A Bio-activity Guided in Vitro Pharmacokinetic Method to Improve the Quality Control of Chinese Medicines

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Background

• The current method of quality control for Chinese medicines (CM) involve stability testing using any designated marker(s) present in the product.

• The shortcoming of the current approach is that these marker(s) are chosen entirely arbitrarily which may have no relevance to the product activity inherent in the herbs.

• Without good QC, the efficacy and safety of TCM products are difficult to establish and world acceptance of TCM is unlikely.

There is a need for an improved method that can provide quality advancement of CM.
Objectives

• To demonstrate the feasibility of a bio-activity guided in-vitro pharmacokinetic method (BAPK) for quality control of both single herb or complex formulae products
  – Si-Wu-Tang (SWT) product

Part I: Bioavailability

Part II: Bioactivity
Part I: Bioactivity Guided Pharmacokinetics (BAPK) Approach

Sample to be investigated (e.g. SWT product)

↓

Dissolution

↓

Gastrointestinal (GI) metabolism

↓

GI absorption

↓

Identification of relevant bioactive marker(s)

→

Stability analysis of the identified markers
Part I: Study Design and Procedures

I. Literature search (data mining) to identify active components in SWT.

II. HPLC-DAD and HPLC-MS/MS assay method development for simultaneous determination of these markers.

III. Preliminary test of raw herbs in accordance with Chinese Pharmacopeia (CP) 2005.

IV. Manufacture of CU-SWT and CU-Si Wu He Ji in accordance with CP 2005 to serve as a “reference” product.

V. Determination of contents of CU-SWT and marketed products.

VI. Identification and comparison of relevant markers via bio-activity guided pharmacokinetics approach.

VII. Stability evaluation of the identified relevant markers in CU-SWT and CU-Si Wu He Ji.

VIII. Establish the most stable marker(s) for SWT product.
### Part I Results: Data Mining

<table>
<thead>
<tr>
<th>Herb</th>
<th>Major active components identified from data mining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angelicae</td>
<td>Ferulic acid</td>
</tr>
<tr>
<td></td>
<td>Ligustilide</td>
</tr>
<tr>
<td>Chuanxiong</td>
<td>Ligustrazine</td>
</tr>
<tr>
<td></td>
<td>Ligustilide</td>
</tr>
<tr>
<td></td>
<td>Ferulic acid</td>
</tr>
<tr>
<td>Butylphthalide</td>
<td>Senkyunolide A</td>
</tr>
<tr>
<td>Paeoniae</td>
<td>Paeoniflorin</td>
</tr>
<tr>
<td></td>
<td>Paeonol</td>
</tr>
<tr>
<td>Rehmanniae</td>
<td>Catalpol</td>
</tr>
<tr>
<td></td>
<td>Gallic acid</td>
</tr>
</tbody>
</table>
Part I Results
Development of HPLC-DAD Assay and Method Validation

HPLC-DAD method development

HPLC-DAD system: Waters 2695 Separation Module
Waters 996 Photodiode Array Detector and Autosampler

LC column: Thermo ODS Hypersil, 4.6 x 250 mm, 5 um, with Waters Delta-Pak C18 guard column, 4.6 x 0.5 mm, 5 um

Mobile phase: 0.04% v/v phosphoric acid and 0.04% v/v diethylamine in water : ACN, gradient elution

Flow rate: 1 mL/min

Temperature of LC column: ambient
Detection: 210 to 400 nm
Quantification: internal standard method
Part I Results

HPLC-DAD chromatograms of the nine analytes of interests

Remark: Gallic acid (GA), senkyunolide A (SA), paeoniflorin (PF), ferulic acid (FA), Z-ligustilide (Lig), butylphthalide (Bu), ligustrazine (TMP) and paeonol (PO)
Part I Results: CP Tests of Raw Herbs

(a) *Radix Paeoniae Alba*

1. Test herb (source 1)
2. Test herb (source 2)
3. Reference herb
4. Paeoniflorin standard

(b) *Radix Rehmanniae Preparata*

1. Test herb
2. Reference herb
3. Catalpol standard

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Part I Results: CP Tests of Raw Herbs

(c) *Radix Angelicae Sinensis*

1. Test herb (root)  
2. Test herb (body)  
3. Test herb (head)  
4. Reference herb

(d) *Rhizoma Chuanxiong*

1. Reference herb  
2. Test herb
**Part I Results:**

**CP Tests of Raw Herbs**

**Content of raw herbs:**

<table>
<thead>
<tr>
<th>Herbs</th>
<th>Batch</th>
<th>FA</th>
<th>Lig</th>
<th>SA</th>
<th>Bu</th>
<th>TMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angelicae</td>
<td>#1</td>
<td>0.058</td>
<td>1.26</td>
<td>0.031</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>0.069</td>
<td>1.82</td>
<td>0.027</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>0.051</td>
<td>0.16</td>
<td>0.027</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#4</td>
<td>0.046</td>
<td>0.69</td>
<td>0.035</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#5</td>
<td>0.036</td>
<td>0.70</td>
<td>0.023</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Chuanxiong</td>
<td>#1</td>
<td>0.130</td>
<td>2.41</td>
<td>0.350</td>
<td>0.063</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>0.025</td>
<td>1.68</td>
<td>0.270</td>
<td>0.049</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>0.081</td>
<td>2.83</td>
<td>0.270</td>
<td>0.028</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#4</td>
<td>0.110</td>
<td>2.60</td>
<td>0.350</td>
<td>0.048</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#5</td>
<td>0.110</td>
<td>1.08</td>
<td>0.300</td>
<td>0.033</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Remark: N.A. = not applicable, N.D. = not detectable

- variation among batches of raw herbs was observed
- batch #4 and #5 of Angelicae do not comply with 0.05% ferulic acid (FA) content as stated in CP (2005)
- Ligustrazine (TMP) was not detectable in Chuanxiong

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### Part I Results: CP Tests of Raw Herbs

#### Content of raw herbs: continued

<table>
<thead>
<tr>
<th>Herbs</th>
<th>Batch</th>
<th>GA</th>
<th>PF</th>
<th>Cat</th>
<th>PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paeoniae</td>
<td>#1</td>
<td>0.120</td>
<td>2.06</td>
<td>N.A.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>0.076</td>
<td>2.11</td>
<td>N.A.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>0.065</td>
<td>0.53</td>
<td>N.A.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#4</td>
<td>0.088</td>
<td>2.36</td>
<td>N.A.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>#5</td>
<td>0.034</td>
<td>1.25</td>
<td>N.A.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Rehmanniae</td>
<td>#1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.004</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.005</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#4</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.004</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>#5</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.001</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

Remark: N.A. = not applicable, N.D. = not detectable

- batch #3 and #5 of Paeoniae do not comply with 1.6% paeoniflorin (PF) content as stated in CP (2005)
- very low content of catalpol (Cat) in Rehmanniae; paeonol (PO) cannot be detectable in Paeoniae
Part I Results – Manufacture CU-Si Wu He Ji

The method was based on the protocol described in CP (2005)

- **A**: Radix Angelicae Sinensis 250 g
- **C**: Rhizoma Chuanxiong 250 g
- **P**: Radix Paeoniae Alba 250 g
- **R**: Radix Rehmanniae Praeparata 250 g

**A+C**
- Soaking in water (RT) 0.5 h

**P + R**
- Steam distillation

**Water Phase**
- Residue
  - Soaking in water (RT) 1 h
  - Filtration 1 h
  - Filtration 1.5 h
  - Filtration 1.5 h

**A+C**
- Concentrated to small volume
- Add ethanol to 55% (v/v)
- Stood for 24 h

**A+C**
- Ethanol phase
- Adjust density to 1.26~1.30 (55~60°C)

**A+C**
- Ethanol phase
- Filtration

**CU-Si Wu He Ji**

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Part I Results – Manufacture CU-SWT

The method was modified from CP (2005)

A: Radix Angelicae Sinensis 250 g
C: Rhizoma Chuanxiong 250 g
P: Radix Paeoniae Alba 250 g
R: Radix Rehmanniae Praeparata 250 g

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Products Manufactured

CU-SWT (batch 1 to 3)
CU-Si Wu He Ji (batch 1)
CU-Chuanxiong (batch 1 to 3)
CU-Angelicae (batch 1 to 3)
CU-Rehmanniae (batch 1 to 3)
CU-Paeoniae (batch 1 to 3)
Part I Results – Contents of SWT

(a) SWT product (solid dosage form)

<table>
<thead>
<tr>
<th>Product</th>
<th>GA</th>
<th>SA</th>
<th>PF</th>
<th>FA</th>
<th>Lig</th>
</tr>
</thead>
<tbody>
<tr>
<td>CU-SWT batch 1</td>
<td>0.987 ± 0.089</td>
<td>0.075 ± 0.002</td>
<td>8.651 ± 0.240</td>
<td>0.525 ± 0.015</td>
<td>1.127 ± 0.224</td>
</tr>
<tr>
<td>CU-SWT batch 2</td>
<td>0.774 ± 0.030</td>
<td>0.049 ± 0.001</td>
<td>6.353 ± 0.057</td>
<td>0.440 ± 0.044</td>
<td>0.473 ± 0.012</td>
</tr>
<tr>
<td>CU-SWT batch 3</td>
<td>0.781 ± 0.101</td>
<td>0.053 ± 0.001</td>
<td>6.362 ± 0.198</td>
<td>0.367 ± 0.015</td>
<td>0.342 ± 0.024</td>
</tr>
<tr>
<td>market-1</td>
<td>0.131 ± 0.009</td>
<td>0.044 ± 0.002</td>
<td>0.054 ± 0.004</td>
<td>0.020 ± 0.002</td>
<td>0.037 ± 0.002</td>
</tr>
<tr>
<td>market-2</td>
<td>0.506 ± 0.070</td>
<td>0.059 ± 0.001</td>
<td>1.930 ± 0.143</td>
<td>0.154 ± 0.041</td>
<td>0.034 ± 0.003</td>
</tr>
</tbody>
</table>

Remark: 1. Gallic acid (GA), senkyunolide A (SA), paeoniflorin (PF), ferulic acid (FA) and Z-ligustilide (Lig).
2. Butylphthalide (Bu), ligustrazine (TMP), paeonol (PO) and catalpol (Cat) are not detectable.
### Part I Results – Contents of SWT

#### (b) Si Wu He Ji (liquid dosage form)

<table>
<thead>
<tr>
<th>Product</th>
<th>Concentration (µg/mL) ± SD (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GA</td>
</tr>
<tr>
<td>CU-SWHJ</td>
<td>307.9 ± 13.9</td>
</tr>
<tr>
<td>market-3</td>
<td>32.7 ± 1.5</td>
</tr>
</tbody>
</table>

**Remark:**
1. Gallic acid (GA), senkyunolide A (SA), paeoniflorin (PF), ferulic acid (FA) and Z-ligustilide (Lig) and butylphthalide (Bu).
2. Ligustrazine (TMP), paeonol (PO) and catalpol (Cat) are not detectable (N.D.).
CU SWT products vs. Commercial SWT

- Among 9 compounds investigated, ferulic acid, gallic acid, paeoniflorin, senkyunolide A and Z-ligustilide are present in all marketed products and CU-SWT & CU-Si Wu He Ji.

- The contents of these components are generally higher in CU SWT products than commercial products (in particular the volatile component Z-ligustilide).

- **Large variations** in contents of each component in marketed products.
Part I: Bioactivity Guided Pharmacokinetics (BAPK) Approach

Sample to be investigated (e.g. SWT product)

↓

Dissolution

↓

Gastrointestinal (GI) metabolism

↓

GI absorption

↓

Identification of relevant bioactive marker(s)  Stability analysis of the identified markers
Part I Results – Dissolution Study

Good dissolution of ferulic acid, paeoniflorin, senkyunolide A, gallic acid and Z-ligustilide in SWT products in the dissolution medium (pH 2) within 30 min.
Part I: Bioactivity Guided Pharmacokinetics (BAPK) Approach

Sample to be investigated (e.g. SWT product)

Dissolution

Gastrointestinal (GI) metabolism

GI absorption

Identification of relevant bioactive marker(s)

Stability analysis of the identified markers
## Part I Results – Metabolism Study

**GI metabolism models:**
- Rat intestine homogenate
- Human intestine homogenate
- Caco-2 cell lysate

**Incubated with markers and SWT products**

Detect metabolites by LC/MS/MS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Possible metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paeoniflorin</td>
<td>Hydrolysis</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>Sulfation, Glucuronation, Methylation</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>Sulfation, Glucuronation, Methylation</td>
</tr>
</tbody>
</table>
Part I:
Bioactivity Guided Pharmacokinetics (BAPK) Approach

Sample to be investigated (e.g. SWT product)

Dissolution

Gastrointestinal (GI) metabolism

GI absorption

Identification of relevant bioactive marker(s)

Stability analysis of the identified markers
Part I Results – Permeability (GI Absorption)

Ferulic acid

Cumulative amount (ug) vs Time (min)

Loading Conc: 19.71 µg/mL
Papp: $1.22 \times 10^{-5}$ cm/s

Z-Ligustilide

Cumulative amount (ug) vs Time (min)

Loading Conc: 62.38 µg/mL
Papp: $1.54 \times 10^{-5}$ cm/s

Senkyunolide A

Cumulative amount (ug) vs Time (min)

Loading Conc: 16.11 µg/mL
Papp: $2.81 \times 10^{-5}$ cm/s

- **Good permeability for** ferulic acid, Z-ligustilide, senkyunolide A
- **Poor permeability for** paeoniflorin and gallic acid
Part I: Bioactivity Guided Pharmacokinetics (BAPK) Approach

Sample to be investigated (e.g. SWT product)

Dissolution

Gastrointestinal (GI) metabolism

GI absorption

Identification of relevant bioactive marker(s)

Stability analysis of the identified markers
Part I Results: – Stability Study

Stability of CU-SWT in various storage conditions

• Temperature at 4, 25 and 40 °C
• Storage period from 0, 1, 2 and 3 months
• Product tested: CU-SWT powder and CU-Si Wu He Ji, both were manufactured by us according to CP 2005
Stability results of CU-SWT (solid dosage form)

Senkyunolide A, ferulic acid and Z-ligustilide in CU-SWT (solid dosage form) are relatively stable over 3-month period at 4, 25 and 40 °C storage temperatures.
Part I Results: – Stability Study

Stability results of CU-Si Wu He Ji (liquid dosage form)

Degradation of senkyunolide A, ferulic acid and Z-ligustilide was observed in CU-SWHJ stored at high temperature.
Part I: Overall Summary in Selection of Relevant Bioactive Markers

Three compounds, i.e. ferulic acid, senkyunolide A and Z-ligustilide, could serve as a more relevant bioactive markers for QC of SWT due to their good dissolution, permeability and stability characteristics.

Paeoniflorin, a marker compound designated by the Chinese Pharmacopeia, is poorly permeable and thus not suitable to be considered as a “relevant bioactive” marker.
Are we done yet?

• **Limitation with these markers**: lack of ability to represent the overall activity of a given SWT product.

• Recently, a landmark study was published in Science showing that differentially expressed genes (DEGs) identified via microarray processing and analysis can serve as signature for wide various of chemical components [1].

• We hypothesize that such DEGs can serve as unique composite fingerprint for a given CM product which could be useful for quality control and identity of such product.

Part II: Objective

• To investigate the applicability of the different expression genes (DEGs) derived from special microarray processing and analysis in serving as unique fingerprint for a given SWT product and its four single herbs.
Part II: Project scheme

- SWT product/herbs samples
  - LC/MS/MS & MTT

- Donor chamber
  - Receiver chamber
  - Caco-2

- Absorbed fraction

- Desalting and concentrate

- MCF7

- Extract RNA

- Microarray processing
  - DEGs

- Multi-gene RT-PCR panel
  - Verification

- Real-time PCR

- Specificity

- Consistency
Experimental design of sample treatment for microarray processing / real-time PCR

Methanol (Control)
1% →
1:2 1:8 1:32

CU-SWT permeates (S)
CU-ChuanXiong permeates (C)
CU-Angelicae permeates (A)
CU-Paeoniae permeates (P)
CU-Rehmanniae permeates (R)

J-SWT permeates (J)

1:2 1:8 1:32

Standard mixture (FA,Lig and SA) permeates (M)
1:2 1:8

1:2 1:8

1:2 1:8

1:2 1:8

1:2 1:8

6hrs

Total RNA isolation

Microarray/Real time PCR
The Principal Components Analysis (PCA) and Venn Diagram of the differentially expressed genes for microarray data of CU-SWT permeates treatment
Identification of differentially expressed genes (DEGs), construction of multi-gene RT-PCR panel and method validation.

**Step I: DEGs selection criteria:**

1. Fold change (FC) greater than a pre-defined threshold (e.g. FC > 1.5 or FC < -1.5);
2. The $p < 0.01$ based on unpaired sample t-test;
3. The expression fold change in a dose-dependent manner;
4. The gene selected was significantly consistent with the drug’s therapeutic use;
5. The expression of genes selected was consistent in three different batches of microarray work.

**Step II: Constructed the multi-gene RT-PCR panel for each treatment group using the DEGs identified.**

**Step III: Verified the expression fold changes of DEGs using real-time RT-PCR.**
# Part II Results

The differentially expressed genes (DEGs) for treatment groups

<table>
<thead>
<tr>
<th>CU-SWT</th>
<th>J-SWT</th>
<th>Standard mixture</th>
<th>CU-Chuanxion</th>
<th>CU-Angelica</th>
<th>CU-Paeonia</th>
<th>CU-Rehmannia</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC7A11</td>
<td>SLC7A11</td>
<td>SLC7A11</td>
<td>SLC7A11</td>
<td>SLC7A11</td>
<td>TRIM65</td>
<td>SLC7A11</td>
</tr>
<tr>
<td>PDK4</td>
<td>CYP1A1</td>
<td>CYP1A1</td>
<td>ALDH1A3</td>
<td>CYP1A1</td>
<td>CYP2B6</td>
<td>CYP1A1</td>
</tr>
<tr>
<td>ST3GAL1</td>
<td>AKR1C1/A</td>
<td>HMOX1</td>
<td>INPP4B</td>
<td>ALDH1A3</td>
<td>OR2H1</td>
<td>HMOX1</td>
</tr>
<tr>
<td>TNFRSF21</td>
<td>SLC7A5</td>
<td>PDK4</td>
<td>MSMB</td>
<td>INPP4B</td>
<td>NAP1L5</td>
<td>CXCR7</td>
</tr>
<tr>
<td>THBS1</td>
<td>GCLM</td>
<td>ALDH1A3</td>
<td>NEDD9</td>
<td>LRP8</td>
<td>TMCO6</td>
<td>ALDH1A3</td>
</tr>
<tr>
<td>PIGW</td>
<td>TXNRD1</td>
<td>PHLDB2</td>
<td>CCNK</td>
<td>TNFSF10</td>
<td>LCE1E</td>
<td>GCLM</td>
</tr>
<tr>
<td>GPER</td>
<td>CDH18</td>
<td>PDE4DIP</td>
<td>HECTD1</td>
<td>SAMD9</td>
<td>OSBPL8</td>
<td>S100A7</td>
</tr>
<tr>
<td>PCDH10</td>
<td>CCL28</td>
<td>MAP3K1</td>
<td>OSBPL8</td>
<td>MBNL2</td>
<td>RBAK</td>
<td>ATRX</td>
</tr>
<tr>
<td>TNFSF10</td>
<td>METTL7A</td>
<td>ZC3H11A</td>
<td>LARS</td>
<td>SAMHD1</td>
<td>ESCO1</td>
<td>CP</td>
</tr>
</tbody>
</table>

Note: Red color: up-regulated genes; Black color: down-regulated genes
Part II Results

Specificity test

Test Products (total 21 different products):

- **Two batches of CU-SWTs** (CU-SWT batch1 and CU-SWT batch2)
- **Three marketed SWTs** (J-SWT, SWT_NBF, SWTHJ)
- **Four single herbal extracts** (CU-Ang, CU-ChuanX, CU-Pae,CU-Reh)
- **Single chemical standards** (ferulic acid, ligustilide, senkyunolide A) and their mixture
- **Eight independent products** either marketed products (Named M1~M8).

Treat MCF-7 cells and extract RNA

Two sets of multi-gene RT-PCR panels for CU-SWT and J-SWT were tested against 21 different products by real-time PCR

Consistency evaluation

- Test Products : Eight -replicate extracts of CU-SWT on 3 separate days
- Treat MCF-7 cells and extract RNA
- The expression fold changes of the CU-SWT DEGs in these products were test using real-time PCR.
Part II Results:

Data analysis of **intra-day and inter-day consistency** by CU-SWT DEGs panel for CU-SWT

<table>
<thead>
<tr>
<th>CU-SWT DEGs</th>
<th>Coefficient of Variations (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-day (8 batches)</td>
</tr>
<tr>
<td>SLC7A11</td>
<td>3.3~5.6%</td>
</tr>
<tr>
<td>PDK4</td>
<td>18.6~26.7%</td>
</tr>
<tr>
<td>ST3GAL1</td>
<td>25.0~29.6%</td>
</tr>
<tr>
<td>TNFRSF21</td>
<td>16.3~31.7%</td>
</tr>
<tr>
<td>THBS1</td>
<td>14.9~17.8%</td>
</tr>
<tr>
<td>PIGW</td>
<td>19.8~30.6%</td>
</tr>
<tr>
<td>GPER</td>
<td>14.1~27.3%</td>
</tr>
<tr>
<td>PCDH10</td>
<td>9.6~22.5%</td>
</tr>
<tr>
<td>TNFSF10</td>
<td>12.6~25.9%</td>
</tr>
</tbody>
</table>
Identify DEGs of two SWTs (CU-SWT, J-SWT), its four single herbal extracts (CU-Angelica, CU-Chuanxiong, CU-Paeoniae and CU-Rehmanniae) and standard mixture (ferulic acid, ligustilide and senkyunolide A) using microarray processing and analysis.

A panel of specific genes can be constructed using real-time RT-PCR to represent unique “fingerprint” of SWT and single herb tested.

Convenient and cost-effective manner for “identity test” of the product during its manufacturing and testing of counterfeit products.
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