New Products From Ancient Remedies:
Chemical Profiling In Cannabis Plant Parts

Dan Jin
May 8th, 2019
01 Introduction

02 Secondary Metabolites Profiling in Whole Cannabis Plant

03 Future Applications and Directions
Future Applications and Directions

Secondary Metabolites Profiling in Whole Cannabis Plant

Introduction
Introduction

Dan Jin

- Bachelor and Master in Engineering at Peking University, China
- PhD candidate at the University of Alberta, Canada

- ISO 17025 and cGMP certified
- Focus on natural health product, dietary supplement, pharmaceutical, and food industries
**Introduction**

Vegetative stage plants

Mature flowering plants

Dried flowers

Cannabis growing site in Vancouver
Introduction

Cannabis as Traditional Chinese Medicine

<table>
<thead>
<tr>
<th>Name</th>
<th>Indications</th>
<th>Methods of preparation</th>
<th>Method of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Malaria</td>
<td>Heat, powder, eat directly or make into pills</td>
<td>Oral administration with tea or warm alcohol</td>
</tr>
<tr>
<td></td>
<td>Asthma</td>
<td>-</td>
<td>Smoking</td>
</tr>
<tr>
<td></td>
<td>Ascariasis</td>
<td>Pulverize</td>
<td>Oral administration juice</td>
</tr>
<tr>
<td></td>
<td>Traumatic injury</td>
<td>Burn to ash, mix with burnt hair and frankincense powder</td>
<td>Oral administration with warm alcohol</td>
</tr>
<tr>
<td></td>
<td>Urinary tract</td>
<td>Mix with liquorice, ground to powder, decoct, mix with yellow or white wine</td>
<td>Oral administration before meals</td>
</tr>
<tr>
<td></td>
<td>infection pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tetanus</td>
<td>Caramelize, mix with wine and water</td>
<td>Oral administration, sweat in bed, 2-3 times a day</td>
</tr>
<tr>
<td>Roots</td>
<td>Gonorrhoea</td>
<td>Boil</td>
<td>Oral administration</td>
</tr>
<tr>
<td></td>
<td>Gynecological</td>
<td>Boil</td>
<td>Oral administration</td>
</tr>
<tr>
<td></td>
<td>disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Traumatic injury</td>
<td>Pulverize</td>
<td>Oral administration juice</td>
</tr>
</tbody>
</table>

The Dictionary of Medicinal Plant, Nanjing University Of Chinese Medicine, Shanghai People's Publishing House, 1st version, 1977
Introduction

Cannabis

- **Flowers**: THC: 10%-12%
- **Leaves**: THC: 1%-2%
- **Stems**: THC < 0.1%
- **Roots**: THC: almost absent

**Cannabinoids**

- **Terpenoids**

- **Flavonoids**

- **Sterols**
Introduction

Plant waste ➔ Wealth

Compounds
What kinds of interesting compounds can be found in cannabis plant parts?

Indications
What kinds of beneficial properties do these compounds have?

Applications
What kinds of natural products can be made using this information?
Secondary Metabolites Profiling in Cannabis Plant Parts

**Strain I (THC dominant)**
*Grand Doggy Purps*

**Strain II (THC dominant)**
*Granddaddy Purple*

**Strain III (Inter. CBD:THC=2:1)**
*CBD Mango Haze*

a. Strain III in a greenhouse

b. Dried cannabis inflorescences

c. Dried cannabis leaves.

d. Fresh cannabis stems

e. Fresh root material
Secondary Metabolites Profiling in Whole Cannabis Plant

a. Chromatogram for 14 cannabinoids by LC-MS

Cannabinoids profiled in each plant part

- Inflorescences
- Leaves
- Stem barks
- Roots

Asterisks indicate statistically significant differences (one-way ANOVA, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). Levels not connected by same letter were significantly different (two-sided Student’s t-test, p < 0.05).

*Chromatograms provided by Labs-Mart Inc.
b. Chromatogram for 44 mono- and sesquiterpenoids by GC-MS

<table>
<thead>
<tr>
<th>Monoterpenoids</th>
<th>Sesquiterpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>1. Eucalyptol</td>
</tr>
<tr>
<td>Camphene</td>
<td>2. Ocimene</td>
</tr>
<tr>
<td>Sabinene</td>
<td>3. γ-Terpine</td>
</tr>
<tr>
<td>(-)-β-Pinene</td>
<td>4. Sabinene Hydrate</td>
</tr>
<tr>
<td>β-Myrcene</td>
<td>5. Terpineolene</td>
</tr>
<tr>
<td>α-Phellandrene</td>
<td>6. Fenchone</td>
</tr>
<tr>
<td>Δ3-Carene</td>
<td>7. Linalool</td>
</tr>
<tr>
<td>α-Terpine</td>
<td>8. Fenchol</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>9. (-)-Isopulegol</td>
</tr>
<tr>
<td>Limonene</td>
<td>10. Camphor</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>11. Borneol</td>
</tr>
<tr>
<td>Camphene</td>
<td>12. Terpin-4-ol</td>
</tr>
<tr>
<td>Sabinene</td>
<td>13. α-Terpineol</td>
</tr>
<tr>
<td>(-)-β-Pinene</td>
<td>14. Sabinene Hydrate</td>
</tr>
<tr>
<td>β-Myrcene</td>
<td>15. Nerol</td>
</tr>
<tr>
<td>α-Phellandrene</td>
<td>16. Pulegone</td>
</tr>
<tr>
<td>Δ3-Carene</td>
<td>17. Carvone</td>
</tr>
<tr>
<td>α-Terpine</td>
<td>18. Geraniol</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>19. (-)-Isopulegol</td>
</tr>
<tr>
<td>Limonene</td>
<td>20. Camphor</td>
</tr>
</tbody>
</table>

Chromatograms provided by Labs-Mart Inc.

Mono- and sesquiterpenoids profiled in each plant part

P < 0.0001

Asterisks indicate statistically significant differences (one-way ANOVA, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). Levels not connected by same letter were significantly different (two-sided Student’s t-test, p < 0.05).

*Chromatograms provided by Labs-Mart Inc.

Information Classification: General
Secondary Metabolites Profiling in Whole Cannabis Plant

c. Chromatogram for 7 flavonoids by HPLC-UV-MS

Flavonoids profiled in each plant part

Note: Mass spectrometry was used for flavonoid identification and UV detector was used for flavonoid quantification.

*Chromatograms provided by Labs-Mart Inc.

Asterisks indicate statistically significant differences (one-way ANOVA, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). Levels not connected by same letter were significantly different (two-sided Student’s t-test, p < 0.05).
Secondary Metabolites Profiling in Whole Cannabis Plant

d. Chromatogram for 3 sterols and 3 triterpenoids by GC-MS

1. Campesterol
2. Stigmasterol
3. β-sitosterol
4. β-Amyrin
5. Epifriedelanol
6. Friedelin

*Chromatograms provided by Labs-Mart Inc.

Sterols profiled in each plant part

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Strain I</th>
<th>Strain II</th>
<th>Strain III</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflorescences</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>0.7589</td>
</tr>
<tr>
<td>Leaves</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>0.0550</td>
</tr>
<tr>
<td>Stem barks</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Roots</td>
<td>b</td>
<td>c</td>
<td>a</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Triterpenoids profiled in each plant part

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Strain I</th>
<th>Strain II</th>
<th>Strain III</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflorescences</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Leaves</td>
<td>b</td>
<td>c</td>
<td>a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stem barks</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Roots</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Secondary Metabolites Profiling in Whole Cannabis Plant

- **Leaf**
  - Flavonoids, 0.40%
  - Sterols, 0.05%
  - Mono- and sesquiterpenoids, 0.28%

- **Root**
  - Sterols, 0.06%
  - Triterpenoids, 0.24%

- **Stem bark**
  - Sterols, 0.08%
  - Triterpenoids, 0.15%
  - Flavonoids, 0.01%
## Therapeutic effects of cannabinoids

*(1.10% - 2.10% in cannabis leaves)*

<table>
<thead>
<tr>
<th>Therapeutic effects</th>
<th>CBD</th>
<th>Δ9-THC</th>
<th>THCA</th>
<th>CBDA</th>
<th>CBC</th>
<th>CBG</th>
<th>CBN</th>
<th>THCV</th>
<th>CBDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-inflammatory</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Pain relieving</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Anti-oxidant and neuroprotective</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beneficial on respiratory system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appetite stimulating</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Anti-depressant</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Anti-anxiety</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedative</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
Secondary Metabolites Profiling in Whole Cannabis Plant

Therapeutic effects of mono- and sesquiterpenoids
(0.13%-0.28% in leaves)

- **Pinene**
  - Anti-inflammatory
  - Anti-oxidant
  - Neuroprotective

- **β-Myrcene**
  - Anti-inflammatory
  - Pain relieving
  - Sedative

- **Limonene**
  - Anti-inflammatory
  - Pain relieving
  - Anti-biotic

- **β-Caryophyllene**
  - Anti-inflammatory
  - Pain relieving
  - Anti-bacterial

- **β-Myrcene**
  - Anti-inflammatory
  - Anti-oxidant
  - Anti-anxiety
  - Anti-microbial

- **Limonene**
  - Anti-inflammatory
  - Pain relieving
  - Anti-biotic

- **β-Caryophyllene**
  - Anti-inflammatory
  - Pain relieving
  - Anti-bacterial

- **Pinene**
  - Anti-inflammatory
  - Pain relieving
  - Anti-oxidant

- **β-Myrcene**
  - Anti-inflammatory
  - Pain relieving
  - Anti-biotic

- **Limonene**
  - Anti-inflammatory
  - Pain relieving
  - Anti-biotic

- **β-Caryophyllene**
  - Anti-inflammatory
  - Pain relieving
  - Anti-bacterial
Secondary Metabolites Profiling in Whole Cannabis Plant

Therapeutic effects of flavonoids
(0.24% - 0.34% in leaves)

Quercetin
- Anti-inflammatory
- Anti-oxidant
- Cardioprotective
- Neuroprotective

Kaempferol
- Anti-inflammatory
- Anti-oxidant
- Cardioprotective
- Neuroprotective

Luteolin
- Anti-inflammatory
- Anti-oxidant
- Cardioprotective
- Neuroprotective

Apigenin
- Anti-inflammatory
- Anti-oxidant
- Anti-allergy
- Protective against diabetes
- Promoting gastrointestinal motility
- Cardioprotective
- Neuroprotective
- Neuroprotective
- Beneficial on liver
- Beneficial on respiratory system

Increase mineral content and bone density
Cardioprotective
Neuroprotective
Beneficial on liver respiratory system
Secondary Metabolites Profiling in Whole Cannabis Plant

Therapeutic effects of triterpenoids
(0.05% - 0.15% in stem barks and 0.13% - 0.24% in roots)

Friedelin
- Anti-inflammatory
- Anti-oxidant
- Pain relieving
- Anti-pyretic

β-Amyrin
- Anti-inflammatory
- Anti-oxidant
- Gastroprotective
- Anti-microbial

Epifriedelanol
- Anti-inflammatory
- Anti-aging
- Anti-fungal

Anti-oxidant
Protective against ethanol-induced gastric ulcer
Estrogenic activity
Secondary Metabolites Profiling in Whole Cannabis Plant

Therapeutic effects of sterols
(0.07% - 0.08% in stem barks and 0.06% - 0.09% in roots)

**β-sitosterol**
- Anti-inflammatory
- Anti-oxidant
- Pain relieving
- Protective against diabetes

**Stigmasterol**
- Anti-inflammatory
- Anti-oxidant
- Pain relieving
- Anti-hypercholesterolemic

**Campesterol**
- Anti-inflammatory
- Anti-oxidant
- Anti-hypercholesterolemic
## Future Applications and Directions

<table>
<thead>
<tr>
<th>Therapeutic effects</th>
<th>Cannabis leaves</th>
<th>Cannabis stem barks</th>
<th>Cannabis roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major metabolite groups</td>
<td>Flavonoids</td>
<td>Triterpenoids and sterols</td>
<td>Triterpenoids and sterols</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Pain relieving</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Anti-oxidant and neuroprotective</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Anti-hypertensive</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardioprotective</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Protective on learning, cognition, and depression</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Protective against obesity and diabetes</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Gastroprotective</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Sedative</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beneficial on liver</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Anti-microbial, anti-bacterial, anti-fungal, anti-viral</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Increase mineral content and bone density</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogenic activity</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-pyretic</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-hypercholesterolic</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Comparison of Flavonoid Content

- Gingko leaves, dried
- Cannabis leaves, dried
- Goji berry, dried
- Cranberry, dried
- Marjoram, dried
- Plums, dried

*Expressed as total aglycones of quercetin, luteolin, kaempferol, and apigenin
Future Applications and Directions

Comparison of Friedelin Content

- Leaves of Bee sting bush, dried
- Cannabis roots, dried
- Cannabis stem barks, dried
- Leaves of Shala tree, dried

Content (%)
Future Applications and Directions

Comparison of Sterols Content

- Corn oil
- Canola oil
- Olive oil
- Cannabis root, dried
- Cannabis stem barks, dried
- Coconut oil
- Chickpeas
- Avocado, dried
- Soybean
- Lentil
- Green pea
- Carrot, dried
- Brown rice
- Broccoli, dried
- Banana, dried

Information Classification: General
Possibility of new products made from cannabis leaves, stems, and roots in the form of:

- Edibles: tea, capsules, oil, spray/tincture/drops
- Topicals: cream, oil, lotion, cosmetics
Thank you for listening!

Questions?
Future Applications and Directions

Health Canada
Natural and Non-prescription Health Product Directorate (NNHPD)

- Vitamins and minerals
- Herbal remedies
- Homeopathic medicines
- Traditional medicines like Traditional Chinese Medicines (TCM) and Ayurvedic (East Indian) medicines
- Probiotics
- Other products like amino acids and essential fatty acids
DIRECTIVE 2002/46/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 10 June 2002
on the approximation of the laws of the Member States relating to food supplements
(Text with EEA relevance)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE
EUROPEAN UNION,

Having regard to the Treaty establishing the European Community, and in particular Article 95 thereof,

There is a wide range of nutrients and other ingredients that might be present in food supplements including, but not limited to, vitamins, minerals, amino acids, essential fatty acids, fibre and various plants and herbal extracts.
Product Licences for Natural Health Products (NHPs)

All NHPs must undergo a pre-market review before they may be sold in Canada. This process requires that evidence supporting the safety, efficacy and quality of the product be submitted to the Natural Health Products Directorate (NHPD) for assessment by means of a product licence application. Those products supported by sufficient evidence to support the safety, efficacy and quality of the product are issued a product licence and corresponding Natural Product Number (NPN) or Homeopathic Medicine Number (DIN-HM), which must appear on the product label (e.g., NPN12345678) as proof that Health Canada has authorized the sale of the product.
General Principles

The following are general principles which should be considered when filing a submission to TPD, BGTD, or NHPD in support of a Schedule A disease preventative claim. These principles are in line with current review practices in the evaluation of claims relating to diseases of a more serious nature.

Evidence submitted by the sponsor in support of a Schedule A disease preventative claim for a nonprescription drug or a natural health product will be evaluated on a case-by-case basis since the context in which these claims are presented will influence the regulatory decision. Granting of market authorization will be based on an evaluation of the totality of the credible scientific evidence. The likelihood of achieving market authorization is commensurate with the strength of the evidence submitted.

Preventative claims for Schedule A diseases will be evaluated in the form of a systematic review assessing the strength of the scientific evidence to support a proposed claim about a product-disease relationship by considering study design types, quality and quantity of evidence (i.e., numbers of studies and study size) for and against the claim, statistically significant outcomes, clinically meaningful differences, relevance to the target population and overall consistency of the results across all studies of acceptable quality. Thus the totality of the evidence with respect to the product will be considered. Data requirements in support of preventative claims for Schedule A diseases embody as much scientific rigour as that applied to treatment claims.
Supplement material

Data Requirements

1. Primary clinical evidence required to support a preventative claim

Such studies take the form of randomized, controlled, and preferably blinded, clinical trials where the substance/product is administered for the purpose and under the conditions of use specified in the submission.

2 Secondary supportive evidence

2.1 Observational studies

2.2 Endpoints to substantiate preventative claims

3 Endpoints to substantiate prevention claims

4 Statistical analysis of data

5 Assessment of safety

6 Studies that serve only as background information

6.1 Traditional use

6.2 Animal and *in vitro* studies

7 Chemistry and manufacturing