A Comparative Pharmacognostic Study of Taiwanese and Commercial Articles of Phellodendri Cortex

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Phellodendri Cortex was first recorded in Shen-nung's Herbal as a medium-grade herbal drug which has been used as a bitter stomachic. The drug is derived from the trunk bard of the rutaceous plants Phellodendron amurense Ruprecht, P. chinense Schneid, P.wilsonii Hay et Kanehira, and closely related plants of the same genus.

In order to understand the commercial articles and to assess their quality for the medicinal use of the herb to base on, in this study we gathered from Taiwan and its nearby areas a total of 31 commercial samples wherefore we used pharmacognostic histological means to identify their botanical origins and used capillary electrophoresis to assay their constituents for assessment of their quality.

Histologic identification showed that among the 31 samples gathered 4 samples belonged to Phellodendron amurense Ruprecht, 14 samples belonged to P.chinense Schneid, 7 samples belonged to P.wilsonii Hay. et Kanehira,and 6 samples belonged to P.amurense Rupr.var.sachalinense Fr.Sch. In these samples the contents of the five quaternary alkaloids — berberine,palmatine,jatrorrhizine,phellodendrine and magnoflorine were determind by capillary electrophoresis whereby it was found that P.wilsonii(4.10 ± 0.78%)and P.amurense var. sachalinense(4.18 ± 1.03)were superior to P.amurense(1.55 ± 0.72%)and P.chinense(1.54 ± 0.60).Berberine was of the highest content in almost all samples It occupied about 80%of the total alkaloids in the first two of the four herbs named above, but only about 40%in the latter two. The Taiwanese articles of P.wilsonii Hay. et Kanehira were of quite good quality and hence worth wide planting and cultivation.

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ABSTRACT

Phellodendri Cortex was first recorded in Shen-nung's Herbal and has been used as a bitter stomachic ever since. The drug is derived from the trunk bark of plants of the rutaceous Phellodendron genus. A total of 31 commercial samples of Phellodendri Cortex from the origins of Plellodendron amurense Ruprecht, P.chinense Schneid, P.wilsonii Hayata et Kanehira and P.amurense Rupr. var. sachalinense Fr. Schn. respectively were collected from the Taiwan and Japan herbal markets. The contents of the five quaternary alkaloids-berberine, palmatine,jatrorrhizine,phellodndrine and magnoflorine in these samples were determined by capillary electrophoresie. It was found that P.wilsonii(4.10 ± 0.78%)and P.amurense var. sachalinense (4.18 ± 1.03%)were superior to P.amurense(1.55 ± 0.72%)and P.chinense(1.54 ± 0.60%).Berberine was of the highest content in almost all damples. It occupied about 80% of the total alkaloids in the first two of the four herbs named above, but only about 40% in the latter two. From the data of chemical analysis of an herb's constituents and from the herb's texture and color, we can postulate the origin and quality of the herb.

Keywords: Phellodendri Cortex, capillary electrophoresis, histological identification, berberine alkaloids.

INTRODUCTION

phellodendri Cortex comes as the dried trunk bark of any of a few rutaceous plants. It is a commonly used Chinese herbal drug possessing the effects of clearing heat, moistening aridity, purging fire, detoxifying toxicosis and clearing deficienncy-fiver[1]. The herb has been known to contain berbering, palmatine, jatrorrhizine, phellodendrine and magnoflorine as its bioactive principles[2-5]. By using high performance liquid chromatography, Chen[6] had determined the content of berberine and the relative amounts of palmatine, magnoflorine and phellodendrine in P.wilsonii Hay.

et Kane., Tosa and coworkers[7] determined the contents of the four aforementioned alkaloids in P.amurense Rupr. From their papers, it was found that there were great differences among the various samples. It has been known that Phellodendri Cortex is very complicated with respect to its sources on the market, thus a systemic comparative study on the commercial articles is important. In this study we collected 31 samples from different herb shops in Taiwan and Japan, identified and categorized them by pharmacognostic histological anatomy, and then assayed the contents of alkaloids by capillary electrophoreses[8]

EXPERINENTAL

Herb Materials

Thirty-one herb samples collected from different herb shops throughout Taiwan and Japan were identified by external appearance and pharmacognostic histolgical anatomy. They were found to have been derived from four species, namely, Phellodendron amurense Ruprecht, P.chinense Schneid, P.wilsonii Hayata et Kanehira and P.amurennse Rupr. var. sachalinense Fr. Schn. Besides, we also obtained the branches of a cultivated species P.wilsonii grown at the National Taiwan University's experimental farm at Meifeng, Nantou Prefecture, Taiwan.

Standards

Berberine chloride was purchased from Sigma(St.Louis, MO.USA).Palmatine, phellodendrine and magnoflorine were isolated from Phellodendri Cortex[4]. Jatrorrhizine was isolated from Coptidis Rhizoma[9].

Sample preparation

Each of the collected samples was embedded in paraffin wax and cut up into thin sections for preparation into slides[10]. The slides were then observed under a microscope for histological features in order to identify the botanical origin for identification purpose.

A 0.5g sample of pulverized Phellodendri Cortex was extracted with 70% methanol(7.5ml)by stirring at room temperature for 30 minutes and then centrifuging at 1500 G for 10 munutes. The extraction was repeated three times. The extracts were combined and filtered through a No.1 filter paper. After the addition of a 2.5-ml initernal standard solution(2 mg brucine in 1 ml 70%methanol),the Phellodendri Cortex extract was diluted to 25ml with 70% methanol. This solution was passed through a 0.45 μm filiter and about 1.1 nl(15-s hydrostatic sampling)of the filtrate was injected into the capillary electrophoresis system directly. The area ratios of the absorption peaks were calculated.

Apparatus and conditions

All analyses were carried out on a Waters Quanta 4000 capillary electrophoresis system equiped with a UV detector set at 280 nm and a 50cmx50 μ mi.d. uncoated capillary(Millipore,USA)with the detection window placed at 42.5cm. The conditions were as follows:samplihg tiime,15 s hydrostatic, run time, 9 min,applied voltage,15kv (constant voltage,positive to negative polarity),temperature,24.5-25.0 ℃. The electrolyte was a buffer solution consisting of 50% 0.5M sodium acteate solution(pH= 4.6,adjusted with acetic acid)and 50% acetonitrile[8]. The electrolyte was filtered through a 0.45 μ m filter before use.

RESULTS AND DISCUSSION

After carefully inspecting the histological sections and surface features of commercial Phellodendri Cortex samples, we found the various Phellodendron species differed mainly in the external surface,texture, fracture surface, the lenticel scar, remnant of coarse skin, presence or absence of cork layer and array of cortical stone cells and phloemr ays(Table 1),and histological identification based chiefly on the features of phloem ray arrangement and the presence or absence of stone cells in the vascular bundle(table 2).

By comparing the features of the external appearances and histological anatomy of the samples with those given in references[11-18],the origins of the 31 commercial samples were identified as follows:fourteen samples belonged to P.chinense Schneid, four samples came from P.amurence Ruprecht, seven samples were of the origin P. wilsonii Hayata et Kanehira,and the other seven samples were derived from P. amurense Rupr. var. sachalinense Fr. Schn.(Tables 3 and 4)

Analyse of these commercial samples with capillary electrophoresis gave the results shown in Tables 3 and 4 ,and the contents of constituents at the various part of the herb weerw calculated sa shown in Table 5.

The data showed great differences among the samples, ranging from the lowest content of 6.9mg/g in sample 11 to the highest 53.3mg/g in sample 22.Among the samples, three samples had contents above 50mg/g, five samples between 40-50mg/g, all of these were found belonging to either P.wilsonii or P.amurense var. sachalinense. Samples with total contents below 10mg/g, however,were those derived from P.chinense or P.amurense. The average total alkaloid contents of the individual species of Phellodendri Cortex were as follows:P.chinense,1.54 ± 0.60%,P. amurense, 1.55±0,72%,P.wilsonii, 4.10±78%, and P.amurense var.sachalinense,4.18 ± 1.03%.From the Taiwan herb market,we collected 25 samples of which 18 samples were imported from mainland China and belonged to P.chinense and P.amurense , seven were of local provenance belonging to P.wilsonii and the other seven from Japan all belonging to P.amurense var. sachalinense.

Investigation of the contents of individual alkaloids found that berberine was the highest, except in some samples of P.chinense and P.amurense which contained higher amount of magnoflorine than of berberine. Berberine occupied 85% of the total alkaloids in P.wilsonii, but 75% in P.amurense var. sachalinense, 40% in P. amurense and 37% in P.chinense. Besides berberine, the second and third highest in P.chinense and P.amurense were magnoflorine and palmatine. The total content of the three alkaloids occupied about 92% of the total alkaloids. However, in P.wilsonii the alkaloid of the second highest content was phellodendrine and the third highest was magnoflorine, which together with berberine occupied 97% of the total alkaloids. In P.amurense var. sachalinense, the second and third highest were magnoflorine and phellodendrine respectively, and the three highest alkaloids occupied 94% of total alkaloids. After careful comparison of the 31 capillary electrophoreograms, we found that smples derived from the same botanical source had almost identical patterns, but there were differences among samples of different species(Fig.1-4). Besides, a comprehensive examination of the statistical data regarding the average values of the contents of total alkaloids, palmatine and phellodendrine, and the ratios of total alkaloid/berberine and total alkaloid/magnoflorine in samples of the same species gave a significant feature as shown in Table 6. Among these statistical data, the most marked distinction lied in the content of total alkaloids and the quotient of total alkaloids divided by magnoflorine. With the former distinction, we can esaily identify samples derived from P.chinense, P.amurense (ca. 15mg/g) or P.wilsonii. P.amurense var. sachalinense (ca. 41mg/g), with the latter distinction we can clearly and assuredly tell which samples belong to P.wilsonii(ca.25) or P.amurense var. sachalinense(ca.8). If we again combine these distinctions with some of the features of external appearance (as shown in Table 1)such as, fracturability and color of fracture surface, we can identify samples belonging to either P. chinense or P.amurense. Thus, by means of the chemical analysis data and external features, we can rapidly identify the sources and assess the quality of Phellodendri Cortex samples.

Although the ratios among the various components were very close to each other, the absolute contents differed greatly from each other. To investigate the reason, we separated the herbs into the inner, middle and outer layers and assayed each layer separately. The results were as shown in Table 5. Data in the table showed no much differences among the different layers,except magnoflorine which was more abundant in the inner layer, and sample 28 in which the various figures in the outer layer were espeically low due to the attachment of coarse skin remnant on the surface of this herb, sample 32 which was a cultivated species grown at Meifeng, Nantou Prefecture,Taiwan and contained low amounts of the individual constituents.

Samples of the various Phellodendron species differed greatly in their external appearance. P.wilsonii and P.amurense var. sachalinense which were of better quality were more solid and dense in texture, whereas the two samples produced in main-

land China were comparatively more porous and lighter. Generally the more yellow colored the fracture surface is, the more excellent the herb'squality will be P.wilsonii and P.amurense var. sachalinense had a more intense yellow color than P.chinense and P.amurense. This is also true for herbs of the same species. The Taiwanese articles of the herb had higher contents and were of quite good quality. Hence, the Taiwanese species is worth wide planting and cultivation in order to explore and develop the resources of medicinal plants.

Table 1 Characteristics of the various commercial articles of Phellodendri Cortex

Item \ Article	*Phellodendron chinense Schneid*	*P. amurense* Ruprecht	*P. wilsonii* Hayata et Kanehira	*P. amurense* Rupr. var. *sachalinense* Fr. Schm.
Lenticel scar on external surface	Visible in some samples	Tiny and scarcely seen	Abundant	Abundant and in dark brown color
Remnant of coarse skin on external surface	Present occasionally, in grey brown color	Present occasionally, in grey white color	Usually present, in grey brown color	None
Character	Light, hard	Light, comparatively harder	Resilient and hard	Light and hard
Fracture surface	Deep yellow color	Fresh yellow or yellow green color	Fresh gold color	Fresh yellow color
Cork layer	Shed, or present in several ten rows	Shed	Shed, or present in several layers, each layer comprising several rows	Shed
Cortical stone cells	Solitary or clustered; globoid or irregular long stick form	Solitary or clustered; globoid or irregular long stick form	Mostly in long stick form; each comprising several thin-membraned stone cells	Solitary in global form, or several ones forming a long stick form
Phloem rays	1–4 rows wide, curved	1–3 rows wide, straight	2–5 rows wide, curved	1–5 rows wide, straight but curved toward the cortex

Table 2 Key to the histological cross sections of Phellodendron species

1. Cork layer is often shedded, inner stratum of cork layer is composed of 1-5 rows of cells containing cubic calcium oxalate crystals.

2. Cortex contains sporadically distributed stone cells that exist solitarily or in clusters, and is scattered with groups of fibers.

3. Phloem rays are each of the width of 1-3 rows of cells, comparatively flat …… P.amurense.

3. Phloem rays are each of the width of 1-4 rows of cells, often flexed …… P. amurense.

1. Cork layer is often shedded, inner stratum of cork layer is composed of 1-3 rows of cells containing no cubic calcium oxalate crystals.

2. Cortex contains sporadically distributed stone cells that exist solitarily in globoid form or with several stone cells linked up into an irregular form, and is scattered with groups of fibers.

3. Phloem rays are each of the width of 1-5 rows of cells, flexed at the part near the cortex and comparatively flat for the remaining part, arrayed in a flame form …… P.amurense var. sachalinense.

1. Cork layer is shedded or partially retained, inner stratum of cork layer is composed of some ten rows of cells with or without cubic calcium oxalate crystals.

2. Cortex contains a plurality of sporadically distributed stone cells mostly of thin-membraned stone cells up into an irregular stripe.

3. Phloem rays are each of the width of 2-5 rows of cells arrayed flexedly in a flame form Occasionally stone cells may appear in clusters or in single pieces, scattered between vascular bundles …… P.wilsonii.

Table 3 Alkaloid contents in the commercial Phellodendri Cortex samples collected from Taiwan herbal market (mg/g)

Original plant	samples	BER	PAL	JAT	PHE	MAG	Total
P. chinense	1	6.0	2.4	0.2	0.7	1.1	10.4
	2	3.4	2.3	0.2	0.7	4.9	11.5
	3	6.7	9.8	0.3	1.5	8.0	26.3
	4	8.9	2.6	0.2	1.3	7.8	20.8
	5	3.1	4.0	0.1	0.8	3.9	11.9
	6	7.2	3.9	0.4	1.3	6.4	19.2
	7	5.4	3.6	0.2	1.2	5.5	15.9
	8	7.7	3.7	0.2	1.1	6.6	19.3
	9	3.0	3.0	0.1	0.5	2.1	8.7
	10	7.5	2.5	0.3	1.1	4.6	16.0
	11	1.5	2.1	0.2	—	3.1	6.9
	12	6.2	3.2	0.3	1.2	8.1	19.0
	13	1.9	2.0	0.1	0.5	3.2	7.7
	14	11.2	2.5	0.2	1.4	6.9	22.2
	average	5.7	3.4	0.2	1.0	5.0	15.4
	(n=14)	±2.8	±2.0	±0.1	±0.4	±2.3	±6.0
P. amurense	15	5.6	1.9	0.2	0.7	0.8	9.2
	16	4.4	2.6	0.1	0.9	3.7	11.7
	17	10.0	4.7	0.3	1.8	8.7	25.5
	18	4.7	3.9	0.2	1.0	5.7	15.5
	average	6.2	3.3	0.2	1.1	4.7	15.5
	(n=4)	±2.6	±1.3	±0.1	±0.5	±3.3	±7.2
P. wilsonii	19	25.5	0.3	0.2	2.4	1.4	29.8
	20	29.7	0.1	0.2	2.4	0.6	33.0
	21	32.9	2.0	0.6	2.9	1.5	39.9
	22	46.2	1.4	0.7	3.6	1.4	53.3
	23	34.5	0.7	0.5	3.1	3.7	42.5
	24	38.4	0.7	0.5	2.6	1.1	43.3
	25	39.5	0.7	0.5	3.0	1.8	45.5
	average	35.2	0.8	0.5	2.9	1.6	41.0
	(n=7)	±6.8	±0.7	±0.2	±0.4	±1.0	±7.8

BER: berberine, PAL: palmatine, JAT: jatrorrhizine, PHE: phellodendrine, MAG: magnoflorine.

Table 4. Alkaloid contents in commercial Phellodendri Cortex collected in Japan herbal market (mg/g)

Original plant	samples	BER	PAL	JAT	PHE	MAG	Total
P. amurense var. *sachalinense*	26	34.9	1.3	0.7	2.6	1.6	41.1
	27	37.5	4.1	0.9	3.2	6.4	52.1
	28	23.3	0.3	0.2	1.5	2.0	27.3
	29	25.3	0.5	0.3	1.8	4.1	32.0
	30	29.9	2.5	1.1	2.8	11.4	47.7
	31	39.2	2.5	0.8	3.5	4.8	50.8
	average (n=7)	31.7 ±6.6	1.9 ±1.4	0.7 ±0.4	2.6 ±0.8	5.1 ±3.6	41.8 ±10.3

BER: berberine, PAL: palmatine, JAT: jatrorrhizine, PHE: phellodendrine, MAG: magnoflorine.

Table 5 Alkaloid contents in different parts of Phellodendri Cortex (mg/g)

Sample	BER	PAL	JAT	PHE	MAG	Total
18 (inner)	4.8	3.4	0.2	0.8	7.9	17.1
18 (middle)	4.5	3.4	0.2	1.3	4.8	14.2
18 (outer)	4.8	4.4	0.2	1.1	5.7	16.2
25 (inner)	42.0	1.4	0.7	2.6	2.9	49.6
25 (middle)	46.0	0.6	0.4	2.9	1.1	51.0
25 (outer)	8.0	0.3	0.1	1.1	1.9	11.4
29 (inner)	20.0	0.4	0.3	1.4	12.2	34.3
29 (middle)	27.7	0.5	0.3	1.9	2.3	32.7
29 (outer)	23.5	1.0	0.3	1.8	2.1	28.7
32[a](xylem)	—	—	—	—	0.6	0.6
32 (cork layer)	—	—	—	—	0.9	0.9
32 (cortical layer)	12.8	0.2	0.7	0.7	1.8	16.2

BER: berberine, PAL: palmatine, JAT: jatrorrhizine, PHE: phellodendrine, MAG: magnoflorine.

[a]Sample 32 was the branch from a cultivated species *P. wilsonii* grown at the National Taiwan University's experimental farm at Meifeng, Nantou prefecture, Taiwan.

Table 6 The statistical data of alkaloid contents and ratios from various *Phellodendron* species.

Original plant	Total alkaloid (mg/g)	Palmatine (mg/g)	Phellodendrine (mg/g)	Total alkaloid / Berberine	Total alkaloid / Magnoflorine
P. chinense	15.4	3.4	1.0	2.7	3.1
P. amurense	15.5	3.3	1.1	2.5	3.3
P. wilsonii	41.0	0.8	2.9	1.2	25.6
P. amurense var. *sachalinense*	41.8	1.9	2.6	1.3	8.2

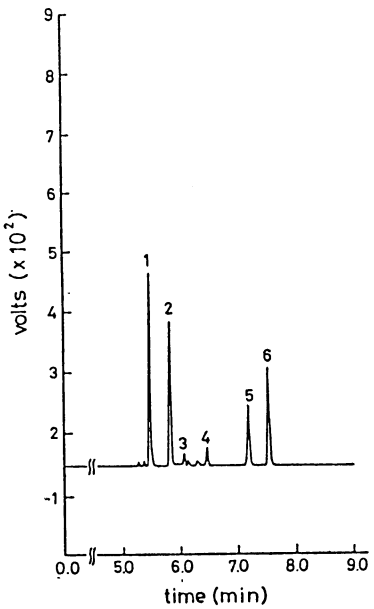


Fig. 1- Capillary electropherogram of the extract of a *P. chinense* Schneid sample (sample 7). Peaks: 1, berberine; 2, palmatine; 3, jatrorrhizine; 4, phellodendrine; 5, magnoflorine; 6, internal standard (brucine).

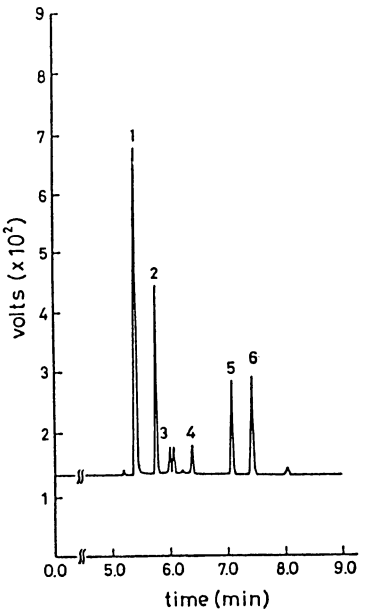


Fig. 2 Capillary electropherogram of the extract of a *P. amurense* Ruprecht sample (sample 17). Symbols as in Fig. 1.

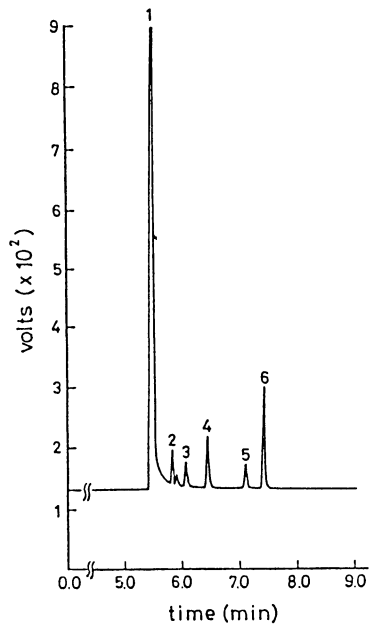


Fig. 3 Capillary electropherogram of the extract of a *P. wilsonii* Hayata et Kanehira sample (sample 25). Symbols as in Fig. 1.

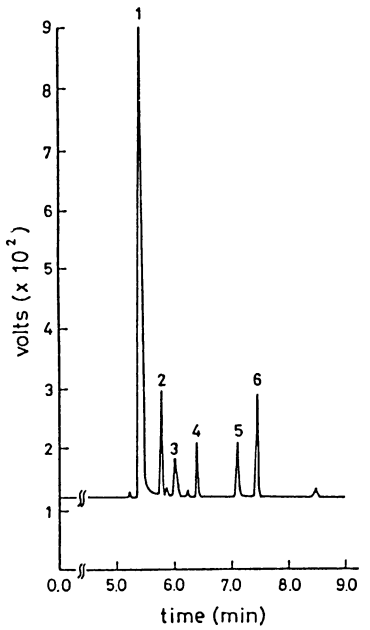


Fig. 4 Capillary electropherogram of the extract of a *P. amurense* Rupr. var. *sachalinense* Fr. Schm. sample (sample 31). Symbols as in Fig. 1.

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台灣黃蘗及市售黃蘗之比較生藥學研究

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黃蘗(Phellodendri Cortex)始載於《神農本草經》列爲中品藥，爲苦味健胃劑，用爲治熱痢泄瀉、黃疸、痔瘡、便血、淋濁、帶下、瘡瘍等症。其來源主要爲芸香料(Rutaceae) 植物黃蘗(Phellodendron amurense Ruprecht)、川黃蘗(P.chinense Schneid)、台灣黃蘗(P. wilsonii Hay. et Kanehira)及其同屬近緣植物之幹皮。

爲了解目前市售黃蘗之種類，並評價其品質，以供爲用藥之依據，本研究由台灣及鄰近地區，搜集市售樣品計31件，以生藥組織學之方法鑑定其來源，並配合毛細管電泳法定量其成分。

由組織之鑑定結果，關黃蘗Phellodendron amurense Ruprecht有4件，川黃蘗P.chinnense Schneid有14件，台灣黃蘗P.wilsonii Hay. et Kanehira有7件，日本黃蘗P.amurense Rupr. var. sachalimense Fr. Schm.有6件。

五種生物鹼成分爲berberine ,palmatine,jatrorrhizine,phellodendrine and magnoflorine等，由毛細管電泳法定量結果，台灣黃蘗（4.10 ± 0.78 %）及日本黃蘗（4.18 ± 1.03 %）含量高於關黃蘗（1.55 ± 0.72 %）及川黃蘗（1.54 ± 0.60 %）。而berberine之含量在這些藥材中均相當高，其中以台灣黃蘗及日本黃蘗含量約佔全生物鹼之80 %，而關黃蘗及川黃蘗則約佔40 %。台灣黃蘗品質頗佳，宜加以推廣栽培。