

מחקר בקנאביס לשימוש רפואי: חומרים פעילים ומנגנוני פעולה

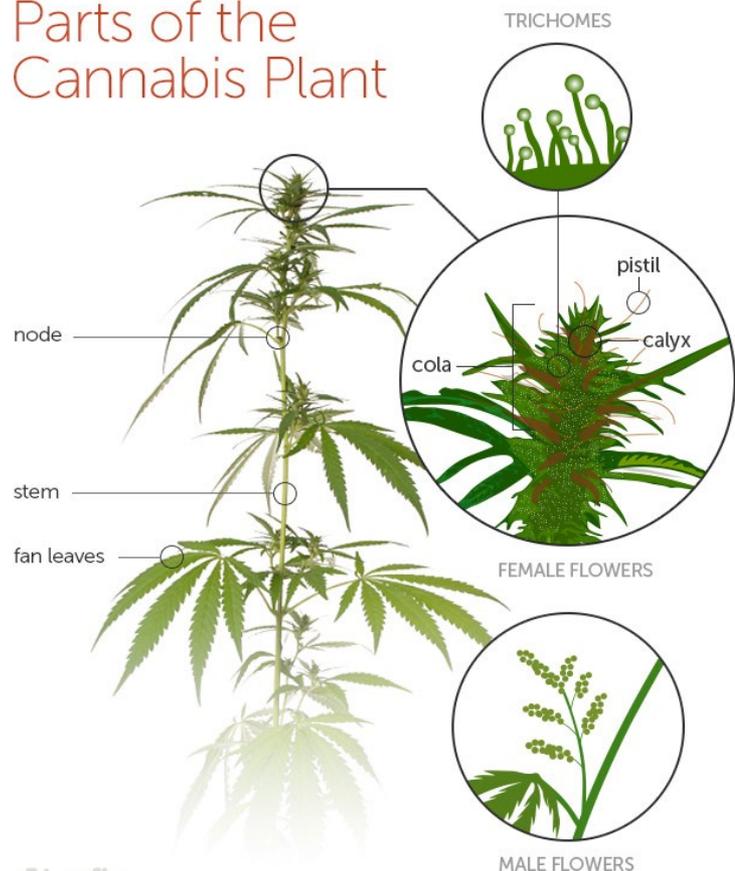
פרופ' חננית קולטאי, PhD

מכון וולקני



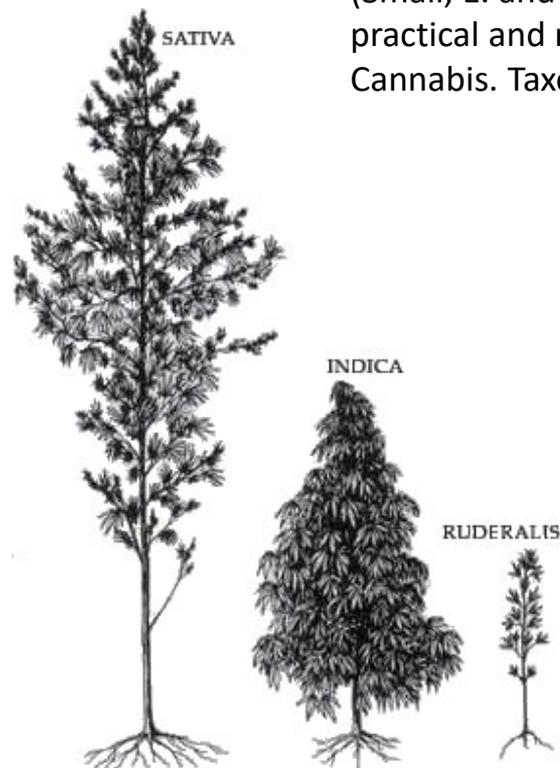
The Cannabis plant: *Cannabis sativa*

Parts of the Cannabis Plant

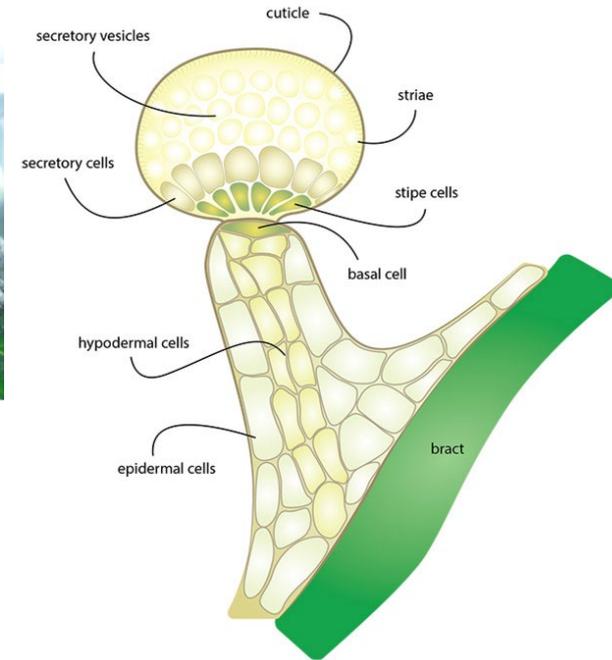


Leafly.com

- Annual, dioecious, flowering herb
- One *Cannabis sativa* species (Small, E. and Cronquist, A., 1976. A practical and natural taxonomy for Cannabis. Taxon, pp.405-435).

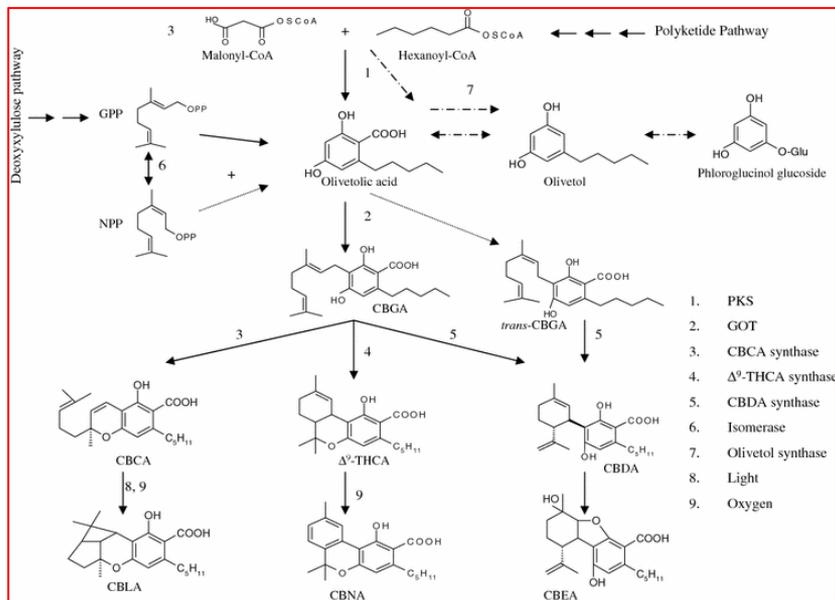


Active compounds- in the female inflorescence

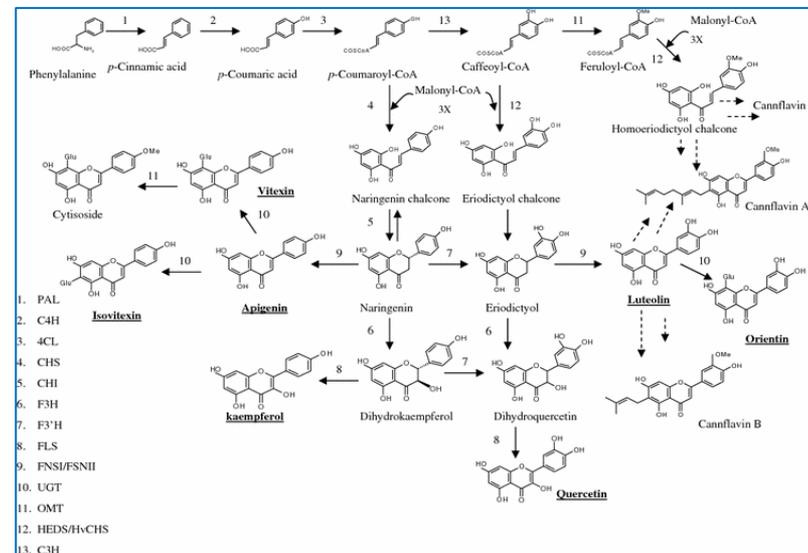


Trichome

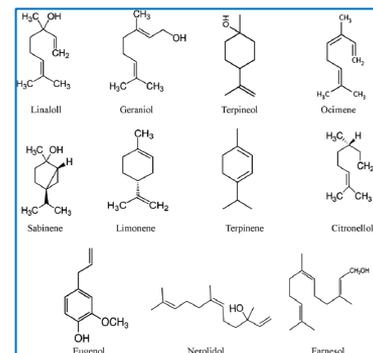
C. sativa produces hundreds of different compounds: phytocannabinoids, flavonoids, phenylpropanoid and terpenoids



phytocannabinoids



flavonoids



terpenes

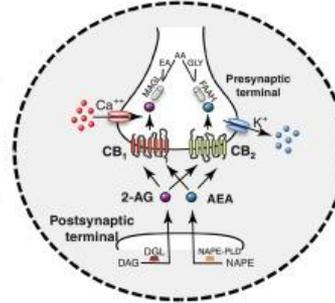
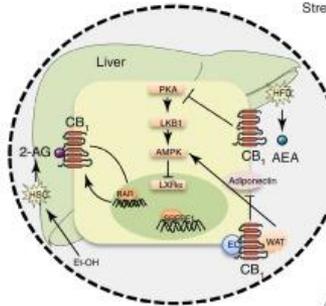
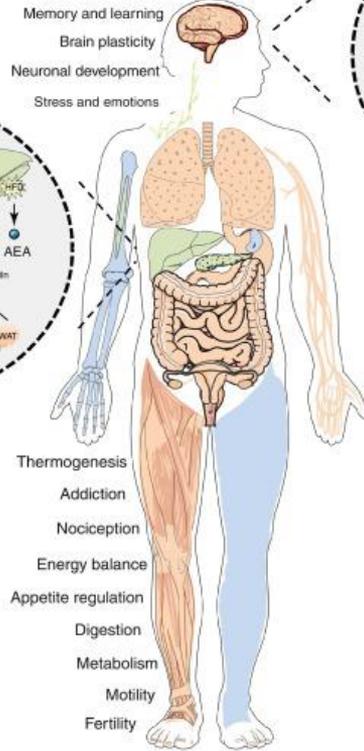
Flores-Sanchez, I.J. and Verpoorte, R., 2008. Secondary metabolism in cannabis. *Phytochemistry reviews*, 7(3), pp.615-639.

Andre, Christelle M., Jean-Francois Hausman, and Gea Guerriero. "Cannabis sativa: the plant of the thousand and one molecules." *Frontiers in plant science* 7 (2016): 19.

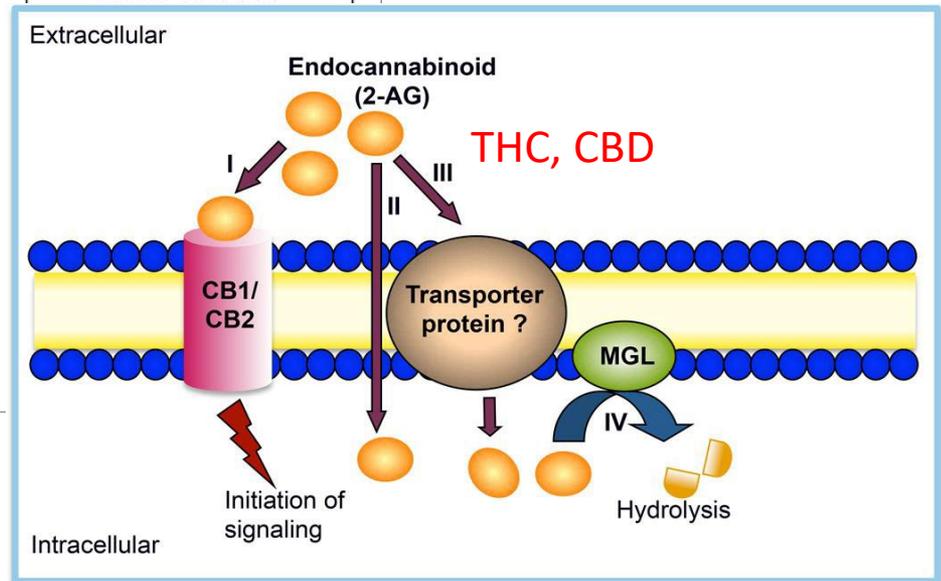


Phytocannabinoids are recognized by the endocannabinoid receptors (CB1, CB2....)

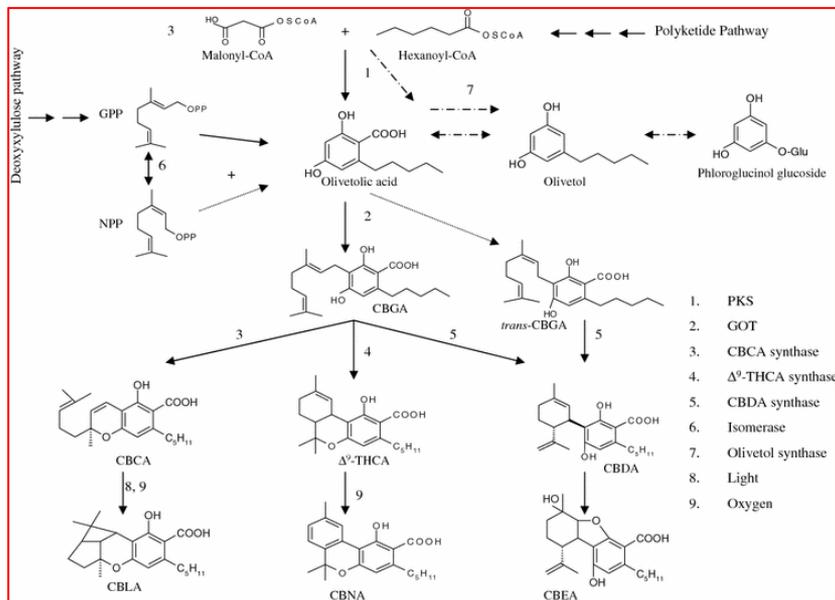
- CB₁ Brain; Lungs; Gastrointestinal tract; Reproductive system; Muscle; cardiovascular system
- CB₂ Bones; spleen; skin
- CB₁ + CB₂ Immune system; Liver Pancreas; Bone marrow



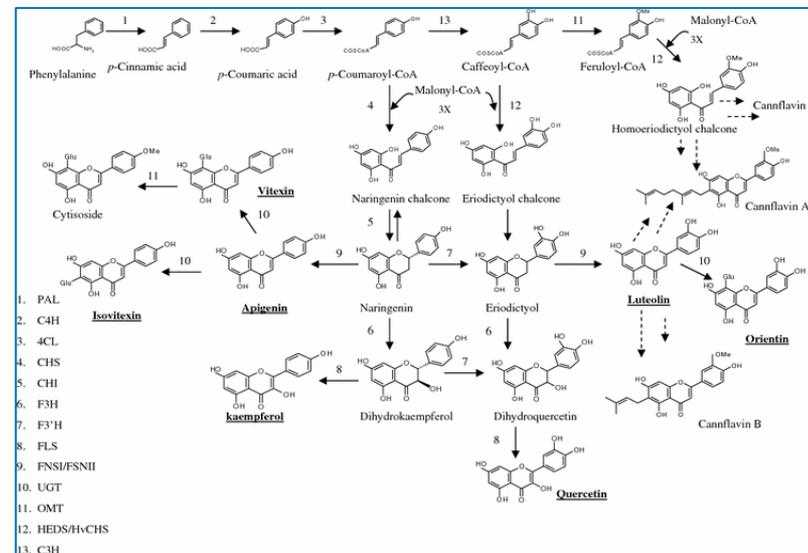
- Receptors**
- CBRs: CB₁, CB₂
 - TRPs: TRPV₁, TRPV₂, TRPV₃, TRPV₄, TRPA₁, TRPM₈
 - Orphan: GPR55; GPR119; GPR18; GPR30
 - EMT
- Endocannabinoids**



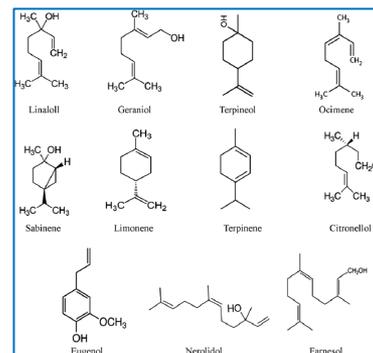
C. sativa produces hundreds of different compounds: phytocannabinoids, flavonoids, phenylpropanoid and terpenoids



phytocannabinoids



flavonoids



terpenes

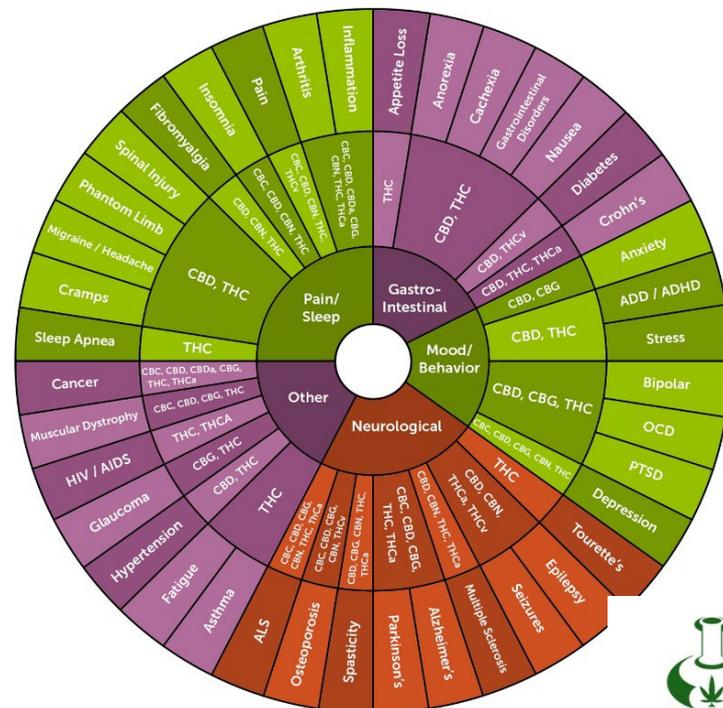
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The “entourage” effect

Ben-Shabat S, Fride E, Sheskin T, et al. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol.* 1998;353:23–31.



A “buzz” word

Hundreds of different “strains”

Inbred lines...
(**sativa vs. indica**).



Sensi Star Indica SAVE

12.7%	0.2%	0.2%
Avg THC	Avg CBD	Avg CBN



Super Sour Diesel Sativa SAVE

22.7%	0.8%	0.1%
Avg THC	Avg CBD	Avg CBN



Blue Dream Sativa SAVE

20.5%	0.3%	0.1%
Avg THC	Avg CBD	Avg CBN



Sour Diesel Sativa SAVE

18.8%	0.9%	0.1%
Avg THC	Avg CBD	Avg CBN



Strawberry Cough Sativa SAVE

16.2%	0.8%	0.1%
Avg THC	Avg CBD	Avg CBN



Afghan Kush Indica SAVE

0.0%	0.0%	0.0%
Avg THC	Avg CBD	Avg CBN



Trainwreck Sativa SAVE

16.1%	0.8%	0.1%
Avg THC	Avg CBD	Avg CBN



LSD Hybrid SAVE

17.2%	1.2%	0.1%
Avg THC	Avg CBD	Avg CBN



American Dream



California Indica



Fruit



Marley's Collie



Sensi Skunk



Shiva Shanti II



ISAIAH 18:4
 For so the LORD said unto me, I will take my rest, and I will consider in my dwelling place like a clear heat upon herbs, and like a cloud of dew in the heat of harvest.



ISAIAH 18:5
 For afore the harvest, when the bud is perfect, and the sour grape is opening in the flower, he shall cut off the strong with pruning hooks, and take away and cut down the branches.



EZEKIEL 34:29
 And I will raise up for them a plant of reed, and they shall be no more consumed with hunger in the land, neither bear the shame of the heathen any more.



EXODUS 30:22
 *Take thou also unto thee three principal spices, of pure myrror five hundred shekels, and of sweet cinnamon half so much, even two hundred and fifty shekels, and of sweet KINEBOISIN two hundred and fifty shekels.



PROVERBS 31:6-7
 Let her make herself like a vine by the fountain of waters, and let her bring forth her fruit like the vine, and her name shall be like the vine, and her fame shall be like the vine.



REVELATION 22:2
 In the midst of the street of it, and on either side of the river, was there the tree of life, which bare twelve manner of fruits, and yielded her fruit every month, and the leaves of the tree were for the healing of the nations.



Shiva Skunk



Skunk #1



Skunk Kush

Hundreds of different “strains”



“It is possible that chemically identical or very closely related plant material is being sold under several different names by different producers and there is no clear definition of the concept of a (cannabis)

strain” Mudge, E.M., Murch, S.J. and Brown, P.N., 2018. Chemometric Analysis of Cannabinoids: Chemotaxonomy and Domestication Syndrome. Scientific reports, 8(1), p.13090.



Current medical use....

Types Of Weed



SATIVA

Cannabis Sativa Sativa is characterized by leaflets that are more narrow, branches that are farther apart, and coloration that tends more toward spring green. Sativa Sativa plants tend to be taller and produce fewer flowers.



INDICA

Cannabis Sativa Indica is characterized by broad leaflets that offer overlap, branches that are closer together, and coloration that tends more toward deep olive green. Sativa Indica plants tend to be shorter and bushier, producing fuller, denser flower buds.

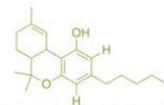


RUDERALIS

Cannabis Ruderalis is characterized by varied leaflets in the mature leaves, a shorter stature and generally small size. This subspecies is used to create S. Sativa or S. Indica hybrids with select desired traits.

www.Types-of-Weed.ORG

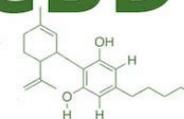
SATIVA				INDICA			
TALLER LESS DENSE & LEAVES ARE LONGER & MORE NARROW				SHORTER BUSHIER & LEAVES ARE SHORTER & WIDER			
ALERTNESS	UPLIFTING & EUPHORIC	CREATIVITY	INCREASED ENERGY	RELAXING	APPETITE STIMULANT	SLEEP AID	PAIN RELIEF
MIND DOMINANT ANTI-ANXIETY ANTI-DEPRESSANT ACTIVATING				BODY DOMINANT SEDATING MUSCLE RELAXANT REDUCE NAUSEA INCREASE DOPAMINE			



THC



CBD



Current medical use....



- Cannabis is used today (medically) mainly as palliative care....
- Can cannabis be used for treatment of medical conditions?



Current medical use....



Primary Condition	Patients, n (%)
	n = 61,379
Unspecified Chronic Pain	23,817 (38.8%)
Anxiety	8280 (13.5%)
Post Traumatic Stress Disorder	5143 (8.4%)
Back & Neck Problems	3969 (6.5%)
Arthritis	2395 (3.9%)
Insomnia	2096 (3.4%)
Cancer Related Pain	1641 (2.7%)
Depression	1249 (2.0%)
Migraines	1245 (2.0%)
Muscle Spasms	1038 (1.7%)
ADD/ADHD	1002 (1.6%)
Chronic Nausea	926 (1.5%)
Fibromyalgia	726 (1.2%)
Headaches	707 (1.2%)
Epilepsy	626 (1.0%)
Other	6519 (10.6%)

Table 5 presents a summary of the individuals reporting each primary condition overall and by gender. Any condition representing less than 1.0% was grouped as an “other”. A list of “other” primary conditions was calculated from the sample size for each respective column. *ADD* attention deficit disorder, *A*



Mahabir, V.K., Merchant, J.J., Smith, C. *et al.* Medical cannabis use in the United States: a retrospective database study. *J Cannabis Res* 2, 32 (2020).
<https://doi.org/10.1186/s42238-020-00038-w>



Current medical use....



- Cannabis is used today (medically) mainly as palliative care....
- Can cannabis be used for treatment of medical conditions?



Alzheimer's disease

- Alzheimer's disease (AD) is the most common type of dementia, accounting for 60 to 80% of cases, and is characterized by the presence in the brain of extracellular **deposits of amyloid-** ($A\beta$), a peptide derived from the aberrant processing of the transmembrane amyloid- protein precursor ($A\beta$ PP);
- AD is also associated with neuroinflammation and oxidative stress, two pathological processes that exacerbate neurodegeneration during AD progression.



Evidences that cannabis and the endocannabinoid system improve some aspects of AD

- Selective synthetic agonists of CB1 and CB2 receptors reduce cognitive impairment and brain alterations associated with A β production, in at least three different animal models of AD
- THC and CBD application reduced the pathological phenotype in mouse models of AD and tauopathy (deposition of abnormal tau protein) when administered at early stages of the disease

Aso E, Palomer E, Juves S, Maldonado R, Muñoz FJ, Ferrer I (2012) CB1 agonist ACEA protects neurons and reduces the cognitive impairment of APP/PS1 mice. *J Alzheimers Dis* 30, 439-459.

Aso E, Juves S, Maldonado R, Ferrer I (2013) CB₂ cannabinoid receptor agonist ameliorates Alzheimer-like phenotype in APP/PS1 mice. *J Alzheimers Dis* 35, 847-858.

Martín-Moreno AM, Brera B, Spuch C, Carro E, García-García L, Delgado M, Pozo MA, Innamorato NG, Cuadrado A, de Ceballos ML (2012) Prolonged oral cannabinoid administration prevents neuroinflammation, lowers γ -amyloid levels and improves cognitive performance in Tg APP 2576 mice. *J Neuroinflammation* 9, 8.

Wu J, Bie B, Yang H, Xu JJ, Brown DL, Naguib M (2013) Activation of the CB₂ receptor system reverses amyloid-induced memory deficiency. *Neurobiol Aging* 34, 791-804.

Aso E, Sanchez-Pla A, Vegas-Lozano E, Maldonado R, Ferrer I (2015) Cannabis-based medicine reduces multiple pathological processes in APP/PS1 mice. *J Alzheimers Dis* 43, 977-991.



Evidences that cannabis and the endocannabinoid system improve some aspects of AD

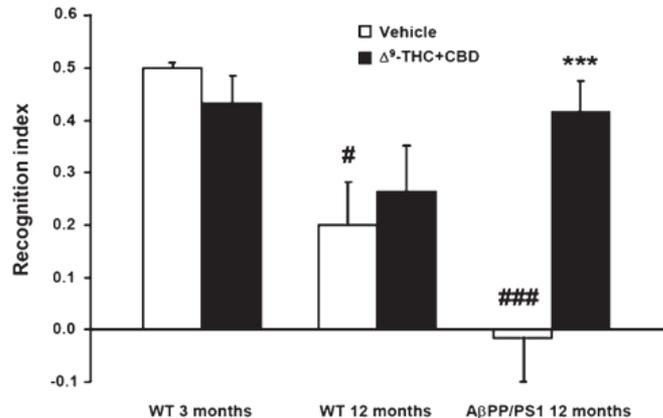


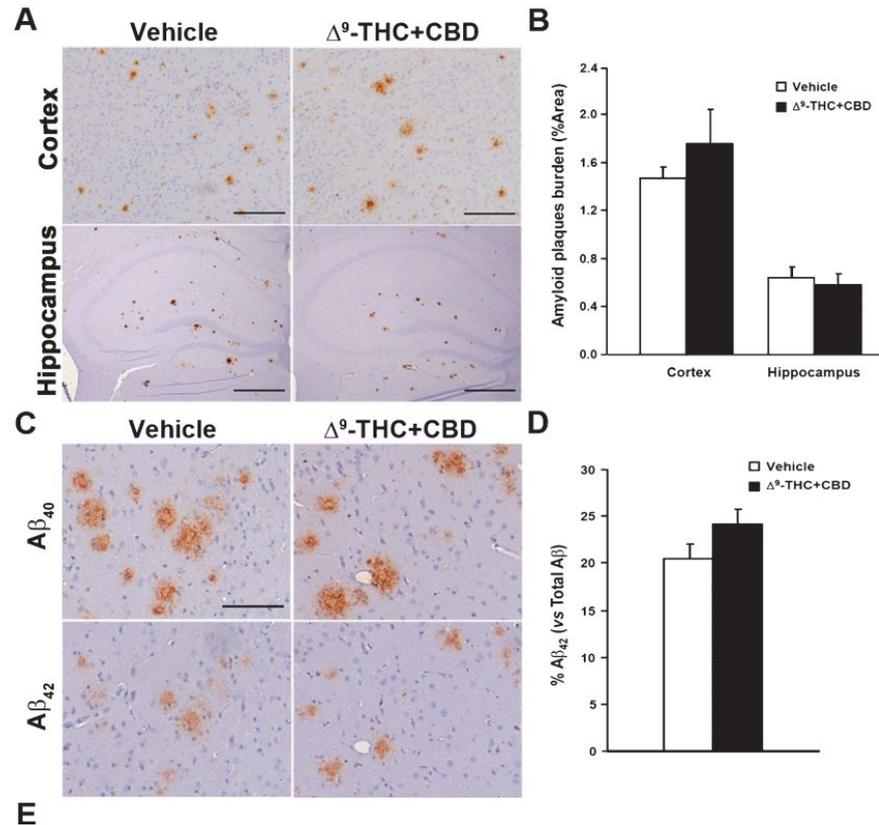
Fig. 1. Memory performance of animals in the two-object recognition test. WT and A β PP/PS1 mice aged 12 months at the beginning of the study exhibit a significant reduction in the recognition index when compared with non-aged control mice (3 months old WT mice). Daily administration of Δ^9 -THC + CBD (0.75 mg/kg each botanical extract i.p.) for 5 weeks blunts memory impairment of A β PP/PS1 mice at advanced stages (12 months). However, it is not effective in reducing memory impairment in 12-month-old WT littermates. Data are expressed as the mean values \pm SEM; *** p < 0.001 treatment effect; # p < 0.05, ### p < 0.001 compared to vehicle-treated non-aged control mice.

- THC and CBD administration blunts memory impairment of A β PP/PS1 mice at advanced stages (12 months)
- But they do not affect cognitive impairment associated with healthy aging in wild-type mice.

Exploring the two objects was recorded and an object recognition index (RI) was calculated, as the difference between the time spent exploring the novel object (TN) and the familiar object (TF) divided by the total time spent exploring the two objects

Aso, E., Andres-Benito, P. and Ferrer, I., 2016. Delineating the efficacy of a cannabis-based medicine at advanced stages of dementia in a murine model. *Journal of Alzheimer's Disease*, 54(3), pp.903-912.

THC and CBD administration do not reduce Amyloid plaques burden...



Representative images of the A β immunoreactivity in somatosensory cortex (top) and hippocampus (bottom) in vehicle- (left) and 9-THC + CBD-treated (right) APP/PS1 mice.

The potential of cannabis treatments

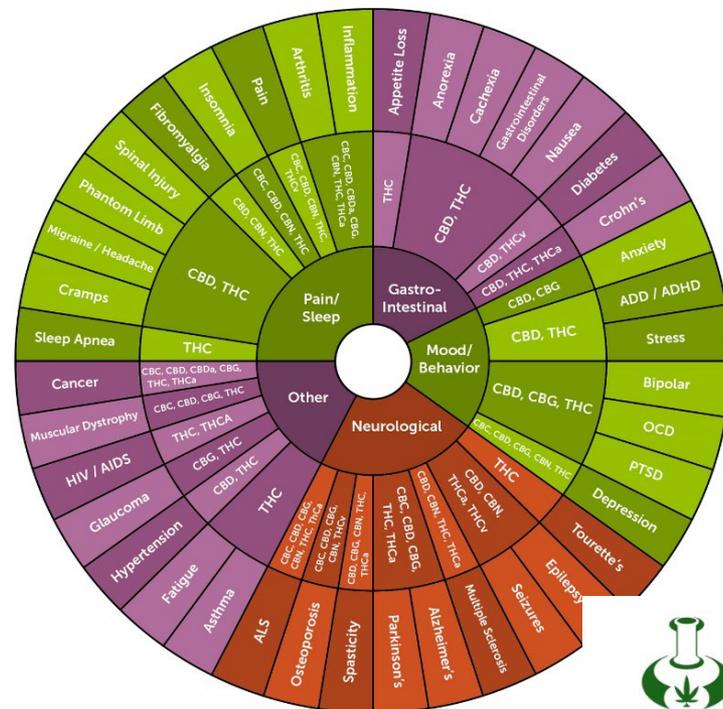
Medical cannabis use provides symptom relief for patients for different medical indications

- However there are above 500 different compounds, so what compounds are the active ones?
- Looking at the effect of THC and/or CBD is not enough.



The “entourage” effect

Ben-Shabat S, Fride E, Sheskin T, et al. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol.* 1998;353:23–31.



A “buzz” word

The potential of cannabis treatments

What is the best **composition** of active compounds for optimized treatment of each medical condition?

- Above 500 different compounds
- “Entourage” effect

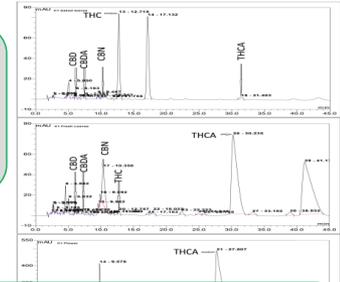


Chain of research and development- KOLTAI LAB

To determine the optimal compositions of *Cannabis* compounds for the treatment of different medical indications



We have an authorized farm in Israel for medical cannabis growth for research purposes and contacts with growers in Israel



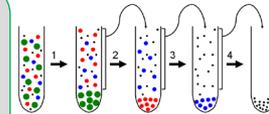
Development of cannabis-based medical products with commercial companies

Extraction protocols from different cannabis lines

Chemical analysis

Examination of medical activity in clinical trials

Separation to fractions



Examination of medical activity in bioassays (*in-vitro*, *ex-vivo*, *in printed tissues*)



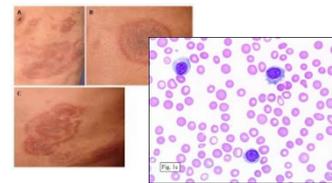
Chain of research and development- KOLTAI LAB

To determine the optimal compositions of *Cannabis* compounds for the treatment of **different medical indications**

Cannabis for the treatment of Inflammatory Bowel Diseases



Cannabis for the treatment of Cutaneous T-cell Lymphoma



Cannabis for the treatment of bladder cancer



Cannabis for the treatment of nervous system- associated disorders



Cannabis for the treatment of the COVID-19 – related acute inflammation

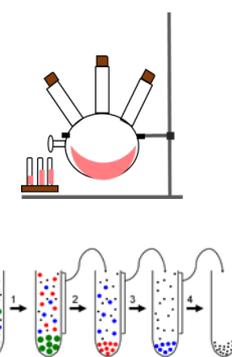
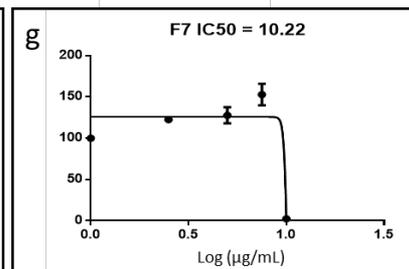
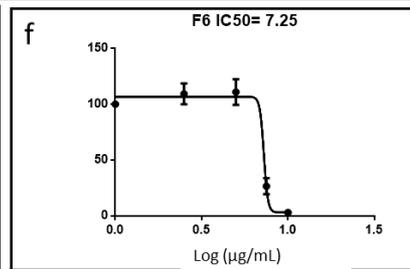
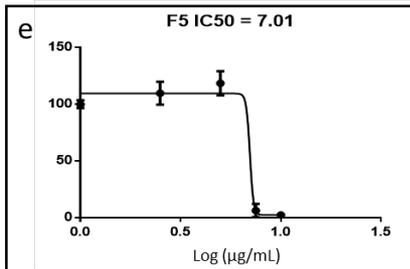
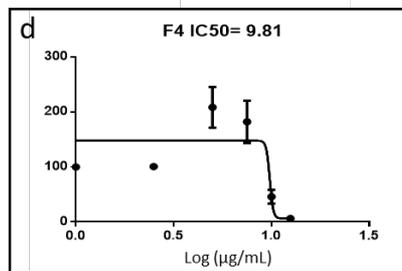
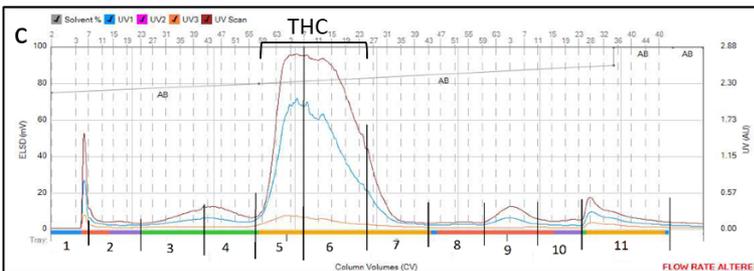
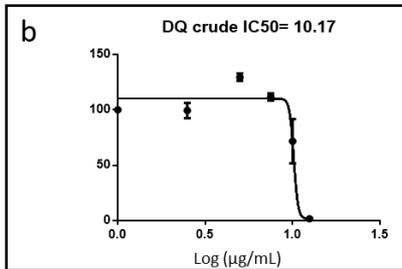
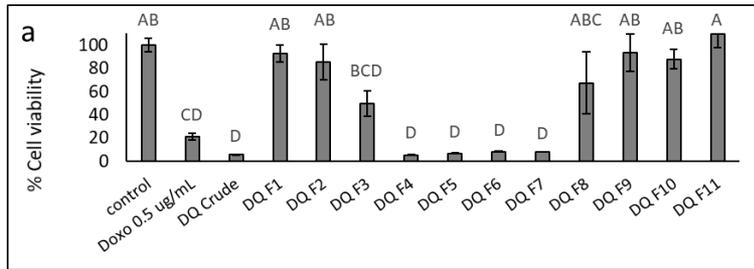


Research towards new cannabis-based drugs: The case of cannabis and glioblastoma (GBM)

- Glioblastoma multiforme (GBM) is the most frequent, invasive, and lethal subtype of glioma brain tumors.
- Cannabis is commonly used for medical treatment, and individual phytocannabinoids have been shown to trigger GBM cell death.
- However, cannabis contains hundreds of different compounds, and the optimal combinations of molecules with anti-GBM activity are unknown.



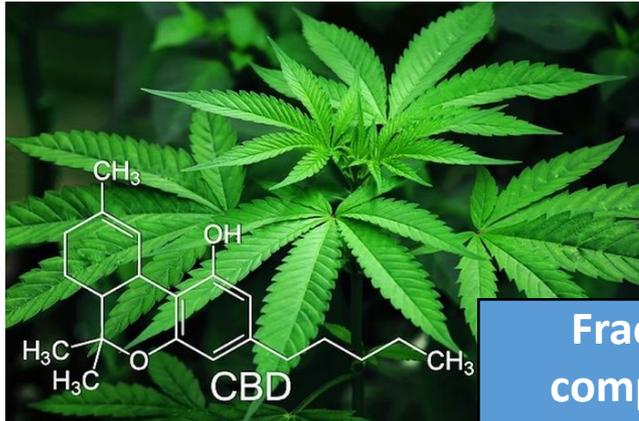
Cannabis and GBM-cell viability



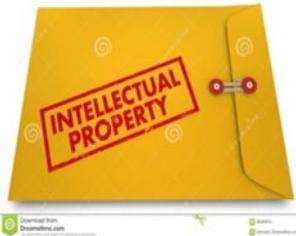
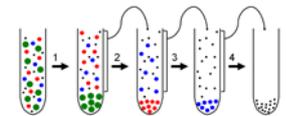
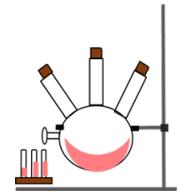
μg/mL

Active fractions were identified, their activity determined

Cannabis and GBM-active composition



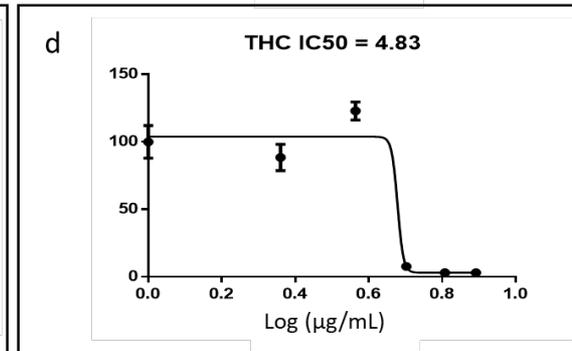
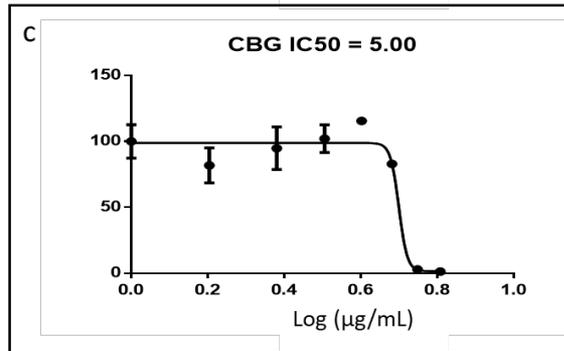
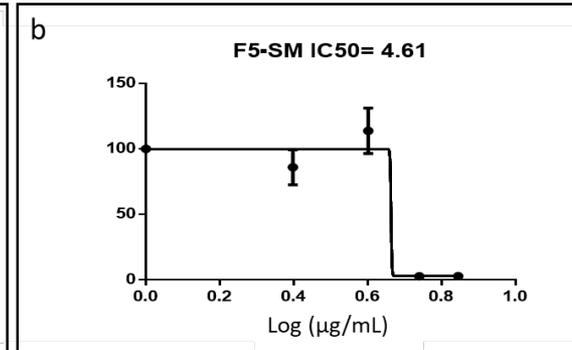
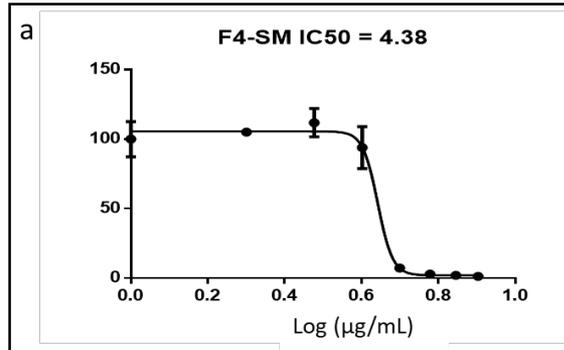
Fraction/ compound	CBD	CBG	CBN	THC	THCV
F4	5.8	80.3	2.8	-	11
F5	-	3.7	4.6	91.7	-



Active fractions composition was determined

Cannabis and GBM-active composition

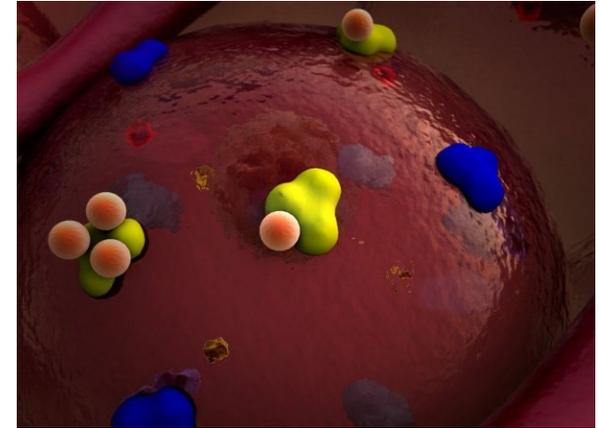
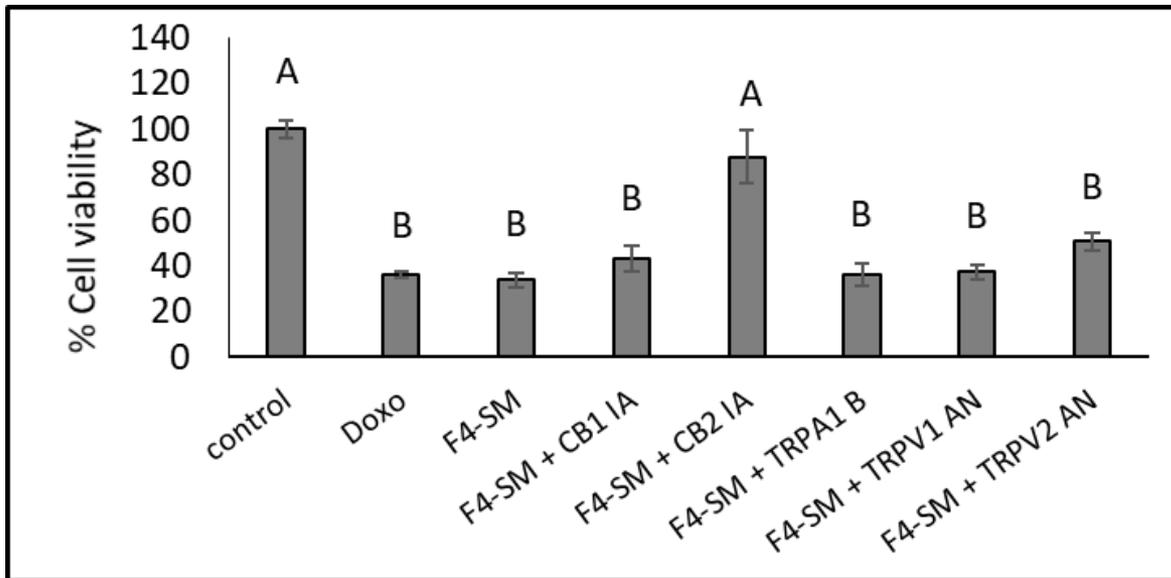
Fraction/compound	CBD	CBG	CBN	THC	THCV
F4	5.8	80.3	2.8	-	11
F5	-	3.7	4.6	91.7	-



$\mu\text{g/mL}$

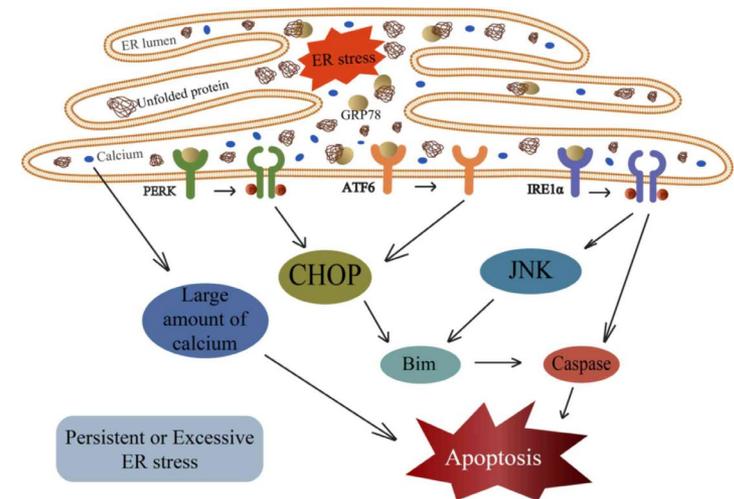
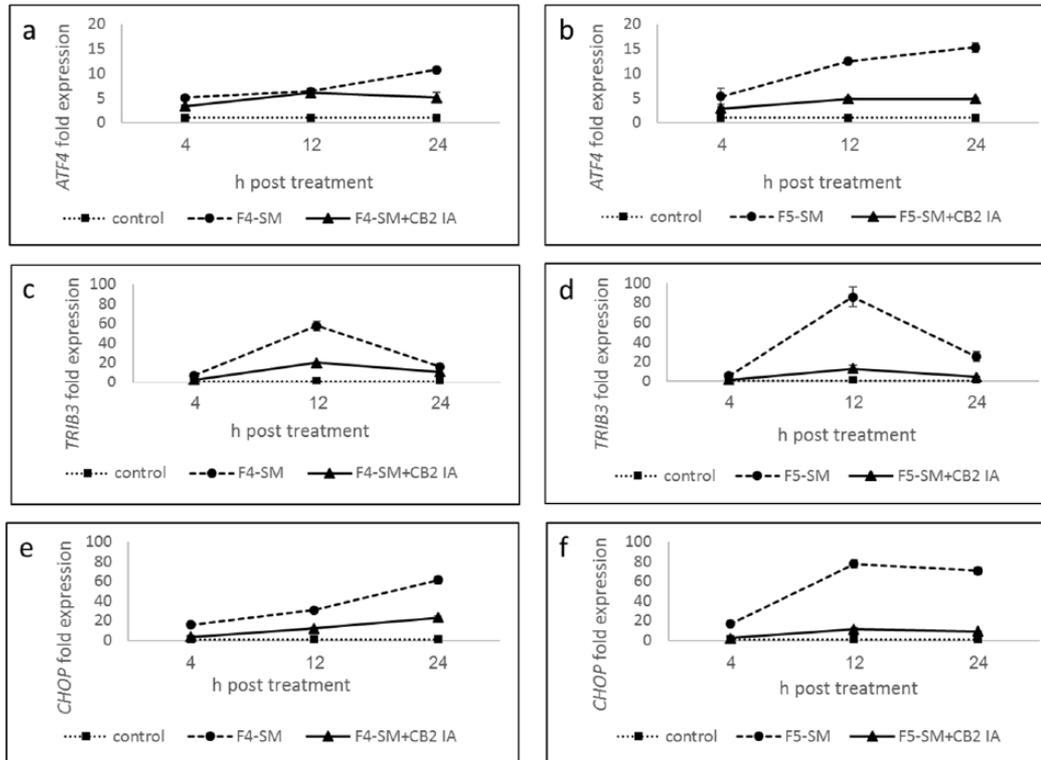
SM (mixture of API) are more active than fractions

Cannabis and GBM- partial mode of action



Activity is mediated via CB2 receptor

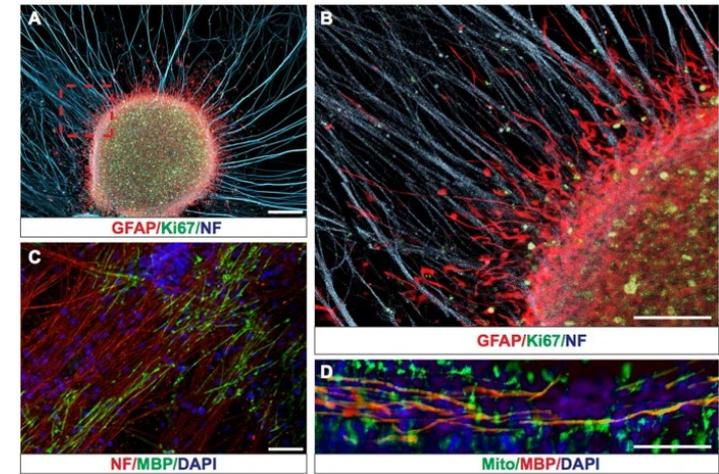
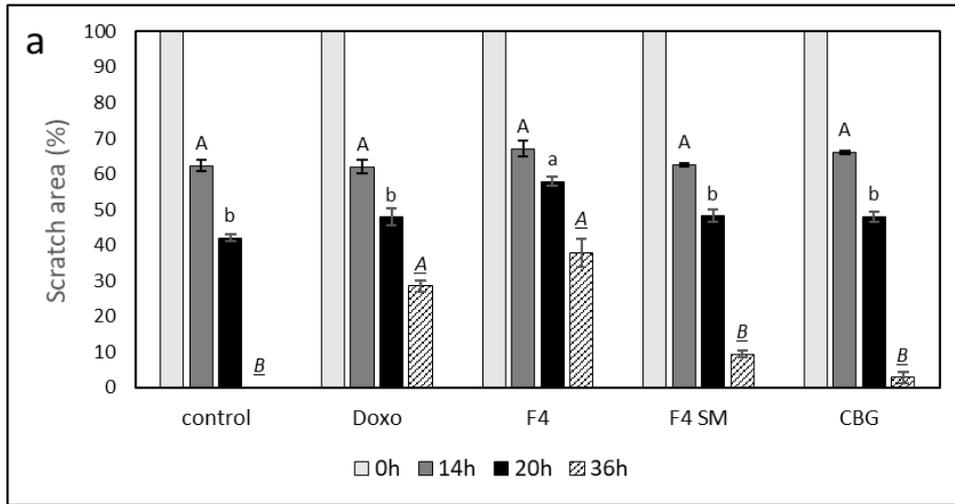
Cannabis and GBM- partial mode of action



Mao, J., Hu, Y., Ruan, L., Ji, Y., & Lou, Z. (2019). Role of endoplasmic reticulum stress in depression. *Molecular medicine reports*, 20(6), 4774-4780.

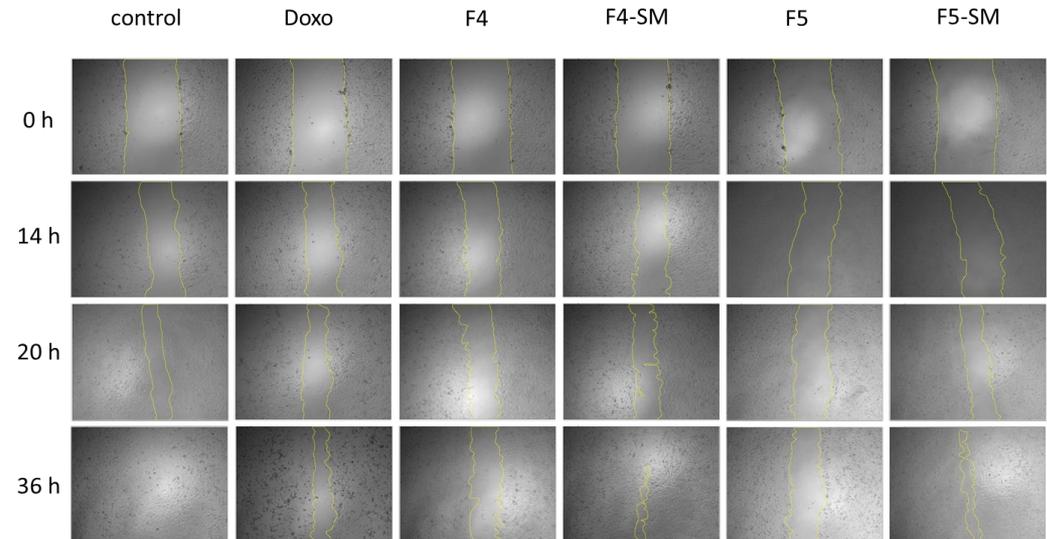
- Cell death is mediated via ER stress
- CBS IA reduced expression of these genes, again suggesting involvement of CB2 in this process

Cannabis and GBM-cell migration

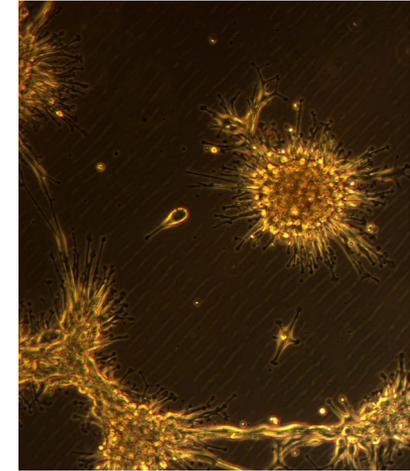
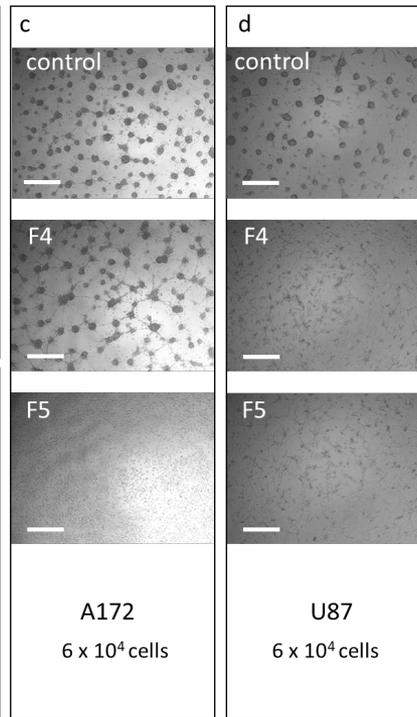
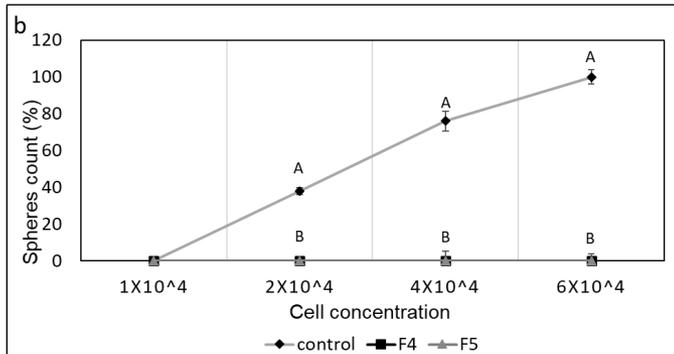
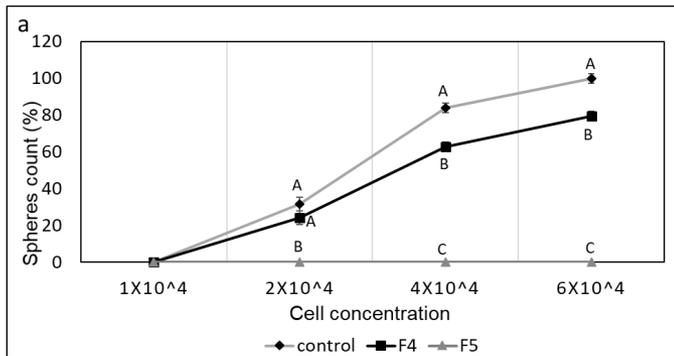


Zepecki et al., *Oncogene* 38, 1734–1750 (2019)

Fractions inhibit cell migration

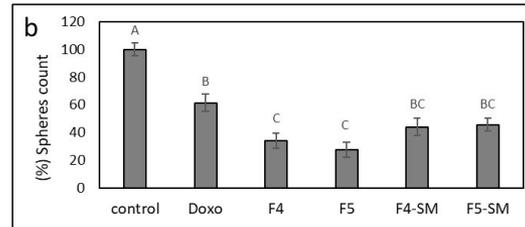
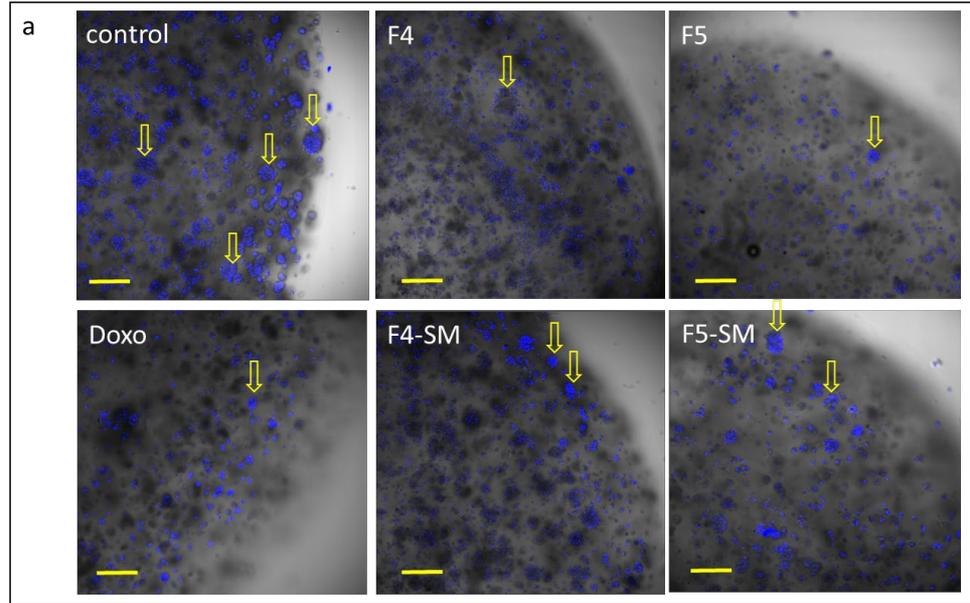
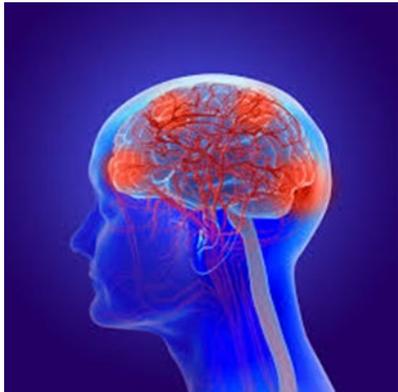


Cannabis and GBM-sphere formation



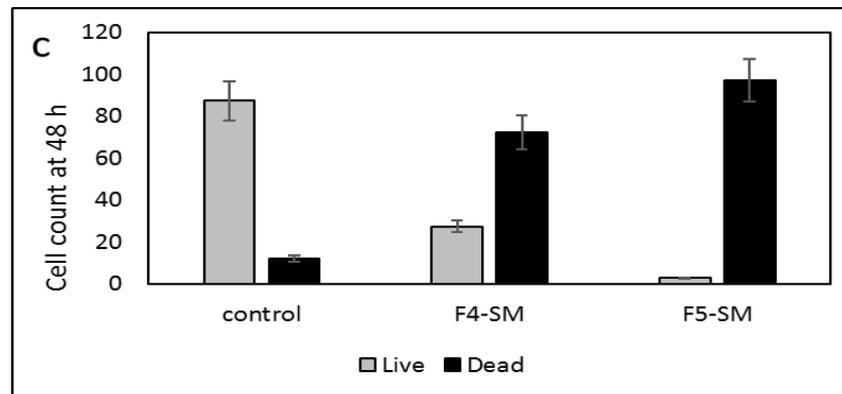
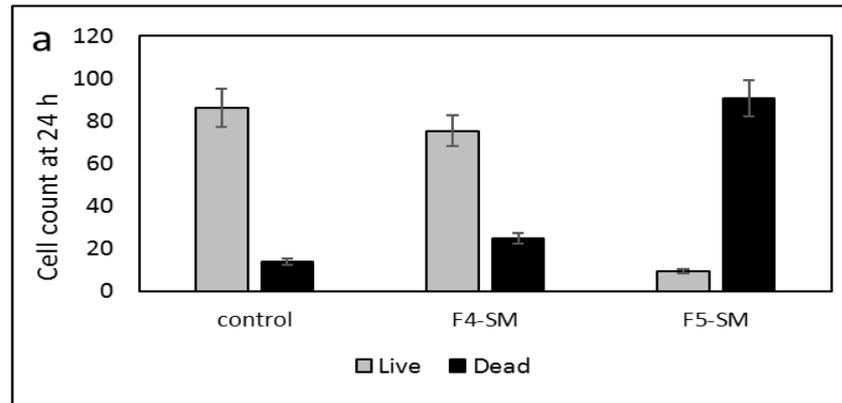
Fractions inhibit colony formation- in 2D model

Cannabis and GBM-sphere formation



Fractions inhibit colony formation- in 3D model

Effect on cells isolated from patients' GBM tumor



In collaboration
with Prof. Chaya
Brodie Lab, Bar Ilan
University

Fractions are cytotoxic to cells isolated from patients' GBM tumor.



From “weed” to molecules to drug development-Koltai lab

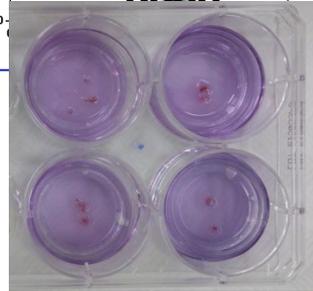
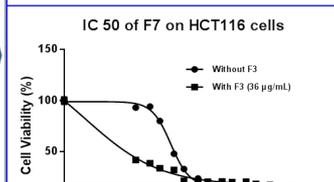
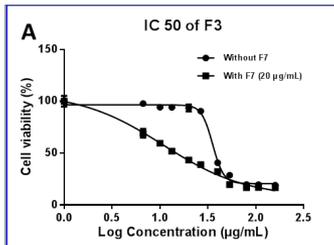
Identification of active compounds and synergies

Types Of Weed

SATIVA
Cannabis Sativa Sativa is characterized by leaflets that are more narrow, branches that are farther apart, and coloration that tends more toward spring green. Sativa Sativa plants tend to be taller and produce fewer flowers.

INDICA
Cannabis Sativa Indica is characterized by broad leaflets that are closer together, and coloration that tends more toward deep olive green. Sativa Indica plants tend to be shorter and bushier, producing fuller, denser flower buds.

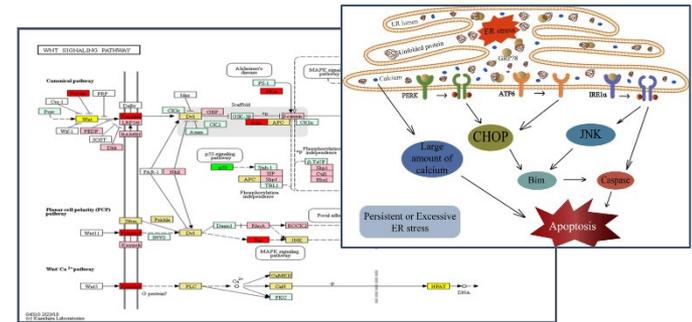
RUDERALIS
Cannabis Ruderalis is characterized by varied leaflets in the mature leaves, a shorter



Formulation API

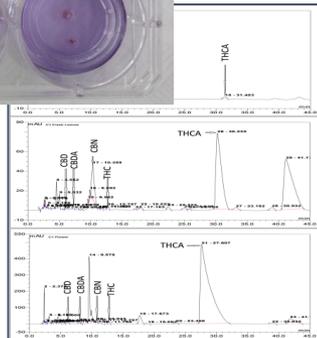
	CBD	CBG	CBN	THC	THCV
	5.8	80.3	2.8	-	11
	-	3.7	4.6	91.7	-

Targets and Mode of action



Toxicity; *in vitro* to *in vivo* extrapolations of cell assays; possible drug–drug interactions; development of drug delivery and clinical studies

Bioassays



Chemical analyses

New generation of cannabis-based products



From “weed” to molecules to drug development-Koltai lab

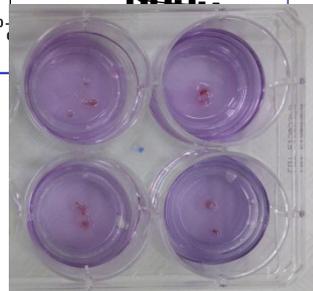
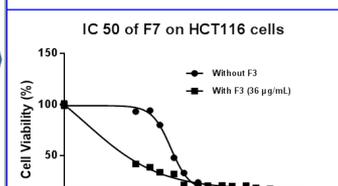
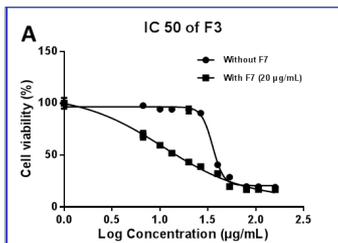
Identification of active compounds and synergies

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Cannabis Sativa Indica is characterized by broad leaflets that often overlap, branches that are closer together, and coloration that tends more toward deep olive green. Sativa Indica plants tend to be shorter and bushier, producing fuller, denser flower buds.

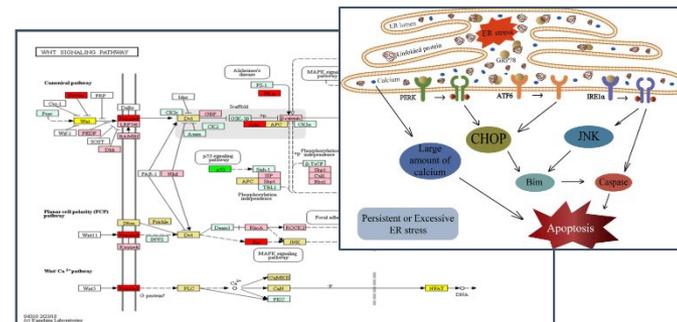
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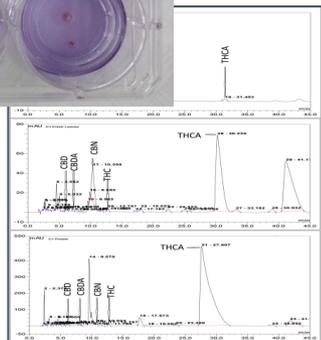
Targets and Mode of action



Development of elite chemovars



Bioassays



Chemical analyses

Acknowledgments

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- Nurit Shalev
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- Dr. Karthik Ananth
- Dr. Stalin N. Rajan
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- Dr. Einav Mayzlish-Gati

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- Rephael Nizar
- Dr. Gila Kazimirsky
- Irit Shoval

Research is evidence-based and unbiased





Thank you