Studies on the Cultivation and Preparation of *Platycodon* Root. III.[1] Effect of Picking Flower and Fruit on the Quality of Skin Peeled Root

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The method for the determination of saponins by thin layer chromatography (TLC)-densitometry and an improved colorimetric method on inulin using Sep-Pak purification were applied on Platycodon root.

Picking the flowers of *Platycodon grandiflorum* A. DC. during cultivation markedly increased the root weight. However, picking flowers or fruit did not affect the content of dilute ethanol-soluble or water-soluble extract defined by JP XII, saponin or inulin in Platycodon root.

It is concluded that picking the flowers of *P. grandiflorum* is favorable for increasing the yield of the root growth without altering the chemical constituents.

Keywords *Platycodon*; cultivation; flower picking; root weight; saponin; inulin; thin layer chromatography-densitometry

The root of *Platycodon grandiflorum* A. DC. (*Platycodon*, 桔梗) is one of the most important crude drugs in kampo, the traditional medicine in Japan. Its crude saponin is well known to have an expectorant activity. We previously reported that the various kinds of soil and their ventilation greatly affected the growth of *Platycodon* root.[1]

Disbudding of *Platycodon* before flowering has been known to increase the root weight.[2] It is also reported that picking the flowers of *Bupleurum falcatum* L.[3] or *Scutellaria baicalensis* GEORGI[4] increases their root growth. However, there is little data which would aid in an explanation of such phenomenon.

The purpose of this study was to investigate the influence of disbudding or flower picking of *Platycodon* on the root weight and the content of chemical constituents in the root.

Experimental

Location of Cultivation with Meteorological Data The experiment was conducted on the same field as described earlier.[1]

The meteorological conditions are: mean annual temperature, 16.7 °C; temperature extremes, −1.5 °C minimum and 34.0 °C maximum; annual precipitation, 2403.1 mm; mean annual sunshine hours, 6.1 h.

Materials *Platycodon* seeds obtained from our field in 1988 were used. Spectrophotometric determination was carried out by Hitachi spectrophotometer UV-220 and thin layer chromatography (TLC)-densitometry using a Shimadzu Dual-wavelength TLC scanner CS-910.

Methods of Cultivation The field was given 200 and 6 kg/a of barnyard compost and a 3-2-3 (N-P-K) fertilizer, respectively. The seeds of *Platycodon* (white flower) were sowed in two line plots 30 cm apart on a ridge bed 90 cm wide, 20 cm high and 25 m long on November 4, 1988. The seedlings were thinned to 20 cm apart on June 14, 1989. Flower picking was done twice on August 28 and September 13, 1989. Fruit picking on another group was done on September 21,1989. The plants were harvested on January 6, 1990. The plant height, weight of aerial parts, diameter of root, root length and weight of fresh root were determined by 10 samples from each group.

Preparation of *Platycodon* Root The epidermis of fresh root was scraped off with a metallic swab. The fibrous and lateral roots were discarded, and the weight of the main root was measured after washing and drying with hot air.[5]

Preparation of Saponin Standard Powdered Platycodon root was extracted with dilute ethanol (dil.-EtOH) by refluxing for 1 h. The solution was filtered and then evaporated *in vacuo*. The residue was dissolved in water and extracted with EtOAc. The aqueous solution was extracted with *n*-BuOH several times and the combined *n*-BuOH solution was evaporated *in vacuo*. The residue was dried in a desiccator (silica gel).

Determination of *Platycodon* Saponins by TLC-Densitometry[5] Powdered Platycodon root (1.00 g) was accurately weighed and extracted twice with MeOH (100 ml each) by refluxing for 1 h each time. The solution was filtered, and the residue was washed with a small amount of MeOH. The combined solutions were evaporated *in vacuo* to give a residue, which was dissolved in water (10 ml) and applied on Sep-Pak C_{18} column (Waters Co., Ltd.). After washing with water (10 ml), MeOH was added to the column to make the filtrate exactly 5 ml, and this solution was used as the sample solution.

Standard solution was prepared by dissolving accurately weighed *Platycodon* saponin mixture (40.0 mg) in 10 ml of MeOH and further diluted with MeOH.

Ten μl of standard and sample solutions was spotted on a TLC plate (Merck, Kieselgel 60 F_{254} precoated) and developed with $CHCl_3$–MeOH–H_2O–HCOOH (50 : 40 : 8 : 1, v/v). The plate was air-dried and heated for 10 min at 100 °C after spraying with 5 ml of 25% H_2SO_4. Densitometric detemination was done on this plate by reflectance and zig-zag modes at 450 nm (sensitivity: CH × 1, linearizer: SX = 3). Linear calibration plots were obtained from the peak areas of 1.0—4.0 mg/ml of standard solution.

Determination of Inulin[5] Powdered Platycodon root (0.250 g) was accurately weighed. Water (45 ml) was added, and the mixture was placed in a water bath at 80—90 °C for 1 h with occasional stirring. After cooling, water was added to the solution to make exactly 50 ml. It was then centrifuged at 3500 rpm for 10 min, and this supernatant fluid was used as the sample solution. About 10.0, 20.0 and 40.0 mg of inulin (Wako Pure Chemical Industries, Ltd., special class, water content, 11.49%) accurately and individually weighed, was dissolved in hot water and, after cooling, water was added to make exactly 20 ml; this solution was used as the standard solution.

Each 0.2 ml of standard and sample solutions were added on Sep-Pak silica (Waters Co., Ltd.) and washed with *n*-BuOH (10 ml) and then $CHCl_3$–MeOH–H_2O (5 : 4 : 1, v/v, 2.5 ml) 6 times. After aeration until the odor of $CHCl_3$ was completely gone, the Sep-Pak column was eluted by water (1 ml) 5 times and all the eluates were combined. One ml of the solution was mixed with 4 ml of 30% HCl·resorcinol (7 : 1, v/w) and warmed for 15 min in a water bath at 80 °C. After cooling, detection was carried out at 480 nm. The mixture of 1 ml water and 4 ml of 30% HCl·resorcinol (7 : 1, v/w) was used as the control.

Statistical Analysis The means of all data were presented with their standard deviation (mean ± S.D.). Student's *t*-test was utilized to determine a significant difference between the groups, $p < 0.05$ being taken as the minimum level of significance.

Results and Discussion

Determination of Saponin in Platycodon Root by TLC-Densitometry Saponin content is widely used for the quality evaluation of Platycodon root and some determination methods are also reported using high pressure liquid chromatography (HPLC).[6] These methods are actually very difficult, however, as pure saponin standard reagent is not easily available. We therefore devised

TABLE I. Effect of Picking Flower or Fruit on the Growth of *Platycodon grandiflorum* A. DC.

	Plant height (cm)	Wt. aerial parts (g)	Diam. root head (mm)	Root length (cm)	Wt. fresh root (g)	Wt. dried unpeeled root (cm)
Flower picked	42.8±3.73	6.7±3.55	15.8±1.94	26.9±2.96	43.9±10.99[a]	10.1±2.70[a]
Fruit picked	39.4±3.78	7.4±8.04	14.7±2.85	22.7±3.51	33.2± 9.43	8.2±2.90
Non-treated	38.9±6.75	4.7±2.83	13.8±3.15	25.1±6.28	30.7±13.48	6.9±3.18

Each value represents the mean±S.D. ($n=10$). *a*) Significantly different from the control ($p<0.05$).

TABLE II. Quality Test of Platycodon Root

	Ash (%)	Ash II[b] (%)	dil.-EtOH ext. (%)	Aq. ext. (%)	Saponin (%)	Inulin (%)
Flower picked	3.59±0.195	0.26±0.108	55.5±8.41	71.8±2.61	1.34±0.371	38.6±5.23
Fruit picked	3.56±0.231	0.22±0.070[a]	53.0±8.72	69.7±6.00	1.29±0.410	41.3±4.89
Non-treated	3.63±0.411	0.41±0.186	58.8±3.11	70.6±2.38	1.67±0.631	38.7±6.70
JP specific.	4.0>	1.0>[c]	25.0<	—	—	—

Each value represents the mean±S.D. ($n=6$). *a*) Significantly different from the control ($p<0.05$). *b*) Acid insoluble ash. *c*) The value for powdered Platycodon root.

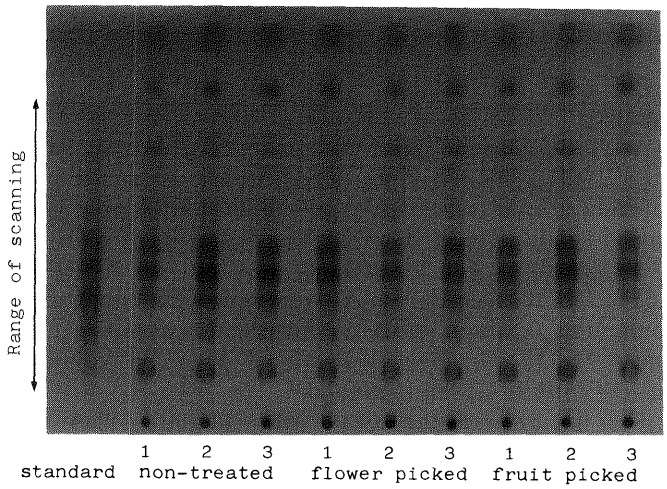

Fig. 1. TLC Profile of Saponin Fraction of Platycodon Root

Developing solvent, $CHCl_3$–MeOH–H_2O–HCOOH (50:40:8:1, v/v); plate, precoated Silica gel $60F_{254}$ (Merck); detecting reagent, 25% H_2SO_4 solution, heated at 100 °C for 10 min.

a convenient determination method using the saponin mixture[7] obtained from *Platycodon* as the standard.

This TLC technique[5] may have rather substantial errors as it includes the densitometry of many spots as shown in Fig. 1. However, it is very useful in studying the effect of cultivation conditions on the saponin content of Platycodon root.

Determination of Inulin in Platycodon Root Ota and Mino have reported on the determination of inulin in Platycodon root using gel-chromatographic separation and coloration with HCl–resorcinol reagent,[8] but this method seems tedious and expensive.

We employed Sep-Pak silica for clean-up of the sample solution as briefly described.[5] This method was shown to be simple and easy for a routine analysis of inulin in Platycodon root.

Effect of Picking Flowers or Fruits on the Growth of *Platycodon* The yield of *Platycodon* root was significantly increased by the picking of flowers, although we found no difference in the weight of aerial parts or in root length among the three groups of different cultivation (Table I).

It is well known that yield of the bulb of *Fritillaria verticilata* WILLDENOW var. *thumbergii* BAKER increases when flowers of the plant are picked.[9] The root weight of *Paeonia lactiflora* PALLAS decreases when flowers are formed.[10] *Scutellaria baicalensis* GEORGI[4] and *Bupleurum falcatum* L.[3] are also reported to have increased root yield when flowers are clipped. Thus, removal of the flowers and fluit appears to make roots grow as the energy harvested during photosynthesis is not used for their development.[11] It is therefore reasonable that root growth is stimulated more by picking flowers than by picking the later fruit.

Effect of Picking Flowers or Fruits on the Quality of Platycodon Root As shown in Table II, we found no variation in the chemical components of ash, dil.-EtOH-soluble extract (dil.-EtOH ext.), water-soluble extract (aq. ext.), or the content of saponin and inulin among the three conditions. The picking of flowers is thus concluded to be a beneficial way of increasing root growth without altering chemical constituents.

Further investigation is necessary to clarify the root growing mechanism.

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