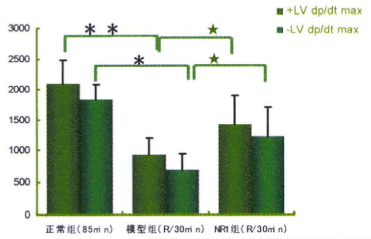
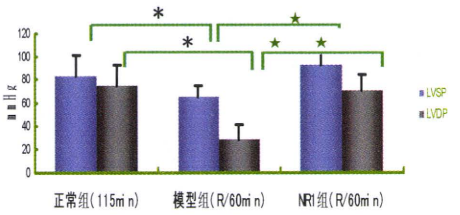
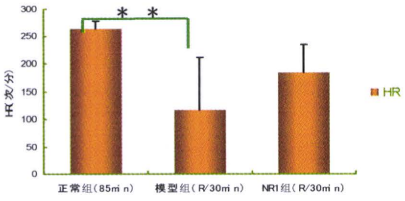
Researches on mechanisms of anti cardiac myocyte apoptosis

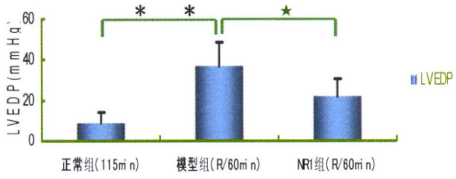
Protective effects on myocardium ⇨ **Protective effects of Notoginsenoside R1 on isolated heart ischemia/reperfusion injuries**





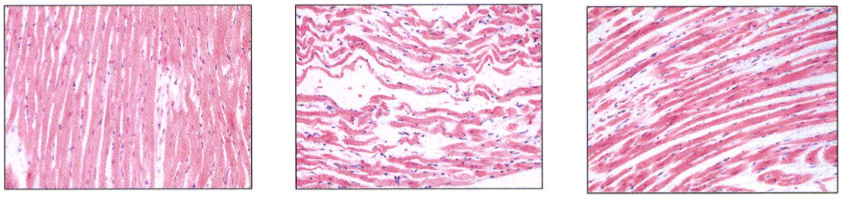
Improvement of heart function





Researches on mechanisms of anti cardiac myocyte apoptosis

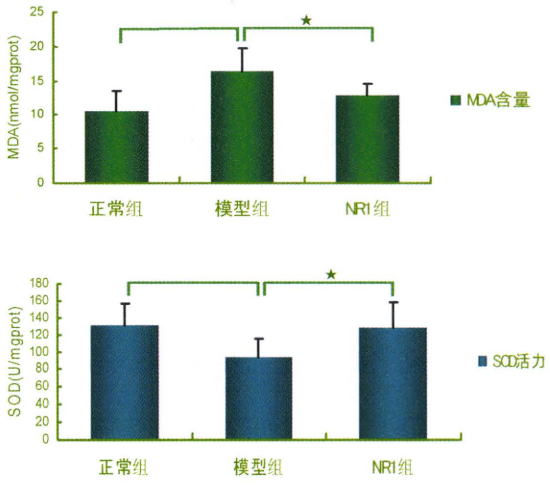
HE staining



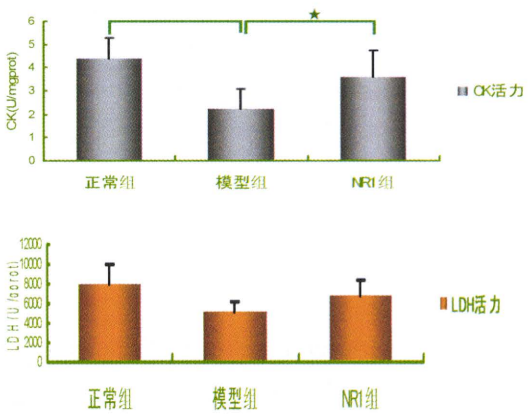
control　　Model　　NR1

Protective effects of Notoginsenoside R1 on isolated heart ischemia/reperfusion injuries

SOD、MDA level in isolated heart tissues

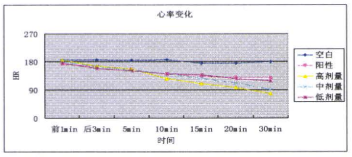


CK、LDH activities in isolated heart tissues

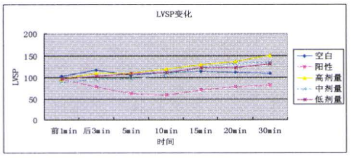


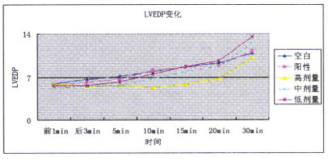
Researches on mechanisms of anti cardiac myocyte apoptosis

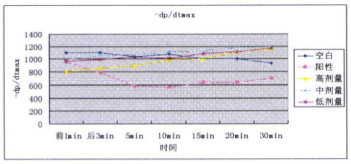
Protective effects → Protective effects on isolated heart ischemia/reperfusion injuries of salvianolic acid B

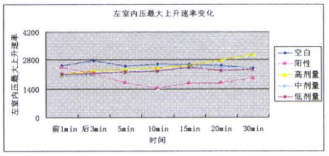


Improvement of heart function









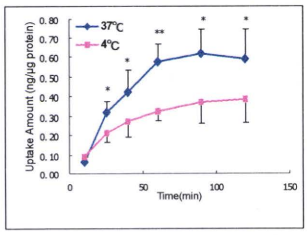
Sorbefacient effect of volatile oil of *Lignum Dallbergiae Odoriferae* on active constituents of *Radix Salviae Miltiorrhizae*

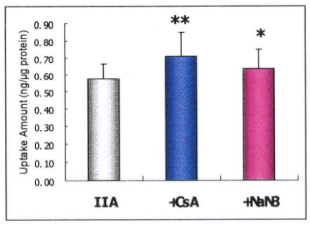
- In cell experiment in vitro, volatile oil of *Lignum Dallbergiae Odoriferae* could increase absorption for active constituents of *Radix Salviae Miltiorrhizae*.
- In animal experiment in vivo, volatile oil of *Lignum Dallbergiae Odoriferae* could increase absorption for active constituents of *Radix Salviae Miltiorrhizae*.
- In molecular experiment, volatile oil of *Lignum Dallbergiae Odoriferae* was with few effect on efflux transporter P-glycoprotein in intestinal epithelial cells.

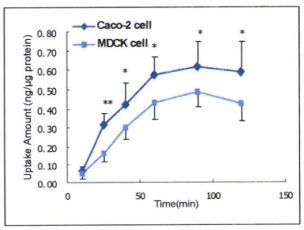
1. In cell experiment in vitro

IIA absorption mechanism: major passive diffusion; minor active transport.

IIA

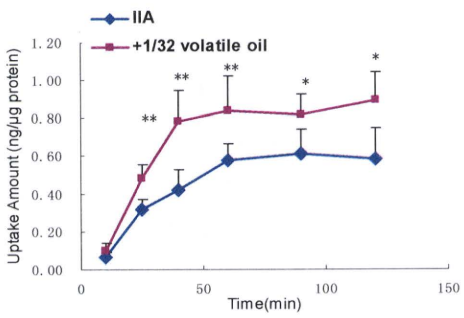






1. In cell experiment in vitro

Caco-2 cell model



increase absorption

Tmax ↓

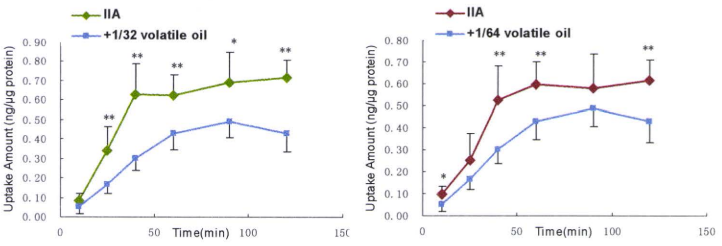
Cmax ↑

Ka ↑

AUC ↑

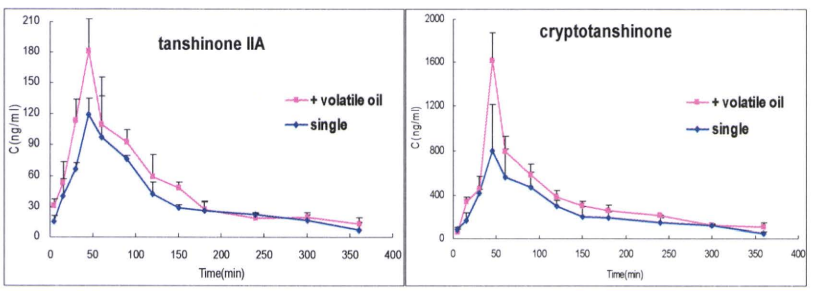
1. In cell experiment in vitro

MDCK cell model



- Volatile oil of *Lignum Dallbergiae Odoriferae* could increase the passive diffusion of absorption for IIA.
- The effect of increase absorption was in a dose-dependent manner.

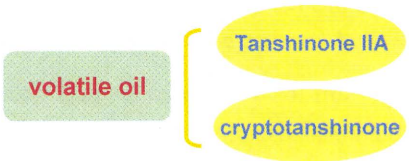
2. In rat experiment in vivo



* AUC ↑ * Cmax ↑ * Ka ↑ * T1/2 ↑ * Ke ↓

2. In rat experiment in vivo

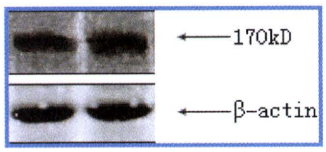
* The PK profile of active constituents of *Radix Salviae Miltiorrhizae* after co-administration of volatile oil of *Lignum Dallbergiae Odoriferae* in SD rat.

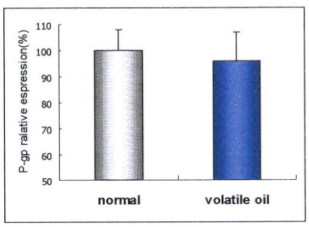


AUC ↑ Cmax ↑ Ka ↑ T1/2 ↑ Ke ↓

3. In molecular experiment

The effect of volatile oil on protein expression of efflux transporter P-glycoprotein in intestinal epithelial cells.





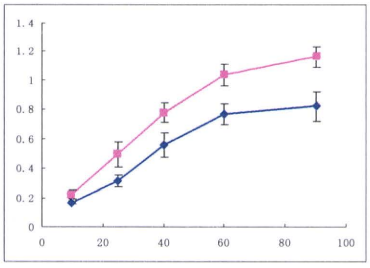
* **Result:** few effect on protein expression of P-glycoprotein.

Sorbefacient effect of flavonoids of *Lignum Dallbergiae Odoriferae* on active constituents of *Radix Salviae Miltiorrhizae*

- In cell experiment in vitro, flavonoids of *Lignum Dallbergiae Odoriferae* could increase absorption for active constituents of *Radix Salviae Miltiorrhizae*.
- In animal experiment in vivo, flavonoids of *Lignum Dallbergiae Odoriferae* could increase absorption for active constituents of *Radix Salviae Miltiorrhizae*.
- In molecular experiment, flavonoids of *Lignum Dallbergiae Odoriferae* could decrease the protein expression of efflux transporter P-glycoprotein in intestinal epithelial cells.

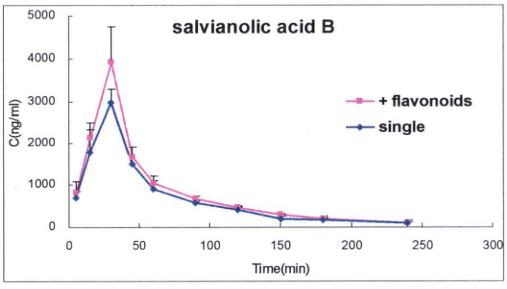
1. In cell experiment in vitro

Caco-2 cell model



- The flavonoids of *Lignum Dallbergiae Odoriferae* could promote the uptake of IIA in Caco-2 cell.

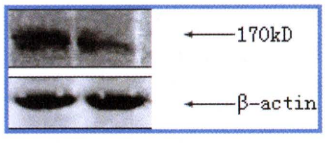
2. In rat experiment in vivo

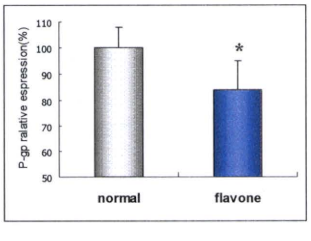


* The flavonoids of *Lignum Dallbergiae Odoriferae* could promote the absorption of salvianolic acid B by increasing the valves of AUC, Cmax and Ka.

3. In molecular experiment

The effect on protein expression of efflux transporter P-glycoprotein in intestinal epithelial cells.

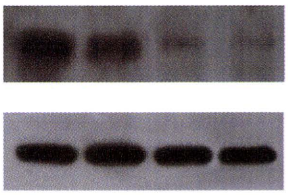




* **Result:** The flavonoids of *Lignum Dallbergiae Odoriferae* could decrease the protein expression of P-glycoprotein.

3. In molecular experiment

The effect of flavonoids on protein expression of efflux transporter P-glycoprotein in intestinal epithelial cells.



* The effect of decrease protein expression of P-glycoprotein was in a time-dependent manner.

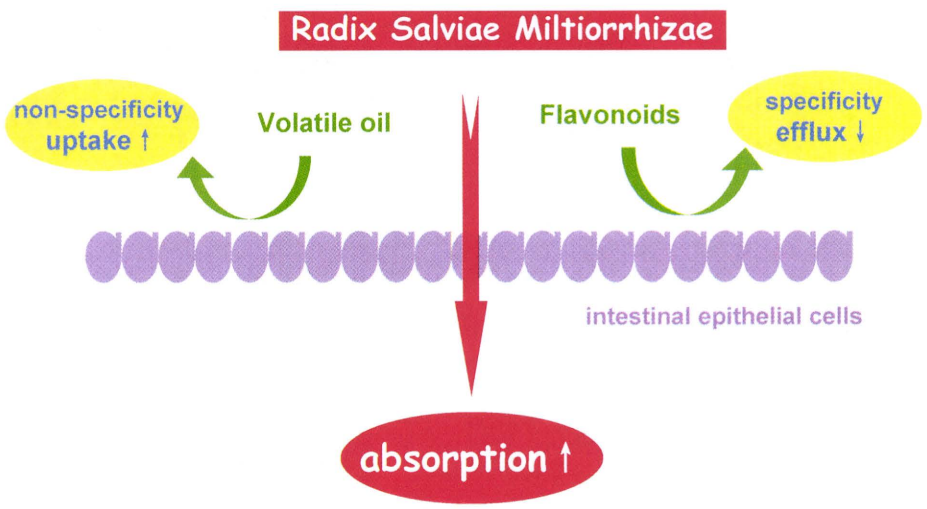
Sorbefacient mechanism

* Volatile oil of *Lignum Dallbergiae Odoriferae* could promote absorption for *Radix Salviae Miltiorrhizae* by increasing the solubility of its active constituents, decreasing the degree of tight junction of intestinal epithelial cells, and facilitating its active constituents cross biomembrane barriers.
* Flavonoids of *Lignum Dallbergiae Odoriferae* could promote absorption for *Radix Salviae Miltiorrhizae* by decreasing the protein expression and activity of P-glycoprotein, decreasing efflux effects and increasing uptake of its active constituents.

Conclusion

- Volatile oil and flavonoids of *Lignum Dallbergiae Odoriferae* could increase absorption for active constituents of *Radix Salviae Miltiorrhizae*, including hydrophilicity constituents and Liposolubility constituents.
- The sorbefacient mechanism of volatile oil mainly was in a nonspecific manner.
- The sorbefacient mechanism of flavonoids mainly was in a specific manner.

***Lignum Dallbergiae Odoriferae*: multipath mechanism of increasing absorption**



- Therefore, traditional medicine achieve its effectiveness by acting on multiple targets to regain the balance of the organism and accomplish the network effects which is hard to be acquired by chemical drugs.
- The single component of a formula shows mild effects with low toxicity, while the overall effects of a formula are much more prominent.

- Traditional medicine can be standardized and internationalized gradually by modern molecular biological techniques and methods from simple to complex, from elementary to profound.
- Traditional medicine will be accepted eventually by more and more people due to its effectiveness and safety.

Thanks for your attention!

The Guidelines for Cultivation and Quality Control of Medicinal Plants in Japan

Osamu IIDA

Tanegashima Division
Research Center for Medicinal Plant Resources
National Institute of Biomedical Innovation

Introduction

The crude drugs used for Kampo medicine in Japan mostly depend on the imports.

↓

1976 Ethical Kampo formulations were approved as drugs covered by national health insurance.

↓

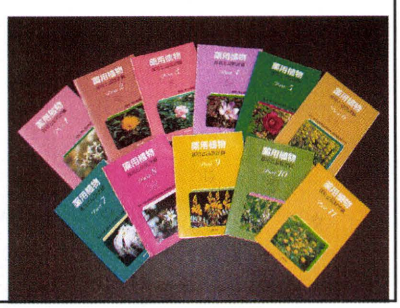
An increase of a demand on the crude drugs

↓

1988 Publishing the monographs on the guidelines was launched.

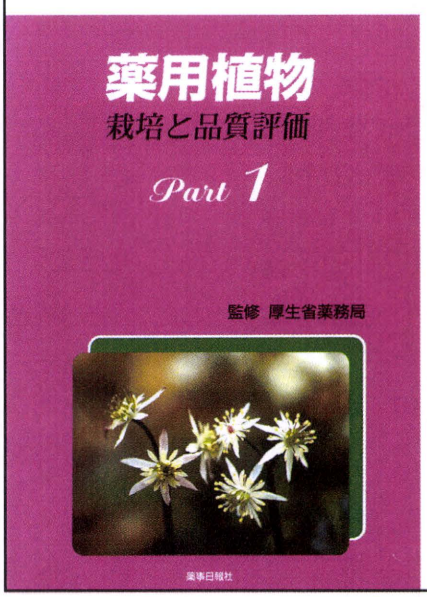
↓

1992 The Part 1 of the series was published. At present, 11 vols. 58 species.



Content

Part 1



★ *Coptis japonica* Makino: (黄連)

★ *Rehmannia glutinosa* Libosch. var. *purpurea* Makino
Rehmannia glutinosa Libosch.: (地黄)

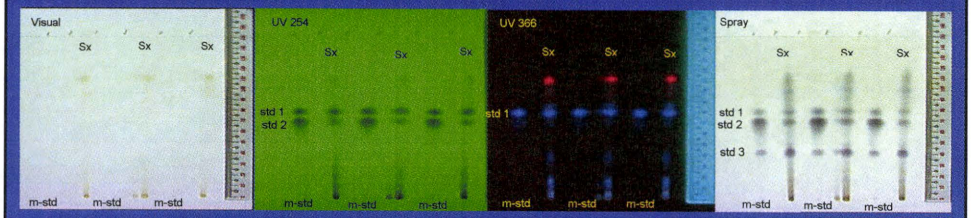
★ *Rheum palmatum* L.
Rheum tanguticum Maxim.
Rheum officinale Baill.
Rheum coreanum Nakai.: (大黄)

★ Angelica acutiloba Kitagawa : (当帰)

★ *Bupleurum falcatum* L. : (柴胡)

SINTA HERB (ANDROGRAPHIDIS PANICULATAE HERB)

Test solution : Weigh approximately 1g of the powdered drug, dissolve in 10 mL of 95% methanol shake using mechanical shaker for 30 minutes and then centrifuge for 10 mins.
Reference solution: # 1) 14-Deoxy-11,12-Didehydroandrographolide
#2) Neoandrographolide
#3) Andrographolide
Loading : 10uL mixed-std & 10uL sample on aluminum back silica gel G
Solvent system : Ethyl acetate : Methanol (10:1), 15 cm run
Spray Reagent : (1:1) 1% vanillin in ethanol & 5% sulfuric acid in ethanol



NOTE: m-std (mixed-std)
Evaluation:

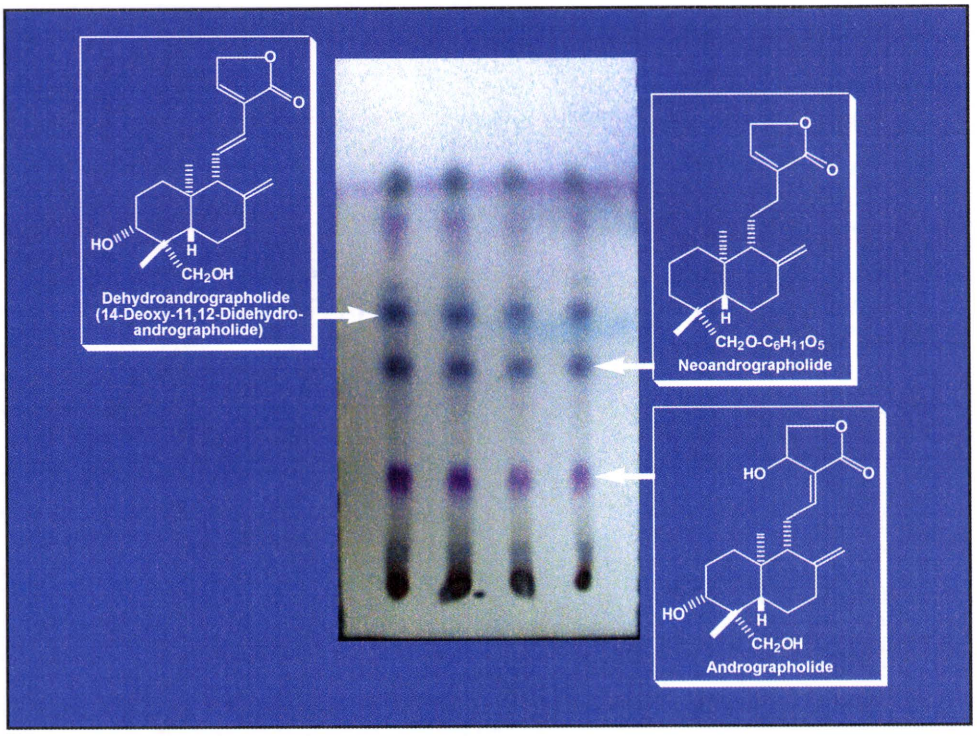
Visual : Std no spot detected , sample, 1 spot detected with Rf 0.73(lt.green)
UV 254: Std 1 Rf 0.53
Std 2 Rf 0.46
Std 3 no spot detected
Sample, 3 major spots detected with Rf 0.53 , 0.58 and 0.73

UV 366 Std 1 detected with Rf 0.53 (blue) , Std 2 & 3 not detected
Sample, 3 spots detected with Rf 0.53 (blue) , 0.58 (lt. Blue) & 0.73 (Red)

Spray Std 1 Rf 0.53 (blue violet)
Std 2 Rf 0.46 (blue violet)
Std 3 Rf 0.27 (violet)
Sample 3 major spots detected with Rf 0.28 (violet) , 0.46 (blue violet) , 0.53 (blue violet) and 3 minor spots with Rf 0.6 (lt.violet) , 0.65 (lt.violet) & 0.72 (lt. Green)

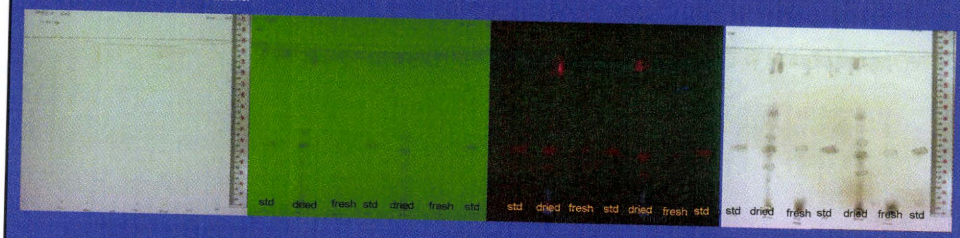
Myanmar Herbal Pharmacopoeia

Andrographis paniculata



SABILA GEL - *Aloe vera* Linne

Test solution 1: 1. Weigh approximately 30 g of the fresh mucilaginous gel, dry in the oven at 60 C for 4-5 hrs. or until all the water content is evaporated.
2. Weigh approximately 0.2 g of the dried mucilaginous gel, dissolve in 4 mL of 95% methanol shake using mechanical shaker for 30 minutes and then centrifuge for 10 mins.
Test solution 2: Weigh approximately 5 g of the fresh mucilaginous gel, dissolve in 5 mL of 95% methanol shake using mechanical shaker for 30 minutes and then centrifuge for 10 mins.
Loading : 30 uL on aluminum back silica gel G
Solvent system : Ethyl Acetate : Acetone : Water : Acetic acid (20:5:2:2), 15 cm run
Spray Reagent : 5% Sulfuric Acid in Ethanol



Evaluation:

Rf	Visual			UV254			UV366			Spray		
	std	dried	fresh	std	dried	fresh	std	dried	fresh	std	dried	fresh
0.260					black			red				
0.400	no spot detected			black	black		red	red	lt. red	brown	brown	lt. Brown
0.490					black							
0.620											red	

Andrographis panioculata (B.F.) Nees, Sinta Herb

Thin-layer chromatographic identification test

Determine the thin-layer chromatogram of Aloe vera Mill. as directed under the Thin-layer Chromatographic Identification Test for Crude Drugs.

Test Solution: To 1 g of pulverized Sinta Herb add 10 mL of 95% Methanol, shake for 30 minutes, filter, and use the filtrate as the sample solution.

Reference Standard: Andrographolide--- Dissolve 1 mg of andrographolide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution.

Loadings: 10 μL (each on silica gel G)

Solvent system: Ethyl acetate / methanol (10 : 1), 15 cm run

Detection: (a) Detected Under UV254,
(b) 1%vanilline-H_2SO_4 reagent

Evaluation: Andrographolide (0.21, –, purple),
Neoandrographolide (0.42, UV254, dark blue),
14-Deoxy-11,12-didehydroandrographolide (0.49, UV254, dark blue)

Philippine Pharmacopoeia 2004

Philippine Pharmacopoeia Project by Japanese ODA Program

Chemical drugs is 30 monographs and crude plants is 33 monographs.

Aloe vera L., Sabila gel

Thin-layer chromatographic identification test

Determine the thin-layer chromatogram of *Aloe vera* Mill. as directed under the Thin-layer Chromatographic Identification Test for Crude Drugs.

Test Solution: To 0.1 g of pulverized Aloe add 2 mL of 95% Methanol, shake for 30 minutes, filter, and use the filtrate as the sample solution.

Reference Standard: Barbaloin (aloin).--- Dissolve 1 mg of barbaloin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution.

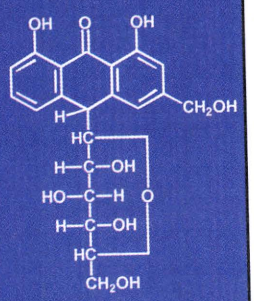
Loadings: 20 µL

Solvent system:

EtOAc / Acetone / H_2O / Acetic acid (100) (20 : 5 : 2 : 2), 15 cm run

Detection: (a) Observe under UV_{366} nm,
(b) 1%vanillin-5%H_2SO_4 Reagent

Evaluation:



Aloin (Barbaloin; 10-D-Glucopyranosyl-aloeemodin-9-anthrone)

The seventh edition (1961):

763 Monographs and 176 crude drugs.

- **In 1955-1960, one of the social accidents was side effect of Chinoform (SUMON;Subacut-Myelo-Optico-Neurophaci) and Tharidomidol (Phocomelia).**
- **Instead of Chinoform, our government was decided to use the traditional medicines so called Kampo Medicines, which was introduced from china and reformed, in Japanese culture.**
- **Pharmacopoeia VII was amended to introduce twice of crude drugs, from 87 to 176 monographs. These medicines were used in modern medical hospitals and these are continued to use broadly.**

The general rules for crude drugs were added in the General Notices on JP 7 (1961)

Achyranthes root, Anemarrhena rhizome, Asiasarum root, Astragalus root, Cassia seed, Belladonna root, Bupleurum root, Cimicifuga rhizome, Cnidium rhizome, Cyperus rhizome, Evodia fruit, Gardenia fruit, Glehnia root, Gypsum, Houttuyinia herb, Imperata rhizome, Japanese angelica root, Jujube, Lithospermum root, Magnolia bark, Montan bark, Morus bark, Nuphar rhizome, Ophiopogon tuber, Oriental bezoar, Oyster shell, Panax rhizome, Peach kernel, Plantgo herb, Plantago seed, Polyporus sclerotum, Poria sclerotum, Prunella spike, Red ginseng, Rehmannia root, Rose fruit, Safflower, Saposhnikovia root, Schisandra fruit, Scutelaria root, Smilax rhizome, Sophora root, Sweet hydrangea leaf, Trichosanthes root

The first edition (1886)

- **The first edition of Japanese Pharmacopoeia had been published on 1886 and this edition was reported 468 monographs included 97 crude drugs.**

- **Draft was introduced from the following Pharmacopoeia;**

  Netherlands Pharmacopoeia II (1871), German Pharmacopoeia II (1882) and USP VI (1886)

Crude Drug of the first edition have been continued to describe 25 monographs in JP14

- **Acacia, Amomum seed, Bearberry leaf, Benzoin, Bitter orange peel, Calumba, Cinnamon bark, Clove, Digitalis, Fennel, Gambir, Japanese gentiana, Ginger, Glycyrrhiza, Honey, Ipecac, Valerian, Mentha herb, Nux vomica, Powdered opium, Rhubarb, Saffron, Senega, Senna, Tragacanth**