Pharmacognostic specifications of five root species in Ben-Cha-Moon-Yai remedy: Thai traditional medicine remedy

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Abstract
Introduction: Ben-Cha-Moon-Yai remedy is one of the Thai traditional medicines notified in Tumrapaad sard song khor. This remedy has been used as an antipyretic, anti-inflammatory and analgesic drug in Thai traditional medicine practice. Establishment of the pharmacognostic profile of five roots species in Ben-Cha-Moon-Yai remedy will be able to guarantee quality, purity and identification of remedy.

Materials and methods: Five root species in Ben-Cha-Moon-Yai remedy were collected from various sources throughout Thailand; then investigated the pharmacognostic specification of each root species following WHO guideline of quality control method for medicinal plant materials. High performance liquid chromatography coupled with photo diode arrays was applied to analyze their fingerprints.

Results: Macroscopic study was demonstrated as whole plant drawing; while microscopic study was investigated in powdered drugs and its transverse section for anatomy and histology evaluation. The comparative characteristics and physico-chemical parameters of five root species were revealed for standardization. The representative fingerprints revealed a common peak of each root species and Ben-Cha-Moon-Yai remedy under the same HPLC condition.

Conclusion: The results obtained from macroscopic and microscopic inspections, physico-chemical parameters, and developed HPLC fingerprints can be used to standardize five root species in Ben-Cha-Moon-Yai remedy.

1. Introduction

Ben-Cha-Moon-Yai remedy is one of the Thai traditional medicines notified in Tumrapaad sard song khor. The remedy is composed of five roots in an equal part by weight, including the roots of Aegle marmelos (L.) Correa ex Roxb. (Rutaceae), Oroxylum indicum (L.) Kurz (Bignoniaceae), Dimocarpus longan Lour. subsp. longan var. longan (Sapindaceae), Dolichandrone serrulata (DC.) Seem. (Bignoniaceae), and Walsura trichostemon Miq. (Meliaceae). This remedy has been used as an antipyretic, anti-inflammatory and analgesic drug in Thai traditional medicine practice.

Previous study showed that Ben-Cha-Moon-Yai remedy extract demonstrated the antipyretic, anti-inflammatory and anti-nociceptive activities in animal models. Apart from the roots, any other parts of these five plant species revealed some biological activities such as the anti-diabetic and anti-inflammatory activities of A. marmelos, the antioxidant activity of D. longan, the anti-ulcer activity of O. indicum, the anti-microbial activity of W. trichostemon.

According to herbal drug market survey, it was observed that five roots species in Ben-Cha-Moon-Yai remedy could be adulterated with upper ground parts of plants or other substances, which resulted in degrading the quality of the remedy. Therefore, the standardization of a crude drug is essential to guarantee the quality and stability of herbal preparation. Many factors such as use of fresh plants, temperature, water availability, period and time of collection, method of collecting, drying, total ash, acid insoluble ash, moisture content and part of the plant collected can greatly affect the quality and consequently the therapeutic value of herbal medicines. Moreover,
Fig. 1. Macroscopic and microscopic characteristics of *Aegle marmelos* (L.) Correa ex Roxb (Rutaceae).

The flowering branch of *A. marmelos*  
1. Flower  2. Fruit  3. Transverse section of fruit

Transverse section of *A. marmelos* root;

The root of *A. marmelos*

Powder of *A. marmelos* root;  
to establish an herbal pharmacopoeia, the pharmacognostic parameters and standardization are also the information incorporated.

In this study, the roots of five species in Ben-Cha-Moon-Yai remedy from various sources throughout Thailand were examined according to WHO guideline of quality control method for medicinal plant materials. Establishment of the pharmacognostic profile of five roots species in Ben-Cha-Moon-Yai remedy was provided to guarantee quality, purity and identification of remedy.

Fig. 2. Macroscopic and microscopic characteristics of Dimocarpus longan Lour. subsp. longan var. longan (Sapindaceae).
2. Materials and methods

2.1. Plant materials

The roots of *A. marmelos*, *D. longan*, *D. serrulata*, *O. indicum*, and *W. trichostemon* were collected from 15 different geographical areas in Thailand. All set of crude drugs were authenticated by Ruangrungsi N. and compared with the herbarium at Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. The roots were shade-dried and grinded to coarse powders. Each root powder was sequentially macerated with ethanol then water until exhaustion. The extracts were filtered, the ethanol extract was evaporated under vacuum whereas the water extract was lyophilized to dryness. The remedy extract was prepared by mixing each extract in the quantity equivalent to the formula. The extract yields were weighted, recorded and stored at -20 °C until use to decrease the possibility of degradation of active compounds.

2.2. Morphological identification

The macroscopic evaluation of medicinal plant materials was illustrated based on the shape, size, colour, surface characteristics,
texture, fracture and appearance of the cut surface. The microscopic identification was performed under microscope to identify the structural features, cells, and ergastic substances of plant samples with application of the knowledge of plant morphology and anatomy so as to authenticate plant species.\textsuperscript{10} Safranin was prepared as a staining solution. The drawing was made using microscope and drawing attachment.

2.3. Physio-chemical identification

The loss on drying, total ash, acid insoluble ash, water content and extractive values parameters were performed to evaluate the pharmacognostic specifications of the five root species in Ben-Cha-Moon-Yai remedy followed by WHO guideline for Quality control methods for medicinal plant materials.\textsuperscript{11} Three grams of powdered...
drug was prepared in a crucible, then dried at 105 °C until the constant weight was obtained to determine loss of moisture and volatile oil matters. Whereas total ash was conducted by burning 3 g of powdered drug at 500 °C for 6 h to observe the carbonless ash which was further weighted, before 25 ml of 2N HCl was added into the remaining ash and gently boiled. It was filtrated and burned at 500 °C for 6 h then measured the amount of silica presented, especially as sand and siliceous earth to obtain the percent of acid insoluble ash. Azeotropic distillation method using water saturated toluene was performed to measure the water presented in the plant materials. The volume of water completely distilled was read off and calculated as a percent of water content. The amounts of

Fig. 5. Macroscopic and microscopic characteristics of Walisara trichostemon Miq. (Meliaceae).

extractable active constituents in ethanol and water were determined. Five grams of powdered crude drugs were macerated in 100 ml of solvent under shaking for 6 h and standing for 18 h before filtration. The filtrate was adjusted to 100 ml, an aliquot (20 ml) was pipetted and evaporated to dryness. The extract was dried in oven at 105 °C until the constant weight was obtained.

2.4. High performance liquid chromatographic analysis

Ten milligrams of the ethanolic extract from each root species and Ben-Cha-Moon-Yai remedy extracts were dissolved in 1 ml of HPLC grade methanol then filtered through a 0.45 µm membrane filter. HPLC-PDA analysis was performed with a SHIMADZU gradient system (Japan) equipped with LC-20AD pumps, a CTO-20AC column oven, DGU-20A3 degasser and a SPD-M20A diode array detector set λ ranged 190–800 nm. Separation was carried out with an Inertsil® ODS-3, C-18 column (particle size of the packing 5 µm, 4.6 × 250 mm) and HPLC guard column (5 µm, 4.0 × 10 mm). The mobile phase was performed by 10 mM phosphoric acid–acetonitrile linear gradient (95:5) over 65 min at flow rate of 0.8 ml/min. The injection volume was 10 µl.

3. Results

3.1. Morphological identification

Macroscopic and microscopic specifications were illustrated in Figs. 1–5. Cytological and histological characterization showed a valuable tool for the identification of each ingredient in Ben-Cha-Moon-Yai remedy. In addition, the comparative macroscopic characters of five species in Ben-Cha-Moon-Yai remedy were presented in Table 1.

3.2. Physio-chemical identification

Table 2 demonstrated the grand average and pooled standard deviation of the quality parameters of each species in Ben-Cha-Moon-Yai remedy.

### Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Deciduous tree to 13 m tall</th>
<th>Deciduous tree to 25 m tall with narrow cylindrical crown</th>
<th>Deciduous tree, 13 m tall</th>
<th>Botany evergreen tree to 15 m tall</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. marmelos</em></td>
<td>An evergreen tree to 30 m tall</td>
<td>Flowers are white, opening at night in small unbranched clusters of 3–7 flowers at end of twigs, 2–3 cm.</td>
<td>Flower is 2–12 cm long, white, opening at night in short unbranched clusters of 3–7 flowers at end of twigs, 2–3 cm.</td>
<td>Flower is mostly white or yellow, regular, bisexual, with branches at upper leaf axils, 4–5 straight, spreading petals, stamens longer than petals, style short, disc ring-like.</td>
</tr>
<tr>
<td><em>D. longan</em></td>
<td>Deciduous tree to 25 m tall with narrow cylindrical crown</td>
<td>Deciduous tree, 13 m tall</td>
<td>Deciduous tree to 25 m tall with narrow cylindrical crown</td>
<td>Flower is 2–12 cm long, white, opening at night in short unbranched clusters of 3–7 flowers at end of twigs, 2–3 cm.</td>
</tr>
<tr>
<td><em>D. serrulata</em></td>
<td>Deciduous tree, 13 m tall</td>
<td>Deciduous tree, 13 m tall</td>
<td>Deciduous tree, 13 m tall</td>
<td>Flower is 2–12 cm long, white, opening at night in short unbranched clusters of 3–7 flowers at end of twigs, 2–3 cm.</td>
</tr>
<tr>
<td><em>O. indicum</em></td>
<td>Botany evergreen tree to 15 m tall</td>
<td>Flower is 2–12 cm long, white, opening at night in short unbranched clusters of 3–7 flowers at end of twigs, 2–3 cm.</td>
<td>Flower is 2–12 cm long, white, opening at night in short unbranched clusters of 3–7 flowers at end of twigs, 2–3 cm.</td>
<td>Flower is mostly white or yellow, regular, bisexual, with branches at upper leaf axils, 4–5 straight, spreading petals, stamens longer than petals, style short, disc ring-like.</td>
</tr>
<tr>
<td><em>W. trichocodon</em></td>
<td>Flower is mostly white or yellow, regular, bisexual, with branches at upper leaf axils, 4–5 straight, spreading petals, stamens longer than petals, style short, disc ring-like.</td>
<td>Flower is mostly white or yellow, regular, bisexual, with branches at upper leaf axils, 4–5 straight, spreading petals, stamens longer than petals, style short, disc ring-like.</td>
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</tr>
</tbody>
</table>

3.3. High performance liquid chromatographic analysis

To develop the representative fingerprint, the ethanolic extract of five root species and Ben-Cha-Moon-Yai remedy were analyzed under the same HPLC condition. In order to obtain the optimal elution conditions for the separation and determination of the constituents, various linear gradients of 10 mM phosphoric acid and acetonitrile at a flow rate of 0.8 ml/min were investigated. The chromatogram revealed a common peak of each root extract and Ben-Cha-Moon-Yai remedy within the retention time of 60 min as shown in Fig. 6.

4. Discussion and conclusion

The quality control methods play an important role in traditional medicine which conserve as a tool for identification, authentication and quality control of herbal drug.10 WHO has published the “Quality control methods for medicinal plant materials” which describes a recommended test procedure to evaluate the identity, purity and quality of the plant materials. These standardization parameters are essential to publish in the pharmacopeia. The majority of the information can be obtained from its macroscopy, microscopy, physio-chemical parameters and chemical fingerprint of medicinal plant materials.

Macroscopic and microscopic methods are the simplest and cheapest methods to establish the correct identity of the plant materials.11 The macroscopic of root indicated that its shape, size, colour, surface characteristics, texture, fracture and appearance of the cut surface. Transverse section was prepared with free-hand section of each root and stained with safranine to confirm its identifications. Microscopy of the powder was also carried out and the specific diagnostic characteristics were recorded. This examination gives a clear idea about the specific histological characteristics of crude drugs, besides the macro-morphological and cytomorphological characters. While these diagnostic features enable the analyst to know the nature and characteristics of the crude drugs, further evaluation of numerical parameters indicate their acceptability by criteria other than the morphological characteristics.10
The physico-chemical parameters are mainly used in judging the purity and quality of the drug. The procedures normally adopted to get the qualitative information about the purity and standard of a crude drug including the determination of various parameters. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drug for marketing. The residue remaining after incineration of plant material indicates presence of various impurities includes both “physiologic ash” which is derived from the plant tissue itself, and “non-physiologic ash” which is the residue of the extraneous matter adhering to the plant surface. Acid insoluble ash is frequently necessary to evaluate the crude drugs, which indicates the residue obtained after treating the total ash with diluted HCL, then weighing the residue. This ash value indicates contamination with siliceous material or acid insoluble matter e.g. earth and sand. These ash values are important quantitative standards which constitutes the inorganic matter after incineration of that particular herbal ingredient, specifications have been set up to limit them.

Moisture is also an inevitable component of crude drugs which should be eliminated as far as practicable. Excess moisture can result in the breakdown of important constituents by enzymatic activity and may encourage the growth of yeast and fungi during storage. Direct measurement of water content and total measurement of water as well as volatile matters in terms of loss on drying is to be tested. Extractive values give an idea about the chemical constituents of crude drug soluble in a particular solvent which yields a solution containing different phyto-constituents.

This study proposed the upper limits for unwanted properties of Ben-Cha-Moon-Yai remedy crude drugs, such as loss on drying, total ash, acid insoluble ash and water contents, together with the lower limits for extractable matters such as the ethanol and water extractive values as shown in Table 2.

The fingerprinting analysis is nowadays getting momentum for the quality control of multi-component herbal medicines and has been widely accepted as a useful tool to determine authenticity and reliability of chemical constituents of herbal drug and formulations. A combination of high performance liquid chromatography and online UV spectrum detection via diode array configuration also adds a value to conventional botanical methods used in the quality assurance of crude drugs and compound preparations. The selection of the HPLC conditions was guided by the requirement for obtaining chromatograms with better resolution of adjacent peaks within short time, especially when large amount of samples were analyzed. In this study, 3D-HPLC profiles of five root species and Ben-Cha-Moon-Yai remedy were established as their characteristic fingerprint and employed to assess their consistency and difference.

However, different herbal materials are traditionally used as the same herbal medicine. The quality is different not only between materials of different species used as the same herbal medicine, but also between materials of the same species growing from different areas. The chemical composition of the same herbal material collected at different times or with different processing methods is different, so the production should be strictly specified in order to control the product quality and minimize variations between different product batches. The results obtained from macroscopic and microscopic inspections, physico-chemical parameters, and development of 3D-HPLC fingerprint can be used to standardize all five root species in Ben-Cha-Moon-Yai remedy.

### Conflict of interest

All authors have none to declare.
Fig. 6. The 3D-HPLC profiles of each species in Ben-Cha-Moon-Yai remedy.
Acknowledgements

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Analysis condition

**Column:** Inertsil® ODS-3, C-18 column (particle size of the packing 5 μm, 4.6 × 250 mm)

**Mobile phase:** 10 mM Phosphoric acid–Acetonitrile

**Linear gradient:** (95:5, 65 min)

**Flow rate:** 0.8 mL/min

**Injection volume:** 10 μL

**Temperature:** 40 °C

**Wavelength:** 190–400 nm.

References