Cannabis as a plant: Taxonomy and Chemistry

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Disclaimer & Disclosure

• I am a member of the faculty at the University of Vermont
• Materials presented here represent my own findings, views and opinions and should not be taken as a statement, position, opinion, or endorsement by the University of Vermont

University of Vermont Health Network

Discussion includes investigational drugs not approved for use in the United States.

DISCLOSURE:

Is there anything to disclose? Yes

Please list the Potential Conflict of Interest (if applicable): Relationships with Vermont Patients Alliance and Phytoscience Institute

All Potential Conflicts of Interest have been resolved prior to the start of this program. Yes

(If no, credit will not be awarded for this activity.)

All recommendations involving clinical medicine made during this talk were based on evidence that is accepted within the profession of medicine as adequate justification for their indications and contraindications in the care of patients. Yes
Cannabis history

Human use
12,000-10,000 years ago Cannabis cultivation begins
1753 Linnaeus classifies Cannabis sativa L.
2011 First Cannabis genome is sequenced

Policy
1930 Harry Anslinger began the process of criminalization and prohibition of drugs including marijuana and by 1931 29 states had outlawed Cannabis.
1970 Controlled Substances Act passed. Cannabis is Schedule one drug categorized to have a high potential for abuse and no medicinal value.
1996 Passage of the Compassionate Use Act initiative

Medical discovery
1964 Tetrahydrocannabinol (THC) is isolated and synthesized
1988 Cannabinoid receptors CB1 and CB2 discovered
1992 Endogenous cannabinoids discovered
Cannabis history

Cultivation for food and fiber
Sacramental use
Medicinal use
Documented in first Herbals
Cannabis sativa classified
Documented in the Americas
Harrison Narcotics Tax Act
THC isolated and synthesized
Controlled Substances Act
Marinol approved
CB1 & CB2 discovered
Endogenous Cannabinoids
Compassionate Use Act
Genome sequenced
Hemp is removed from CSA

10,000 BCE - 0
100
1500
1700
1930
1964
1970
1985
1988
1992
1996
2011
2018

Used as a therapeutic drug
Criminalized
Gains popularity as a therapeutic drug
Cannabis history
The ancestral distribution of *Cannabis* is central Asia (Li, 1974)

*Cannabis* arrived in the Americas after 1545 (Small et al., 1975)

Currently *Cannabis* is distributed world-wide

Photo credits: Hillig and Mahlberg, 2004; Angiosperm Phylogeny Group, 2015
Cannabis plant biology

Macroscopic morphological characters
- Leaves, flowers, and fruit

Photo credit: http://en.wikipedia.org/wiki/Cannabis
Cannabis plant biology

Microscopic morphological characters
- Trichomes and glands

FIGURE 3 | Hemp trichome types. (A) Unicellular non-glandular trichome; (B) cystolytic trichome; (C) capitate sessile trichome; (D) capitate-staked trichome; (E) simple bulbous trichome; (F) complex bulbous trichome. Images kindly provided by Dr. David J. Potter.
Reproductive characters

- Male and female flowers are found on different plants
- Wind Pollinated

Plants produce chemical compounds, phytochemicals, as part of their normal metabolic activities.

Phytochemicals are divided into two groups:

1. Primary metabolites
   - found in all plants, serve basic plant functions
   - i.e. sugars and fats

2. Secondary metabolites
   - found in select plants, serve in a more specific function
   - i.e. opium, phytocannabinoids

Slide content contributed by Dr. Wolfgang Dostmann, UVM LCOM
Cannabis plant biology

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Cannabis glandular trichomes containing phytocannabinoids

Delta-9-tetrahydrocannabinol, one active principle of Cannabis

Slide content contributed by Dr. Wolfgang Dostmann, UVM LCOM
Cannabis chemicals

Slide content contributed by Dr. Wolfgang Dostmann, UVM COM; source Potter, 2009
The main biologically active phytocannabinoids

**Delta-9-tetrahydrocannabinol (THC)**
- Partial Agonist CB1/CB2 receptors
- Psychoactive
- Anti-pain, anti-nausea
- Anti-spasm, anti-immune

**Cannabidiol (CBD)**
- Antagonist CB1/CB2 receptors
- Not psychoactive
- Anti-seizure activity

Activated by heating the plant

Slide content contributed by Dr. Wolfgang Dostmann, UVM LCOM
What are Phytocannabinoids?
How are Phytocannabinoids manufactured by the plant?

Phytocannabinoid biosynthesis

1. Chemical precursors are absorbed through the roots from the soil

2. CBD & THC derive from CBG compounds, catalyzed by the enzyme THCA synthase or CBDA synthase
   - CBDA synthase is dominant
     - Hemp has THCA & CBA synthase
     - Drug-type (Marijuana) has THCA & CBDA synthase
   - nonfunctional CBDA synthase

Source: Weilben et al., 2015
Phytocannabinoids evolved in *Cannabis*

Herbivory defense?
Environmental response?
## Cannabis chemicals

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Number of Known Compounds</th>
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<tbody>
<tr>
<td>1</td>
<td>CBG type</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>CBC type</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>CBD type</td>
<td>8</td>
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<tr>
<td>4</td>
<td>$\Delta^9$-THC type</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>$\Delta^8$-THC type</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>CBL type</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>CBE type</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>CBN type</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>CBND type</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>CBT type</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td>Miscellaneous</td>
<td>22</td>
</tr>
<tr>
<td>12</td>
<td>Total cannabinoids</td>
<td>104</td>
</tr>
<tr>
<td>13</td>
<td>Total noncannabinoids</td>
<td>441</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>545</td>
</tr>
</tbody>
</table>
What are the non-cannabinoids?

Terpenes (Myrcene)

Slide content contributed by Dr. Wolfgang Dostmann, UVM LCOM
Myrcene has an analgesic effect

“Terpenes such as myrcene may constitute a lead for the development of new peripheral analgesics with a profile of action different from that of the aspirin-like drugs.”
α-Pinene appears to be a broad-spectrum antibiotic

• Does α-Pinene in three hemp cultivars have an inhibitory activity of microbial growth?
• Yes, results demonstrate α-Pinene (in hemp essential oils) significantly inhibits microbial growth
  • Gram (+ and -) bacteria
  • Yeasts
• Conclusion: industrial hemp essential oils applications could control spoilage, food-borne pathogens, and phytopathogen microorganisms.
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Boiling point</th>
<th>Partition Coefficient, logP</th>
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</thead>
<tbody>
<tr>
<td>Myrcene</td>
<td>166 °C (330 °F)</td>
<td>4.30</td>
</tr>
<tr>
<td>Limonene</td>
<td>176 °C (349 °F)</td>
<td>3.40</td>
</tr>
<tr>
<td>Linalool</td>
<td>198 °C (388 °F)</td>
<td>2.65</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>155 °C (311 °F)</td>
<td>2.80</td>
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<tr>
<td>Geraniol</td>
<td>230 °C (446 °F)</td>
<td>3.28</td>
</tr>
<tr>
<td>THC</td>
<td>157 °C (315 °F)</td>
<td>6.97</td>
</tr>
<tr>
<td>CBD</td>
<td>180 °C (356 °F)</td>
<td>6.33</td>
</tr>
<tr>
<td>CBN</td>
<td>185 °C (365 °F)</td>
<td>6.93</td>
</tr>
</tbody>
</table>
Why does Cannabis produce Phytocannabinoids?

Cannabis chemicals

Level of Phytocannabinoids

photosynthesis & breathing

storage of sugars or starch

light-energy

water vapor

sugars & starch

C₆H₁₂O₆ — sugars & starch

breathing

H₂O and minerals through the root hairs

O₂ ↔ CO₂
Cannabis chemicals

Why does Cannabis produce Phytocannabinoids? What we know....

Plant organ matters!

Highest amount of THC & CBD is found in flower (Andre et al., 2016)
Size matters!
The amount of THC is correlated to the size of resin gland (Small and Naraine, 2015)
**Cannabis chemicals**

Why does *Cannabis* produce Phytocannabinoids? What we know....

**Light quality matters!**

- **Magagnini et al. (2018)** Phytocannabinoid yield changes with different light sources
  - Perhaps complex mechanisms mediated by the UV-A and blue wavelengths that induce CBG accumulation (CBG is precursor of other phytocannabinoids)

- **Lydon et al. (1987)** In “drug-type” plants, increasing levels of UV-B radiation corresponded with increased levels of THC
  - Potential for selective pressure to favor “drug-type” plants in environments with high levels of UV radiation
Desiccation Prevention?

• Major Phytocannabinoids increased when intentional drought applied seven weeks into reproductive stage (Caplan, 2018)

• Wild plants in cool humid environments had fewer glandular hair compared to plants in hot dry environments (Sharma, 1975)

• Higher THC levels measured in drier continental climate compared to maritime climate (Murari et al., 1983)
Cannabis chemicals

Why does Cannabis produce Phytocannabinoids?

Stress?

- Correlation between low plant height and high Phytocannabinoid content
- Low Mg, higher THC & CBD content

(Coffman and Gentner, 1975)
Cannabis chemicals

Why does Cannabis produce Phytocannabinoids?

Insecticide?

• THC-rich Mexican (vs. CBD-rich Turkish) Cannabis was reported fatal to tiger moth (Arctia caja) larvae (Rothschild et al., 1977)
Cannabis chemicals

Why does Cannabis produce Phytocannabinoids?

Chemical plant defense?

- Cannabis extract inhibited germination of Lettuce (*Lactuca sativa*)
  
  (Mahmoodzadeh et al., 2015)

- Cannabis extract decreased the germination of monocots
  
  (Pedulko, 2014)
Why does *Cannabis* produce Phytocannabinoids?

Disease prevention?

- Harbor antibiotic properties (ElSohly *et al.* 1982)

- Antibiotic properties assist in the overwintering of seed (Ferency, 1956)
Cannabis chemicals

Why are the amounts of Phytocannabinoids inconsistent?

Plant age changes Phytocannabinoid content

- THC increases throughout the life cycle of Cannabis before reaching a plateau (Debacker et al., 2012)

Why are the amounts of Phytocannabinoids inconsistent?

Phytocannabinoid difference due to changes in:

- Genetics
- Plant Organ
- Light
- Soil composition
- Available moisture
- Age

Aizpurua-Olaizola et al., 2016
Is it possible to grow Cannabis with reproducible terpene and phytocannabinoid levels?

- Yes! As long as environmental conditions and genetics are standardized
  - Fischedick et al. (2010)
    - 11 “strains”
    - 36 compounds
    - Control for grow cycle time, plant stress, different genotypes
How is Phytocannabinoid and terpene production related?

- Levels of cannabinoids and terpenoids correspond (Fischledick et al., 2010)
Are there variations in the chemical composition of *Cannabis* grown under Title 18 of the Vermont Statutes?
Are there variations in the chemical composition of *Cannabis* grown under the Industrial Hemp Program in Vermont?

**Parameters controlled:**
- Organ harvested
- Light
- Soil composition
- Available moisture
- Harvest Age

**Variables:**
- Genetic composition

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*Cannabis chemical research*
Are there variations in the chemical composition of *Cannabis* grown in different environmental conditions under Title 18 of the Vermont Statutes?
Are there variations in the chemical composition of *Cannabis* grown in different environmental conditions under the Industrial Hemp Program in Vermont?

**Cannabis chemical research**

Parameters controlled:
- Organ harvested
- Genetic composition
- Soil composition
- Available moisture
- Harvest Age

Variables:
- Light

![Graph showing variations in CBD content for outdoor and indoor growth conditions.](image-url)
Does *Cannabis* grown with different nutrients yield different chemical compositions?

- Different fertilization concentrations produced changes in vegetative growth, biomass yield, and phytocannabinoid concentrations
  - Increasing fertilizer rate had a dilution effect on THC, THCA, and CBGA, despite increased biomass yield (Caplan et al., 2017; doi: 10.21273/HORTSCI12401-17).
Cannabis chemical research

Does Cannabis grown with different nutrients yield different chemical compositions?

Dried flower weight

Percent Phytocannabinoid by weight

Parameters controlled:
- Organ harvested
- Light
- Available moisture
- Harvest Age
- Genetic composition

Vegetative growth curve
Cannabis: the name

Taxonomy

- Family: Cannabaceae
- Genus: Cannabis
- Species?
  - At least 13 Cannabis species have been described

Why is the scientific name important?
Cannabis: the name

Why worry about using a scientific name?

Consistency!

“hemp” usually = *C. sativa*, but the term has been applied to many fiber crops

- Manila hemp = *Musa textilis* (banana anyone?)
- Sisal hemp = *Agave sisalana* (related to tequila!)
- Sunn hemp = *Crotolaria juncea* (pea family)
What is the difference between hemp and marijuana?

Plants can generate different chemical constituents due to the environment in which they grow.

The different chemical compositions within the same plant species are called chemical phenotypes, or “chemotypes.”

Cannabis: the name

Chemical content!

< 0.3% THC content = Hemp
**Cannabis: the name**

Is there a genetic difference between hemp and marijuana?

*Cannabis* genome and transcriptome

- Published 2011
- Sequenced DNA and RNA with Illumina Next-generation technology
- SNP identified differences in “hemp” and “marijuana genome

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**Figure 5 Neighbour-joining tree for two hemp cultivars and two marijuana strains.** The tree was plotted in MEGA5 [71] using the maximum composite likelihood of SNV nucleotide substitution rates, calculated based on the concatenated SNV sequences in each variety, as a distance metric. The topology of the tree reveals a distinct separation between the hemp and marijuana strains.

Van Bakel et al. (2011)
Cannabis: the name

Is there a genetic difference between hemp and marijuana?

Phylogenetic neighbor network of ~3000 SNP alignment from the Cannabis genome

Lynch et al., 2015
**Cannabis: the name**

Is there a chemical difference between hemp and marijuana?

Yes, if chemotypes are based on CBD/THC ratio

- Hillig and Mahlberg (2004) suggest distinct groups

![Graph showing distribution of THC/CBD ratios with histograms and scatter plots.](image)
Cannabis: the name

Is there a chemical difference between hemp and marijuana?

Yes, if chemotypes are based on average percentage of major Phytocannabinoid mass for dried and un-pollenated female flowers (Lynch et al., 2015)
Cannabis: the name
Cannabis: the name

Are the drug-type names *Cannabis sativa* and *Cannabis indica* viable?

No, *Cannabis indica* should not be used

- Plants grouped morphologically as *C. indica* (red) or *C. sativa* (blue) do not form distinct groups based on chemical composition
  - one species concept *Cannabis sativa* L.
Do drug-type *Cannabis* “strains” have different chemotypes?

No, “strains” do not have not consistently reproducible chemical compositions.

*Figure 5: PCA scoring plot of full cannabis flower data set with scaling.*

Elzinga et al. (2015)
Cannabis names and chemicals

What’s Next?

Source: http://galaxy.phylosbioscience.com/?source=website
**Cannabis names and chemicals**

What’s Next?

- *International Code of Nomenclature for Cultivated Plants*
  - Established in 1953
  - Rules
    - Name is unique
    - Follows principle of priority
  - Operates within the Botanical code (International Code of Nomenclature for algae, fungi, and plants)
  - Does not regulate trademarks or marketing names
    - trade designations
    - *Cannabis* “strains” should be identified as cultivars once stabilized
Translational *Cannabis* Science and Medicine at the University of Vermont College of Medicine Department of Pharmacology

Through education we help turn observations in the laboratory, clinic and community into interventions that improve health and bridge scientific discoveries in medical *Cannabis* with the needs of health care providers, researchers, students, and professionals.
Cannabis impacts humans through the endocannabinoid (eCB) system.

The eCB system consists of:
- cannabinoid receptors (CBs)
- endogenous agonists
- agonist-metabolizing enzymes

Slide content contributed by Dr. John McPartland, UVM LCOM
Why does Cannabis impact humans?

- **CB$_1$** is primarily expressed in neurons
- **CB$_1$** is found in adipose tissue, blood vessels, gut, testes, uterus
- **CB$_1$** is *not found* in the brainstem’s cardiorespiratory drive centers – which explains the lack of lethal overdoses from cannabis
  - Unlike opioid receptor distribution

CB$_2$ is primarily expressed in the immune system

Autoradiograph of rat brain exposed to $[^{3}H]$CP55,940 (Herkenham *et al.*, 1990).

- Orange = highest densities in memory centers, limbic system, basal ganglia, cerebellum
- Red = lower densities in cerebral cortex
- Pink = lowest densities in the brain stem

Slide content contributed by Dr. John McPartland, UVM LCOM
**Methods**

- compilation of data from randomized clinical trials (RCTs) comparing cannabinoids to placebo for many conditions
- information about potential for adverse events (AEs) with cannabis

**Results**

- Improvement in pain when compared to placebo overall (Odds Ration 1.41 [0.99-2.00])
- Cannabinoids were associated with approximately 3x increased odds of any AE compared to placebo
FDA Approved: Synthetic THC

- Dronabinol (Marinol™) for Pain or Nausea
- Nabilone (Cesamet™) for Pain or Nausea

FDA Approved: CBD

- Cannabidiol (Epidiolex®)
• Risk of pediatric exposure
• Risk of diversion for recreational use
• No known risk of death from the drug itself
  • Margin of exposure (MOE) is the ratio between toxicological threshold (benchmark lethal dose) and estimated human intake based on individual and population scenarios
  • MOE analysis shows that by a wide margin, cannabis is the least risky recreational drug (Lachenmeiera DW and Rehm J., 2015)
Cannabidiol (CBD) activates non-endocannabinoid receptors

- TRPV1 is found in nervous tissue
- TRPV1 is involved in the feeling of pain
- TRPV1 activity can be decreased with overactivity = desensitization
  - Can lead to analgesic effects

Iannotti, F.A. et al., 2014; doi: 10.1021/cn5000524
Why not just pharmaceutical products?

1. Dronabinol not well tolerated, Epidiolex just became available, and Sativex is not yet approved in USA
2. When approved, costs could be exorbitant
3. “Whole plant” or “Full spectrum” extracts have other cannabinoids and terpenes with effects on CB receptors

Are plant-based products more effective (and less expensive) than pharmaceuticals?

Can they be provided in a safe and consistent fashion?

Cannabis clinical research

Slide content contributed by Dr. Kalev Freeman, UVM LCOM
Cannabis research

Label accuracy on CBD products
Cannabis research

Label accuracy on CBD products
All products contain CBD!

Only one product had levels above 0.3% THC

50% of products were labeled accurately

17% of Vermont products were labeled accurately

**Cannabis research**

**Label accuracy on CBD**

- **capsules**
  - n=7
  - dose range: 5-30mg
  - mean: 13.4mg

- **tinctures**
  - n=11
  - dose range: 1-25mg
  - mean: 12.9mg

- **concentrates**
  - n=3
  - dose range: unlabeled
  - % potency range: 47.99-77.07%
  - mean: 63.81%

- **cartridges**
  - n=3
  - dose range: unlabeled
  - % potency range: 14-64%
  - mean: 32%

![Graph showing label accuracy on CBD products](chart.png)
Questions?