# Kampo traditional ointments for wound healing

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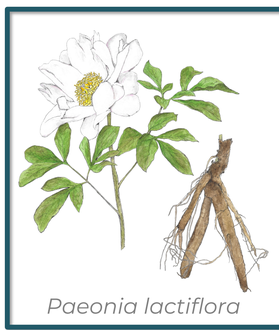
## Introduction


*Paeonia lactiflora*

Japanese herbal medicine, named **Kampo**, covers numerous therapeutic indications including dermatological ones. Three ointments are used for skin wound healing: ***Shiunkō, Chuōkō*** and ***Shinsen taitsukō***. All have in common **sesame oil** in which crude drugs are extracted. Herbs are representatives of the botanical genera *Angelica, Lithospermum, Curcuma, Phellodendron, Paeonia, Rheum, Rehmannia, Scrophularia* and *Cinnamomum*(1).

The aim of this study on Kampo ointments is to better understand the **chemical diversity** of **oily herbs extracts** and to correlate this with **biological effects on wound healing**. The study takes up the strengths of Kampo as a traditional medicine integrated into the Japanese health care system.

This study combines the **therapeutic tradition** of Kampo with the **innovation of modern analytical techniques** including biology (**scratch assay**) and **metabolomics** bioinformatics by **W4M - WorkflowforMetabolomics**(2,3).

## Materials & methods

### Required elements

- **Inventory** the **Kampo therapeutical practices**
- Information from **Japanese pharmacopoeia**(4)
- Use **different extraction methods: M**aceration**, LH** (Low-Heating) and **HH** (High-Heating)
- Produce extracts **with and without beeswax** to facilitate analysis
- Make **single herbs** and **combined extracts**
- **Explore multiple species, origins** or **processes carried on crude drugs**

### Metabolomics

- **LC/MS (ESI)+** on quadrupole time-of-flight (**QTOF**) high-resolution mass spectrometer (HRMS) Agilent
- **XDB-C18 column** (1.0 mm x 30mm 3.5 µm) Zorbax®
- Oils solubilized using **isopropanol** (IPA) Samples = 1% oil by weight in IPA
- Analysis with **Galaxy** platform, **W4M**(2,3) with **pre-processing steps** producing **3 matrices**: variable metadata, data matrix and sample meta data, organizing the m/z values

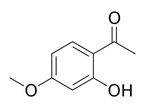
### Scratch assay

= Evaluation of cells' **migration** and **proliferation**(5)

- **Immortalized keratinocytes** (N-tert-2g) on 96-well plates
- 5-10% Kampo oils dissolved in **DMSO**
- [DMSO] final in wells < 1%
- **Live/dead** cells **fluorescent** labelling
  Positive control = **EGF** 10 ng/ml
- **Incucyte® ZOOM Live-Cell Analysis System Essen**

Biotic / abiotic factors → e.g. botanical species, varieties, origins harvesting / processing steps…

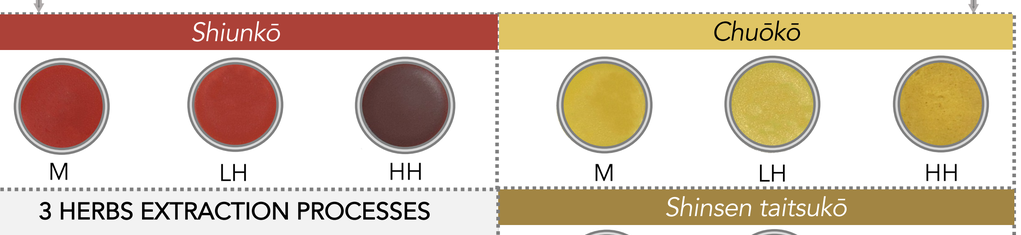
Herbs metabolites



KAMPO CRUDE DRUGS + SESAME OIL + BEESWAX → KAMPO OINTMENTS FOR WOUND HEALING

| *Shiunkō* 紫雲膏 | *Chuōkō* 中黄膏 | *Shinsen taitsukō* 神仙太乙膏 |
|---|---|---|
| = *Angelica* root + *Lithospermum* root | = *Curcuma* rhizome + *Phellodendron* bark | *Angelica (acutiloba + dahurica)* roots + *Cinnamomum* bark + *Paeonia, Rheum, Scrophularia* and *Rehmannia* roots |

VARIABILITY OF EXTRACTION TECHNIQUES + CRUDE DRUGS




**3 HERBS EXTRACTION PROCESSES**

- Maceration 7 days - room temp (M)*
- Low Heating 80 °C - 30min (LH)*
- High Heating (HH) using polymerized oil

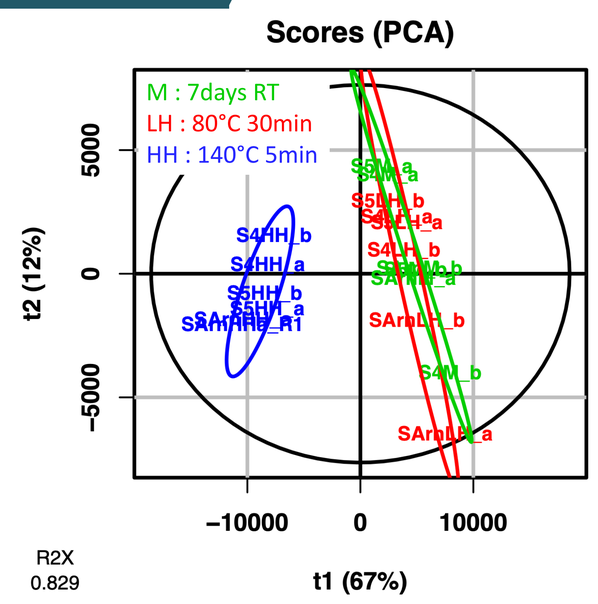
*Beeswax is usually add after the extraction

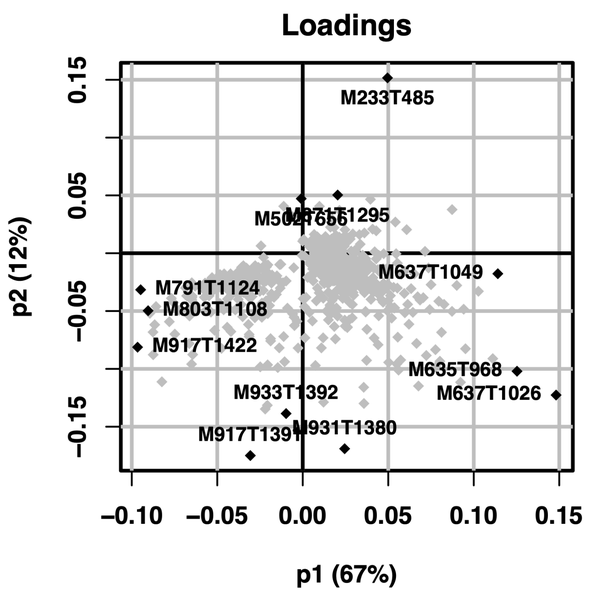
KAMPO OINTMENTS • DIVERSITY OF INGREDIENTS AND EXTRACTION TECHNIQUES

## Results

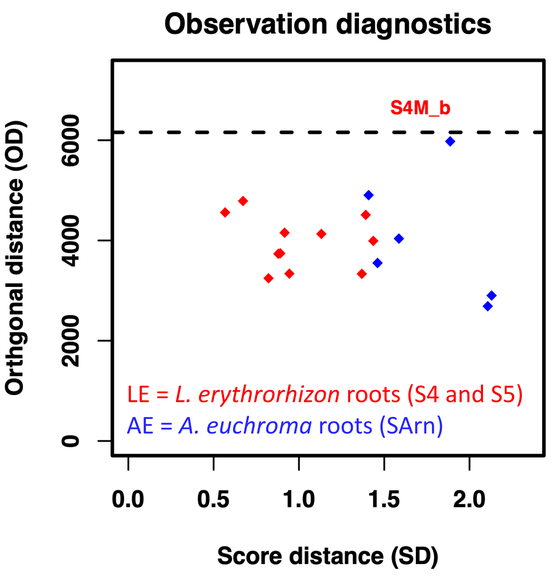
### Metabolomics

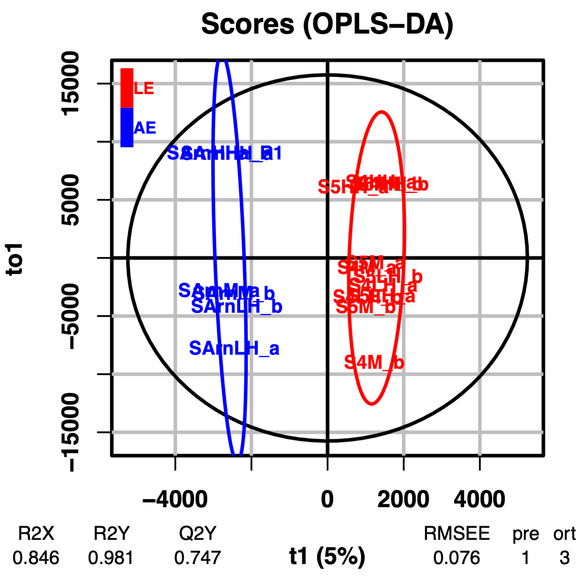
- **Multivariate analyses** using **PCA** and **OPLSDA** show separations into distinct groups according to **loadings (ions)** dispersion:
  - ACP represents the **extraction temperature as main discriminant** parameter. **HH** extraction type is highly different from **M** and **LH**
  - OPLSDA spotlight differences among **similar crude drugs** e.g. ***L. erythrorhizon*** and ***A. euchroma*** root might be switched depending of traditional uses
- Multivariate analyses enable to describe the **geographical origins** or **species effects** as **biotic/abiotic factors**
- After correlation with the biology, **univariate analyses** on specific **metabolites** allow identification of compounds involved in wound healing




**EFFECT OF HERBS EXTRACTION TEMPERATURE ON METABOLITES**
Principal component analysis (PCA) score plots showing extraction process separation of oily samples analyzed by LC/MS. Corresponding loadings and R2X are shown – made with W4M(2,3)




**EFFECT OF BOTANICAL SPECIES ON METABOLITES**
Orthogonal Partial Least Square Discriminant Analysis (OPLSDA) score plots showing oily samples analyzed by LC/MS separation depending of species
*A. euchroma* (SArn) and *L. erythrorhizon* (S4 = Chinese and S5 = Japanese origin)
Score distance, R2X and other parameters are shown – made with W4M(2,3)

### Scratch assay

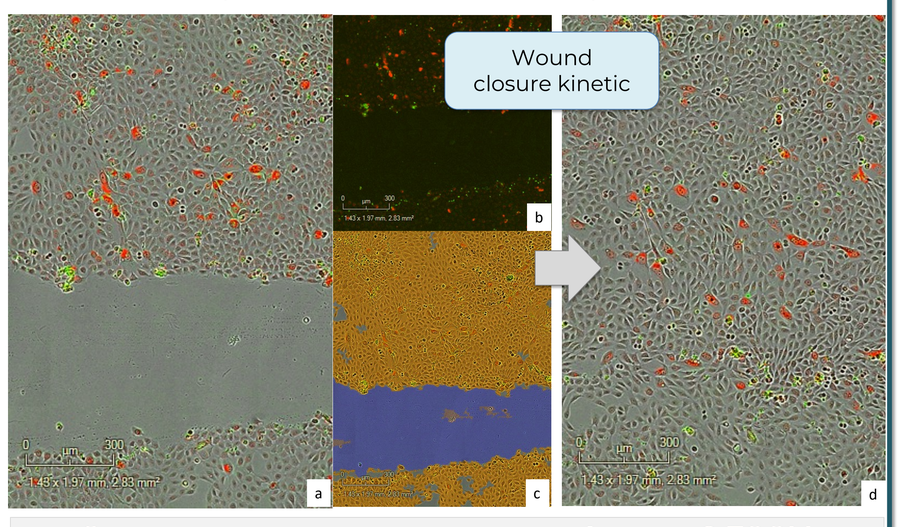
- Different **wound closure times** for Kampo herbs oils extracts compared to oil control

**(+) effects**

- *Angelica acutiloba + A. dahurica*
- *Scrophularia ningpoensis*
- *Rehmannia glutinosa*
- *P. lactiflora* roots

**(-) effects**

- High Heating extracts (HH)
- *Curcuma longa* rhizoma




**SCRATCH ASSAY ON KERATINOCYTES USING INCUCYTE® ZOOM**
Before: a=composite, b=fluorescent and c=with wound mask
After: d= wound closure after *S. ningpoensis* extract exposure

## Conclusion

Both the metabolomics and scratch assays carried on oily extracts from Kampo ointments ***Shiunkō, Chuōkō*** and ***Shinsen taitsukō*** suggest a **great diversity** of metabolites and **wound healing effects.**

This research into the **rationalization** of traditional uses of Kampo topical remedies allows a better understanding of these treatments which could offer different **therapeutic solutions** to patients suffering from **chronic wounds or severe burns.**

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