase antigen using microtiter plates. A new method for enzyme immunoassay of the tuber extract was developed with an antiserum to the tuber and a solid-phase antigen as immunological reagents, and β-D-galactosidase-labeled anti-rabbit IgG as the tracer. The method was successfully applied to the quantitative analysis of the extracts of Pinellia tuber contained in various chinese traditional medicines. The specificity of the assay to the extracts was also demonstrated.

KEYWORDS Pinellia tuber; new enzyme immunoassay; selected antibody assay; chinese traditional medicine; chinese crude drug; extract content

Many traditional chinese medicines consist of the extract of a mixture of various chinese crude drugs. It has been impossible to determine the exact content of an extract of a particular chinese crude drug in a traditional chinese medicine. In the present paper we report a new method to determine the content of the extract of Pinellia tuber contained in several traditional chinese medicines using an enzyme immunoassay method named selected antibody enzyme immunoassay (SAEIA).

Although development in immunoassay methods have been rapid,[1-7] application of the methods have been limited to water soluble antigens and antibodies. Water insoluble material has been outside of the limitation of immunoassay because antisera specific to insoluble materials, which are the key reagents for immunoassay, have been difficult to obtain. The chinese crude drug, Pinellia tuber, is insoluble in water. Consequently a novel method for preparation of antiserum specific to the chinese crude drug was needed. Recently we introduced a novel procedure for preparation of antibodies specific to two kinds of insoluble fungi.[8,9] The procedure was modified and applied for preparation of rabbit antiserum specific to Pinellia tuber. Powder of the tuber was prepared, suspended in a saline solution, and sonified at 60 W for 3 min using a Branson Sonifier (Model W 185, U.S.A.). One ml of the resulting suspension containing 1 mg of the tuber powder was emulsified in an equal volume of Freund's complete adjuvant and injected subcutaneously into a female rabbit at multiple points. Three booster injections of half the first dose were given at biweekly intervals. The rabbit was bled from the ear vein two weeks after the final injection and the serum specific to Pinellia tuber(anti-PT) was stored at -30℃ pending use.

Immunoassays have been limited to use for assay of certain antigens or antibodies, but not to an unknown population of antigens. We, therefore, planned to develop a new immunoassay that would selectively use the population of antibodies specific to the extracted materials of Pinellia tuber. The principle of the new assay is as follows: rabbit anti-PT antiserum should contain a population of various antibodies specific to

various parts of Pinellia tuber, and when the extract of Pinellia tuber was used for the solid-phase antigen, the population of antibodies specific to the mixture of extracts should bind to the solid-phase antigens, but other populations of antibodies should not. After the antibodies are bound, it should be possible to assay the extract by measuring the amount of bound rabbit antibody using a tracer, β-D-galactosidase-labeled goat anti-rabbit IgG,[10] prepared according to the reported method.

The wells of a microtiter plate (Nunc Immunoplate II; Roskilde, Denmark) were coated by loading 0.2 ml of the sonified suspension of 10 μg of the extract of Pinellia tuber, prepared as above, for 20 min at 37℃. Then the plate was washed with buffer B (0.06 M phosphate buffer containing 0.1% bovine serum albumin [BSA] and 1 mM EDTA), and used as the solid-phase antigen.

After extensive trials, the assay procedures for the extract of Pinellia tuber were established as follows: the microtiter wells were incubated for 3 h at 25℃ with 0.1 ml of a 10,000-fold diluted anti-PT and 0.1 ml of either a buffer B solution of the extract of Pinellia tuber or phosphate-buffered saline as the control. After washing three times with 0.2 ml of buffer A (0.02 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl, 1 mM $MgCl_2$, 0.1% BSA and 0.1% NaN_3), 0.2 ml of pooled β-D-galactosidase-labeled goat anti-rabbit IgG which was diluted 1:200 with buffer A was filled in the wells and incubated for 3 h at 25℃. The amount of enzyme conjugate bound to each well was measured by adding 0.2 ml of 0.1% o-nitrophenyl-β-D-galactoside to buffer A, and the wells were incubated at 37℃ for a suitable period. The enzymatic activity was stopped by adding 25 μl of 1 M glycine-NaOH buffer, pH 10.6, to each well, and the resulting color intensity was measured spectrophotometrically at 414 nm using an ELISA Analyzer (SLT Labinstruments, Austria).

A typical dose-response curve for EIA of the extract of Pinellia tuber with a working range between 100 ng and 100 μg is shown in Fig. 1. This assay is specific to the extract of Pinellia tuber and there was little cross-reactivity for extracts of other chinese crude drugs such as Ginseng and Glycyrrhiza (Fig. 1).

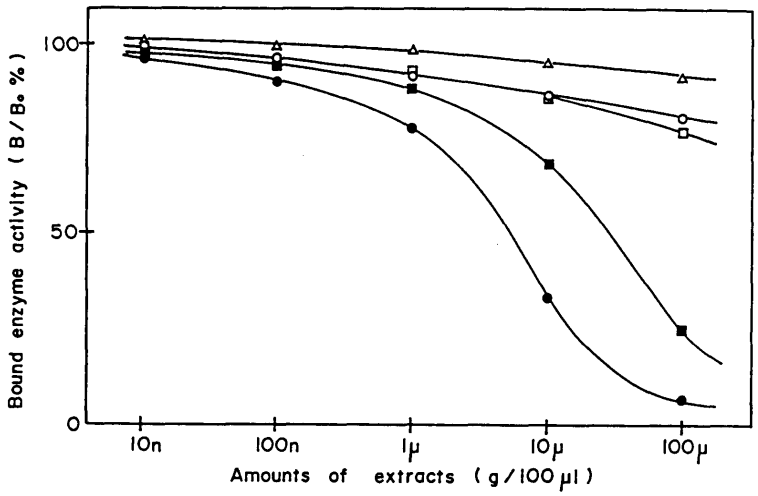

Fig. 1. Dose Response Curves for Enzyme Immunoassay of the Extract of Pinellia Tuber(●), Ginseng(△), Glycyrrhiza(○) and Saiko-ka-ryukotsu-borei-to with(■) or without(□) Pinellia Tuber, Determined by SAEIA for the Extract of Pinellia Tuber

Applications of the EIA for assays of the contents of the extract of Pinellia tuber in three chinese traditional medicines, Saiko-ka-ryukotsu-borei-to, Ryo-kan-kyo-mi-sin-ge-nin-to, and Hange-syasin-to, were examined. The contents of chinese crude drugs in these medicines before extraction are listed in Table I. For comparison, two pseudo - traditional chinese medicines from which a component of a chinese crude drug, either

Pinellia tuber or Bupleurum Root was omitted - from the components of Saiko-ka-ryukotsu-borei-to, were also prepared (Table I) and measured by the present method. The data obtained are summarized in Table II, showing that it is possible to measure quantitatively the extract of the Chinese crude drug, Pinellia tuber, selectively contained in several Chinese traditional medicines with the new SAEIA method.

Table I. Composition (g) of Chinese Crude Drugs in Three Kinds of Chinese Traditional Medicine before Extraction

Saiko-ka-ryukotsu-borei-to		Ryo-kan-kyo-mi-sin-ge-nin-to	
Bupleurum Root	5.0	Hoelen	4.0
Pinellia Tuber	4.0	Pinellia Tuber	4.0
Hoelen	3.0	Apricot Kernel	14.0
Cinnamon Bark	3.0	Schisandra Fruit	3.0
Scutellaria Root	2.5	Glycyrrhiza	2.0
Jujube	2.5	Ginger	2.0
Ginger	0.7	Asiasarum Root	2.0
Longgu	2.5		
Oyster Shell	2.5	Hange-syasin-to	
Ginseng	2.5	Pinellia Tuber	5.0
Rhubarb	1.0	Scutellaria Root	2.5
		Ginger	2.5
		Ginseng	2.5
		Glycyrrhiza	2.5
		Jujube	2.5
		Coptis Rhizoma	1.0

Table II. Measurement of the Extract of Pinellia Tuber in Eight kinds of Normal- and Pseudo-Chinese Traditional Medicines

C.T.M.[a]	Measured amount of the extracted PT[b] contained in either 100 μg or 10 μg of C.T.M. (μg/100μg)	(μg/10μg)
Saiko-ka-ryukotsu-borei-to	17.5	1.8
Without Pinellia tuber[c]	N.D.[d]	N.D.
Ryo-kan-kyo-mi-sin-ge-nin-to	8.6	1.1
Without Pinellia tuber	N.D.	N.D.
Without Glycyrrhiza	7.6	1.2
Hange-syasin-to	16.5	1.2
Without Pinellia tuber	N.D.	N.D.
Without Glycyrrhiza	17.0	1.6

a) Chinese traditional medicines.
b) Pinellia tuber.
c) This was the extract of pseudo-Saiko-ka-ryukotsu-borei-to without Pinellia tuber.
d) Not detected by using the sensitive SAEIA.

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