The research of sustainable utilization on Traditional Chinese medicine resources

China Academy of Chinese Medical Sciences
Huang Luqi
1. Introduction on the pilot work of the national survey on TCM resources
Conduct TCM resources survey and method study; Grasp the resources background information of TCM resources.

31 provinces 922 counties
28 provincial centers
65 county-level monitoring stations
28 breeding bases
2 germplasm resources banks
TCM resources survey

- A large number of data from TCM resources survey has been stored
  - The number of quadrat: 687024 (more than 6 hundred thousand)
  - The number of the medicinal resources varieties: 16048 (the 3rd national survey: 12807 varieties)
  - The species of those have exploitable reserves: 1995 (The country focus the research on 563 varieties)
  - Cultivar: 631 varieties
  - Appropriate techniques for planting: more than 320 techniques
  - Traditional knowledge: 9658 items
  - The number of photos: 4323186 (more than 4300 thousands photos)
  - Specimens: 1 million specimens have been collected, and 130 thousand have been submitted to Beijing

2016. 8
The amount of data for 31 provinces (regions, cities) and pilot counties

Each province submits the survey data successively, the total number of records has been increasing.
The integrity rate of data from 31 provinces (regions, cities) and pilot counties

Based on the verification on the data submitted by each county, the proportion of available data from each province is increased and some decreased.
Regionalization on species richness of TCM resources

The TCM resources varieties were abundant in Yun Nan Province, Hu Nan Province, Si Chuan Province, etc.
The number of TCM resources varieties in each county

The top 10 counties which have the highest number of TCM resources varieties throughout the country:

- Hu Bei Province: Zhu Xi County, Li Chuan County
- Hu Nan Province: Yong Ding County, Sang Zhi County
- Guangxi Zhuang Autonomous Region: Huan Jiang County
- Hai Nan Province: Wan Ning County
- Si Chuan Province: Chong Qing City Feng Du County
- Gui Zhou Province: Wei Ning County
- Yun Nan County: Lu Quan County, Yuan Jiang County
Provide the service to the acceptance check, scientific research and long-term storage for the national survey on TCM resources

- Submitting
- Verification
- Acceptance check
- Data mining and discovery

Herbarium in Da Xing District

Collating on provincial level
Carry out the basic condition construction and appropriate technology research;
Promote the effective supply for the raw materials of Chinese herbal medicine

- **2012-5**
  - 90% completed
  - Got the preliminary service ability

- **2013-11**
  - Under construction according to the planning

- **2015-12**
  - Construction begin

28 bases, breeding & production focusing on seeds & seedlings of 160 varieties of Chinese medicinal materials
Appropriate technology for Chinese medicinal materials production

Techniques and information of seeds & seedlings are needed
Study on dynamic monitoring system construction and the technical methods research;
Grasp the variation trend of the quantity, quality and the price of Chinese medicinal materials

- Monitoring system starts operating
- Information platform operates stably
- Information released regularly
- Start to provide testing and technical guidance services gradually
Joint application of monitoring devices and unmanned aerial vehicle (UAV), in order to monitor *Scutellariae radix*.
Technical specifications for the national survey on TCM resources

7 industry standard drafts have been formed
A new species of *Trichosanthes Linn.* has been discovered. The seed morphology proved that the classification (*Subsect. Hemsleyanae Ku* belongs to *Sect. Involucraria (Ser.) Wight et Arn.*) is correct.
New understanding

*Lycii fructus*: 【flavour】 bitter, cold.
Revision on “Compendium of Materia Medica”

有关《本草纲目》中北艾产地修订

黄璐琦¹，邱功²

(1. 中国中医科学院 中药资源中心 道地药材国家重点实验室培育基地, 北京 100700；
2. 中国中医科学院 中国医史文献研究所, 北京 100700)

【摘要】《本草纲目》载北艾产地为汤阴“伏道”。查汤阴仅有“伏道”一地，是否“伏道”即为“伏道”？作者通过梳理艾叶道地沿革，考证“伏道”地名，同时结合全国中药资源普查资料，得出“伏道”一词首次出现在宋代苏颂《本草图经》，历代沿用，但所指均不甚清楚。至《本草纲目》始出现“汤阴”与“伏道”并提。伏道为扁鹊墓地之一，扁鹊时代存在，沿袭至今，素称变化，汤阴艾因伏道扁鹊墓而得名，可推测李时珍认为“伏道”即为“伏道”，北艾产地应为“汤阴伏道”。

【关键词】 本草纲目；北艾；汤阴；伏道；伏道
2. TCM resources identification
The advantages of molecular identification

Morphological identification
Morphological identification
Microscopic identification
Microscopic identification
Physiochemical identification
Physiochemical identification

破碎 不易识别
原料 来源复杂
化学 分析繁琐 且不全面
检测 周期长
Applied range of molecular identification

Original animals and plants/germplasm resources/seedlings

Crude medicine/prepared drug in pieces

Extractive/Formula-Granule

Compound prescription/Chinese patent medicine
Crude medicine / prepared drug in pieces

- Molecular identification on prepared drug in pieces of snakes

PCR identification for the specificity of *AGKISTRODON* and *Zaocys* is the first molecular identification method recorded in "Chinese pharmacopoeia".
Germplasm resources / seeds and seedlings

- **Lonicerae japonicae flos** germplasm identification

1. 河北红绣球

- **采集日期**: 2013.5.31
- **采集地**: 河北省邢台市巨鹿县姬堤村

**生境**: 平原，土质为黄土质，较干旱，采用地下水源灌溉。

**性状特征**: 株型圆形, 平均株高为 87.5 cm, 至于较细, 为黄色或黄白色; 分枝少, 上部短小; 花色为红色至紫红色, 花冠, 平均直径为 0.165 cm, 花瓣, 平均直径长为 2.813 cm; 叶片为椭圆形, 较厚, 平均厚度为 0.039 cm, 绿叶, 叶尖呈绿色, 平均叶长为 5.272 cm, 平均叶宽为 2.889 cm, 叶片和叶脉颜色有被疏被毛, 叶脉和叶柄均为红色, 叶柄较长, 平均长 0.397 cm; 花瓣较少, 散生, 平均单枝花蕾数为 28 个, 花梗较长, 平均长度为 0.633 cm, 花瓣为披针形, 堆花对生或二元型; 花茎较短, 花径平均长 2.545 cm, 平均直径为 0.325 cm, 红色, 翅瓣和柱头不弯曲, 花丝和花药, 花药较大, 平均花药长 0.349 cm, 平均花药宽 0.08 cm, 花瓣较短, 平均直径为 0.027 cm, 开花早, 花期短, 盛花期为 5 月中下旬。
Mixed product identification technology

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>M</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>M: DL2000 marker; 1: Negative control; 2-7: Different proportion of <em>Lonicera confusa</em> (Sweet) <em>DC.</em> blended in <em>Lonicera japonica</em> Thunb.</td>
<td>1</td>
<td>1%</td>
<td>3</td>
<td>5%</td>
<td>4</td>
<td>10%</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixing</td>
<td>Intensity (a.u.)</td>
</tr>
<tr>
<td>450</td>
<td>455</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>C</td>
</tr>
</tbody>
</table>

CCP-FRET/pg
(Apply to the identification on the Extractive / Formula-Granule)
Compound prescription / Chinese patent medicine

- Molecular identification on snakes raw materials in Chinese patent medicine

DNA identification fluorescence spectrum of snakes medicinal materials of CCP-FRET: (a) Identification on Bungarus parvus and its adulterants; (b) FRET rate of CCP-FRET identification on AGKISTRODON, Zaocys, and Bungarus parvus; (c) Identification threshold value of AGKISTRODON, Zaocys, Bungarus parvus and their adulterants; (d) Test results by visual inspections under the 365 nm uv lamp.

Rapid assessment on growth year of the Chinese medicinal materials

Introducing the telomeres theory

Fitted curve of ginseng telomeres and growth year has been established, based on the find of that the length of ginseng telomere will grow along with the age growth. Took a 5 years raw ginseng randomly, and measured the telomeres length is 12.56kb. Put it into the fitted formula, and got the result of that the growth year of ginseng is 5.23, which is consistent with the actual value.

qPCR rapid identification

Could measure the relative length of ginseng telomeres in 2 hours. 50mg samples are enough to be tested.

On-site and fast demand

Accurate, fast, high throughput, low cost
On-site operation of molecular identification

- DNA rapid extraction technique—DNA alkali pyrolysis
  - Simple reagent:
    1. Extracting buffer (A): NaOH (KOH) and additive agent KCl, NP-40, β-mercaptoethanol, PEG, glycocoll, Tween 20, PVP; 2. neutral buffer (B): HCl, NaAc, Tris
  - The operation is simple, and no instrument needed:
    Put medicinal powder into solution A, vibrate for 1 minutes, and then put into solution B, vibrate for 1 minutes, get supernatant for future determination.

- Rapid PCR technique
  - PCR amplification time can be reduced to 30 minutes;
  - Without using electrophoresis equipment, visual inspection is ok for getting the result.

On-site operation of molecular identification

1. DNA extraction
   - DNA alkaline lysis method
   - 5-10 min

2. Amplified reaction
   - Rapid PCR technique
   - 30 min

3. Test and authenticity
   - Fluorescent dye technique
   - 1-2 min

3. Research on TCM resource protection
Wild provenance is not clear

Rare TCM resources

A shortage of wild resources

Endangered TCM resources

Raw materials for industrial extraction

Provenance protection

Population protection

Plant cultivation protection

The development and protection on new medicine resources

Biological technology protection

5 types of TCM resources

Generic technology, protection strategy, sustainable use

“The research on 5 protection model for rare, endangered and commonly used TCM resources” won the second class prize of national award for progress in science and technology (the 1st author, 2008)
Sustainable Utilization of the Traditional Chinese Medicine Resources

Active ingredients of medicinal plant

Secondary metabolites

Biosynthetic pathway

Improve by metabolic engineering

Produce by synthetic biology

Artemisinin
High-throughput Sequencing

Abundant Data Base

High Resolution Mass Spectrum

Genomics
- Phylogeny and evolution
- GWAS
- Gene cluster

Transcriptomics
- Deep transcriptome
- Co-expression network

Metabolomics
- Metabolite annotation
- Change in development, stress and genotype

Data processing, databasing, integration

Hypothesis generation by systems analysis

Reverse genetics/biochemistry/chemistry, mathematical modeling

Functional genomics (hypothesis validation) and biotech application (synthetic biology)

Heterologous production of high value natural products

Synthetic Biosystems for the Production of High-Value Plant Metabolites

The total project budget is $13,602,100 over 4 years.
75 plants that produce high-value natural products.

<table>
<thead>
<tr>
<th>Products</th>
<th>Microorganisms</th>
<th>Progress</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td><em>Escherichia coli</em></td>
<td>Taxadiene 1g/L</td>
<td>Ajikumar et al. <em>Science</em>, 2010</td>
</tr>
<tr>
<td>Opioids</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Hydrocodone 0.3μg/L</td>
<td>Galanie et al. <em>Science</em>, 2015</td>
</tr>
<tr>
<td>Ginsenosides</td>
<td>Curcumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Paddon et al. 2013, *Nature*
Biosynthesis and heterologous production of tanshininones

Three genes involved in the upstream terpenoid bio-pathway
HMGS, FPPS, GGPPS

Six genes involved in tanshinone biosynthesis
SmCPS1, SmKSL1, CYP76AH1, CYP76AH3, CYP76AK1, SmCPR1

Four engineered yeast strains for tanshinone production.

Guo, Ma, Zhao, Huang et al. 2016, New Phytologist
Cui G, Huang L, Peters, R. J., Qi, X. et al. 2015, Plant Physiol
Guo, Zhou, Peters, Zhao, Huang et al. 2013, PNAS
Zhou, Gao, Huang, Zhao et al. 2012, JACS
Dai, Huang, Zhang et al. 2012, Biotech and Bioeng

The First Prize in Science and Technology Awards of the Chinese Pharmaceutical Association
Ginsenosides production: 1.3 g/L PPD and 10 g/L dammarenediol-II(DD)
Finally, the biosynthetic pathways of Rh2 and Rg3 were constructed by introducing the UDP-glycosyltransferases genes from P.ginseng, with fermentation titres of 0.5mg/L Rg3 and 0.8mg/L Rh2. which realized the microorganism production of ginsenosides.
Thank You!