Herbal extraction & isolation

Nattaya Lourith, Ph.D.
13 Feb 10

Herbal extraction & isolation

Contents:
- category of herbal use
- consumption value & trend
- data requirements
- forms used
- herbal selection
- herbal extraction
- herbal extract's quality

Herbal uses
- Food herbs
  - garlic, lemon, peppermint, ginger, etc.
- Medicinal herbs
  - aloe, ginkgo, ginseng, etc.
- Poisonous herbs
  - some mushrooms, etc.

Herbal products in each culture

<table>
<thead>
<tr>
<th>Type</th>
<th>Source</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayurvedic</td>
<td>PAM</td>
<td>India</td>
</tr>
<tr>
<td>Chinese</td>
<td>PAM</td>
<td>China</td>
</tr>
<tr>
<td>Indusynunic</td>
<td>PAM</td>
<td>Pakistan</td>
</tr>
<tr>
<td>Islamic : Unani-Tibb</td>
<td>PAM</td>
<td>Middle East</td>
</tr>
<tr>
<td>Kampo</td>
<td>PAM</td>
<td>Japan</td>
</tr>
<tr>
<td>Oriental</td>
<td>PAM</td>
<td>Other Asians</td>
</tr>
<tr>
<td>Herbalism/Homeopathy/</td>
<td>P</td>
<td>European + African</td>
</tr>
<tr>
<td>Botanical</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P - plants; A - animals; M - minerals

Current herbal use

Complementary and alternative medicine (CAM)
Fast growing over-the-counter products
- US$ 23 billion by 2010
Beauty aliments
Skin rejuvenation

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>Description</th>
<th>Example</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Single ingredient; plant derived</td>
<td>Vinblastine, taxol or aspirin</td>
<td>Rx or OTC</td>
</tr>
<tr>
<td>Botanical drugs</td>
<td>Clinically validated and standardized phytochemical mixtures</td>
<td>CAM</td>
<td>Rx or OTC</td>
</tr>
<tr>
<td>Dietary supplements/nutraceuticals</td>
<td>Plant component with health benefits</td>
<td>Garlic</td>
<td>OTC</td>
</tr>
<tr>
<td>Functional/medicinal foods</td>
<td>Food engineered or supplemented to provide health benefits</td>
<td>Canola oil; golden rice</td>
<td>OTC, Rx, Grocers</td>
</tr>
<tr>
<td>Recombinant proteins</td>
<td>Plant isolated proteins</td>
<td>During clinical trials</td>
<td>Rx</td>
</tr>
</tbody>
</table>
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**Function/Activity**

1. **Plant properties**
   - exp. sebum regulating, anti-bacterial, anti-inflammatory/anti-irritant, soothing/calming, skin healing & regeneration, stimulate skin regeneration, stimulate collagen production or inhibit breakdown, protect against UV & harsh conditions, anti-oxidant/free radical scavenger, phytopheromones, moisturising/nourishing/smoothing, circulatory stimulant, anti-swelling, astringent, etc.

2. **Plant activities**
   - Identification of plants high in specific actives
   - Analytical data
   - Claims substantiation:
     - chemical measurement
     - *in vitro*
     - *in vivo*

**Herbal forms used**
- Total extract or crude
- Selected active principles
- Isolated pure compounds

**Herbs**
- Primary extraction
  - Crude
    - Secondary extractions
      - Selected active principles
        - Extractions
          - Pure compounds

**Herbal Quality Analysis**
- Chromatographic Methods
- Spectral Methods
- Bioassays

**Plant derived ingredient**
- Botanical name
- Part of used
- Preparation method
- Characteristic composition
- Physicochemical specification
- Microbiological quality
- Allergenic potential
Herbs selection

- Traditionally used
- Safety
- Active compounds
- Clinical proven
- Literatures

Sample preparation for extraction

- Drying
  - Hot
  - Cold
- Grinding

Extraction/Isolation/Purification techniques

Bad sample preparation & extraction: loss of actives

Drying: hot

Drying: cold

Grinding

powder pulverata pulverized
Extraction techniques

- Cold extraction
  - Maceration
  - Percolation

- Hot extraction
  - Decocation
  - Distillation
  - Infusion
  - Soxhlet

- Accelerated extraction
  - Microwave assisted process
  - Sonication
  - Supercritical fluid

Proper solvent

Solvent used

Apolar

- C₆H₄
- C₆H₆
- Ether

Polar

- EtOAc
- CHCl₃
- AcCN
- PrOH
- ETOH
- MeOH

H₂O

Alkaloids
Terpenoids
Coumarins
Fatty acids
Flavonoids
Terpenoids

Maceration: soaking in solvent

Macerate with gradient solvent of different polarity
With or without stirring

Percolation

- Passing solvent through sample
- Continuously flow of solvent
- Large volume of solvent used

Decoction: extract in boiling water

Infusion: treat with hot water or EtOH + water
Soxhlet: reflux temp.

Thimble

- Cellulose thimble
  - Organic compounds
- Glass thimble
  - Inorganic compounds: high temp. up to 550 °C

Microwave assisted process

H₂O vibration

Supercritical fluid extraction

Fluid as solvent
No solvent residue
No need to concentrate
Fast & effective
Compounds selectable
High pressure
Low temperature
High cost

CO₂
31 °C
74 bar

Comparison of accelerated extraction methods

<table>
<thead>
<tr>
<th></th>
<th>Soxhlet</th>
<th>Sonication</th>
<th>Microwave</th>
<th>Supercritical Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample weight (g)</td>
<td>5-10</td>
<td>5-30</td>
<td>0.5-1</td>
<td>1-10</td>
</tr>
<tr>
<td>Solvent</td>
<td>*</td>
<td>*</td>
<td>Hexane/EtO</td>
<td>CO₂</td>
</tr>
<tr>
<td>Solvent Volume (ml)</td>
<td>&lt;300</td>
<td>300</td>
<td>10-20</td>
<td>5-25</td>
</tr>
<tr>
<td>Vessel Volume (ml)</td>
<td>500-1000</td>
<td>500</td>
<td>-100</td>
<td>5-25</td>
</tr>
<tr>
<td>Temp. (°C)</td>
<td></td>
<td></td>
<td>R.t.</td>
<td>40, 70, 100</td>
</tr>
<tr>
<td>Time</td>
<td>16 h</td>
<td>30 m</td>
<td>30-45 s</td>
<td>30-60 m</td>
</tr>
<tr>
<td>Press. (atm)</td>
<td>Ambient</td>
<td>Ambient</td>
<td>1-5</td>
<td>150-650</td>
</tr>
<tr>
<td>Relative energy consumption</td>
<td>1</td>
<td>0.05</td>
<td>0.05</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*= CH₂Cl₂, Acetone, hexane, cyclohexane, toluene, etc.

Extractions

Protocol may be modified depending on the extracted molecules

Concentrations

Reduced pressure
Concentrations

- Evaporation
- Spray dry
- Freeze dry
- N₂ blown down

Isolation/ Purification techniques

- Crystallization
- Sublimation
- Distillation
- Liquid-liquid extraction: Partition
- Chromatography

Crystallization
Sublimation

Distillation

Liquid-liquid extraction
Polarity + gravity employments
Like dissolve like

Chromatography
- Stationary phase
- Mobile phase
  + partition
  + adsorption
  + dissolution

Chromatographic extraction
Extraction tracking
Thin layer chromatography: TLC

TLC tank preparation

TLC plate preparation

TLC spotting

TLC visualizing
UV: 254, 366 or 365
I₂ vapour
Spraying agents

R_f calculation

solvent front
new position of compound
origin

\[ R_f = \frac{2.1}{2.8} = 0.75 \]
**High performance liquid chromatography**

![Diagram of high performance liquid chromatography](image1)

**Gas chromatography**

**Table 1: Recommended Linear Velocities and Flow Rates**

<table>
<thead>
<tr>
<th>Diameter (mm)</th>
<th>Linear Velocity (cm/sec)</th>
<th>Flow Rate (mL/min)</th>
<th>Hydrogen</th>
<th>Hydrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.18</td>
<td>30.45</td>
<td>45-60</td>
<td>0.1 0.7</td>
<td>0.7 0.2</td>
</tr>
<tr>
<td>0.25</td>
<td>30.45</td>
<td>45-60</td>
<td>0.9 1.3</td>
<td>1.3 1.5</td>
</tr>
<tr>
<td>0.32</td>
<td>30.45</td>
<td>45-60</td>
<td>1.4 2.2</td>
<td>2.2 2.9</td>
</tr>
<tr>
<td>0.53</td>
<td>30.45</td>
<td>45-60</td>
<td>4.6 6.8</td>
<td>6.0 7.9</td>
</tr>
</tbody>
</table>

![GC chromatogram for organochlorine pesticides residue in Chinese herbs](image2)

**Supercritical fluid chromatography**

**Diagram of supercritical fluid chromatography**

![Diagram of supercritical fluid chromatography](image3)

**Supercritical Fluid Chromatogram for achiral screening in drug discovery compounds**

![Supercritical Fluid Chromatogram for achiral screening in drug discovery compounds](image4)
Fraction collector: HPLC, SFE

Bioassay-guided fractionation
- Total extract or crude
- Selected active principles
- Isolated pure compounds

Bioassays
- Biological target specific
  - Lower organism: microorganisms (bacteria, fungi, viruses)
  - Invertebrates: insects, crustaceans, molluscs
  - Isolated subcellular systems: enzymes, receptors
  - Animal or human cell cultures
  - Isolated organs of vertebrates
  - Whole animals

Biological Activities
- MIC: minimum inhibitory concentration
- MLC: minimum lethal concentration
- MFC: minimum fungicidal concentration
- MBC: minimum bactericidal concentration
- LC_{50}, LC_{90}: Lethal concentrations/doses
- 95% Confidential limits

Biological activity
- Antioxidant
- Anti-inflammatory
- Tyrosinase inhibition
- Melanogenesis inhibition
- Keratinolysis
- Collagen induction
- Blood circulation
- 5α-reductase inhibition

Antioxidant
- Exp.
  - DPPH assay
    - 2,2-diphenyl-1-picryl-hydrazyl + Vitamin C
  - TEAC assay
    - 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) + Vitamin C
  - β-carotene bleaching assay
    - β-carotene + Linoleic acid
  - FRAP assay
    - 2,4,6-tripyridyl-S-triazine + FeCl₃

Cosmetics: Radical scavenger (2,2'-diphenyl-1-picrylhydrazyl: DPPH)
Tyrosinase inhibitor
Propionibacterium acne

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Anti-inflammatory

- Free radicals
- Acne
- Hair loss
- Keratin
  - interleukin (IL)-1α
  - tumor necrosis factor (TNF)-α
  - granulocyte-macrophage colony stimulating factor (GM-CSF)

Keratinolysis

- Hard keratin
  - Hair & nail
- Soft keratin
  - Skin

Disulfide bonds content

Keratinolytic agent = proteolytic enzymes exp. Trypsin, pepsin, papain

Collagen induction

- Radicals
- Skin elasticity
- Skin sagging
- Skin wrinkling

Blood circulation

Toxin drainage
Inflammatory reduction

5α-reductase inhibition

Antiandrogenetic

- Hair loss treatment
- Acne
- Sebum hypersecretion
- Hirsutism
**Additional benefit**

Surfactant properties:
- Detergency
- Foaming
- Chelating
- Surface tension
- Emulsifying

Humectancy
Etc.

**Adverse effects**

- Allergy
- Toxicity

**Doses & efficacy : Threshold**

**Adverse effects**

Allergen
- Skin prick test
- Patch test
  - Atopic dermatitis
  - Irritation

**Adverse effects**

Toxicity : positive false result
- Cytotoxicity
- Human skin cell

**Standardized & Qualified extracts**

Minimum level of active ingredients assured
Quality consistency
Safety confirmatory

Identity, purity, content or assay
Monograph or Pharmacopeia

**Standardized extracts: exp**

Gingko extracts:
standardized extracts (dry extracts from dried leaves)
acetone + water, drug: extract = 35-67:1

Contain:
22-27% flavone glycosides
5-7% terpene lactones
2.8-3.4% ginkolides A, B, C
2.6-3.2% bilobalide
Standardization

prevent
- biological & chemical variations
- adulteration
- contamination
  + microbial
  + metal
- toxicity

Identification

1. Pharmacognostic characteristics
   - Macroscopical description
   - Microscopical description

2. Chemical characteristics
   - Preliminary test
   - Confirmatory test

Microscopical description: Purity

1. Foreign matter
2. Volatile & non-volatile
3. Moisture
4. Microbial contamination
5. Ash content
6. Pesticide & Fungicide residue contamination
7. Metal contamination
8. Radioactive contamination

Microbial contamination

Total aerobic microbial count
Total fungal count
Total Enterobacteriaceae count
Prohibited microbes
  - Staphylococcus aureus
  - Escherichia coli
  - Pseudomonas aeruginosa
  - Candida albicans
  - Aspergillus niger

Specification of herb

- Identification
- Purity

Analytical methods

- Physical Tests
- Chemical Tests
- Chromatography
- Spectroscopy
**Microbial test**

- **Pour plate**
- **Disc diffusion**

**TLC; bioautography**
- Contact
- Immersion
- Direct

**Test kit**

**Ash**

- Total ash = organic + inorganic matters
- Acid-insoluble ash = inorganic matters

**Pesticide & Fungicide residue**

**Toxicity from agricultural practices**
- Spraying
- Cultivation soil
- Fumigant storage

**Extraction**

 Partition/adsorption for impure movement

**Analyze**
- GC
- MS
- GC/MS

**Metal contamination**

**Toxic metal**: Pd, Cu, Cd, As

**Analyze**
- Inductive couple plasma: ICP
- Neutron activation analysis: NAA
Radioactive contamination

Nuclear exposure
- Skin
- Hair
- Brain
- Thyroid
- Blood system
- Heart
- Gastrointestinal track
- Reproductive track

Chemical characteristics: Chemical tests

- **Tannins**
  - FeCl₃: blue-black = gallitannins, ellagitannins
  - brownish-green = condensed tannins
  - Gelatin + 10% NaCl: precipitate = pseudotannins
  - Matchstick + HCl conc. + warm: pink or red = catechin
  - NH₄OH + air: green = chlorogenic acid
- **Alkaloids**
  - Mayer’s reagent = cream
  - Hager’s reagent = yellow
  - Wagner & Dargendorff reagents = reddish-brown
- **Anthraquinones**: NH₄OH or NaOH = pink, red, violet
- **Coumarins**: NH₄OH = blue, blue-green, violet

Herbal extract’s quality

+ efficacy
+ stability
+ safety
+ usability

**GOOD PRACTICE**

The Five “G’s”

- Good Agricultural Practice (GAP)
- Good Harvesting Practice (GHP)
- Good Clinical Practice (GCP)
- Good Laboratory Practice (GLP)
- Good Manufacturing Practice (GMP)

References


Thank you for your attention