Kampo traditional ointments for wound healing
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Introduction

Japanese herbal medicine, named Kampo, covers numerous therapeutic indications including dermatological ones. Three ointments are used for skin wound healing: Shinkō, Chuōkō and Shinzen taisetsukō. All have in common sesame oil in which crude drugs are extracted. Herbs are representatives of the botanical genera Angelica, Lithospermium, Curcuma, Phellodendron, Paeonia, Rheum, Rehmannia, Scrophularia and Cinnamomum3.

This aim of the study on Kampo ointments is to better understand the chemical diversity of oily herb 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 – WorkflowforMetabolomics5,6.

Materials & methods

- **Required elements**
  - Inventory the Kampo therapeutic practices
  - Information from Japanese pharmacopoeia7
  - Use different extraction methods: Maceration, 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, organs 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 × 30 mm, 3.5 μm) Zorbax®
  - Oils solubilized using isopropanol (IPA) Samples = 1% oil by weight in IPA
  - Analysis with Galaxy platform, W4M5,6 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 proliferation8
  - Immortalized keratinocytes (N-tet-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

Kampo OINTMENTS FOR WOUND HEALING

- **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

Results

- **Observation diagnostics**
  - LE = L. erythrorhizon roots (54 and 55)
  - AE = A. euchroma roots (56 and 57)

- **Scores (OPLS-DA)**
  - Score distance, R2X and other parameters are shown – made with W4M5,6

- **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 (56m) and L. erythrorhizon (54m + Chinos and SS: Japanese origin)
  - Score distance, R2X and other parameters are shown – made with W4M5,6

Conclusion

Both the metabolomics and scratch assays carried on oily extracts from Kampo ointments Shinkō, Chuōkō and Shinzen taisetsukō 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.

References

5. The Analytical Platform of the Faculty of Pharmacy (APFP, Université Libre Bruxelles, Belgium).
6. UC, Grenoble Alpes TIMC EESP UMR 5225 and CEA Grenoble BIG BGE Biomiques (France).
7. Yokohama University of Pharmacy, Laboratory Eli Kampo Natural Products Chemistry Japan.