Scientific evidence to support the use of phytochemicals for Lyme Borreliosis

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AONM
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Outline

1. EBM
2. Phytochemicals
3. Why Research Borreliosis
4. Phytochemicals and Borreliosis Research
5. Our Current Research
6. Future Prospects

Disclosure Statement

I am the CEO and co-founder of TriTest Oy and co-inventor of TICKPLEX and TOXIPLEX.

I have no other statement of disclosure.

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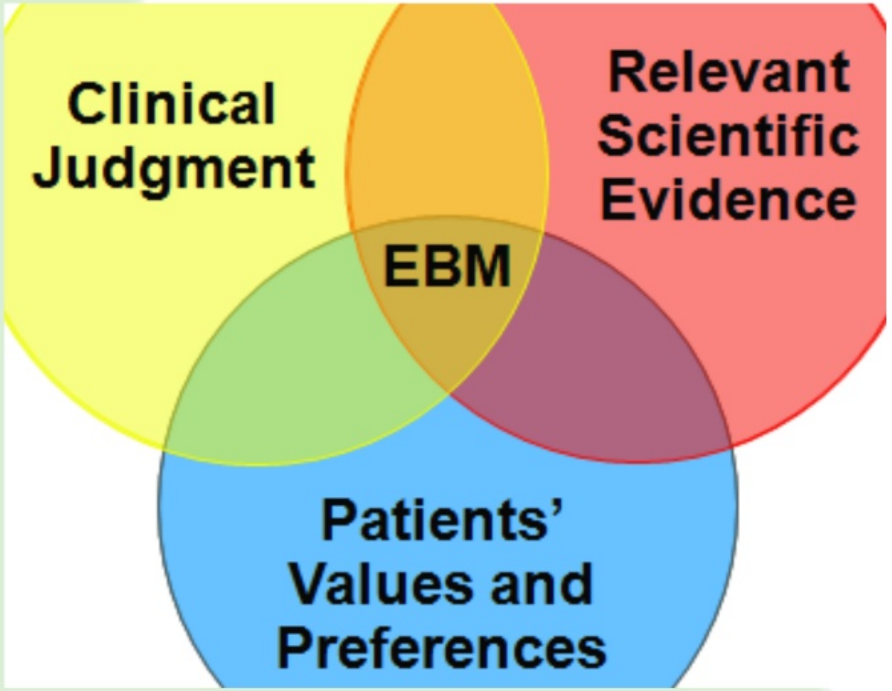
I have no other statement of disclosures.

1. Evidence Based Medicine

-conscientious, explicit, and reasonable use of modern and best research in making decisions about patient care



Sackett et al 1996

2. Phytochemicals

Medicinal plants are traditionally used all over the world.

https://www.pinterest.de/pin/120752833738224103/?lp=true

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties; especially as an antioxidant.

They protect cells by defending them against harmful free radicals.

3. Why Research Tick-borne Diseases

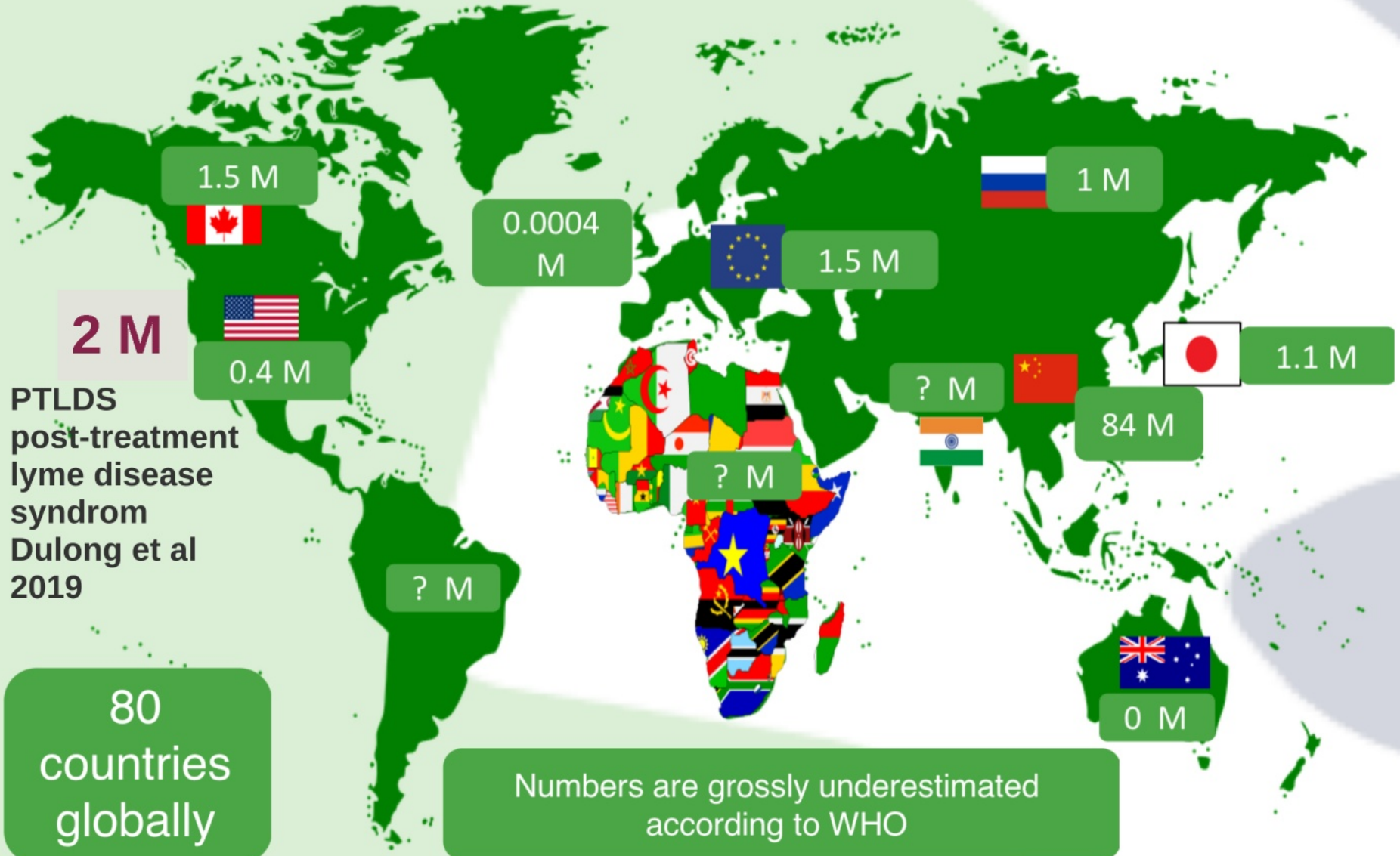
120 sec
Europe

96 sec
USA

2 sec
China

sec = seconds

3. Why Research

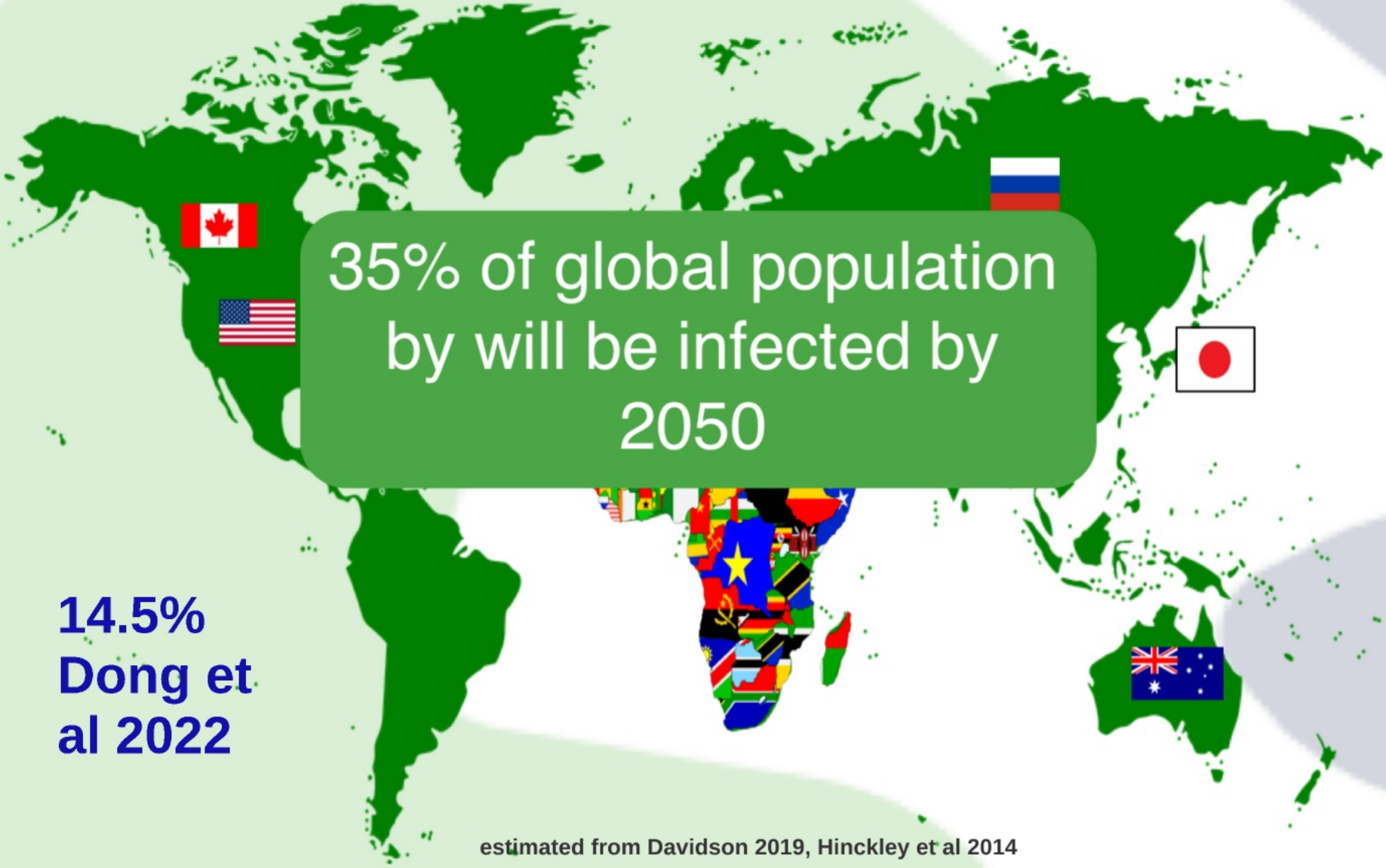


PTLDS post-treatment lyme disease syndrom Dulong et al 2019

80 countries globally

Numbers are grossly underestimated according to WHO

3. Why Research Borreliosis



14.5% Dong et al 2022

estimated from Davidson 2019, Hinckley et al 2014

3. Why Research

11 visits

Under Your Skin

20 B € to manage patients

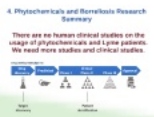
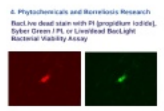
Davidsson 2018, Adrion et al 2015

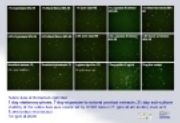
4. Phytochemicals and Borreliosis Research

-20 peer reviewed publications with experiments
-17 non-peer reviewed publications on anecdotal accounts

-1 BSc thesis (Kerns 2019)
-4 other peer reviewed publications:
reishi mushroom (Sherr 2006, anecdotal)
bee venom (Socarras et al 2017)
fish oil (Dumlao et al 2012: mouse study)
Review Thompson et al 2023

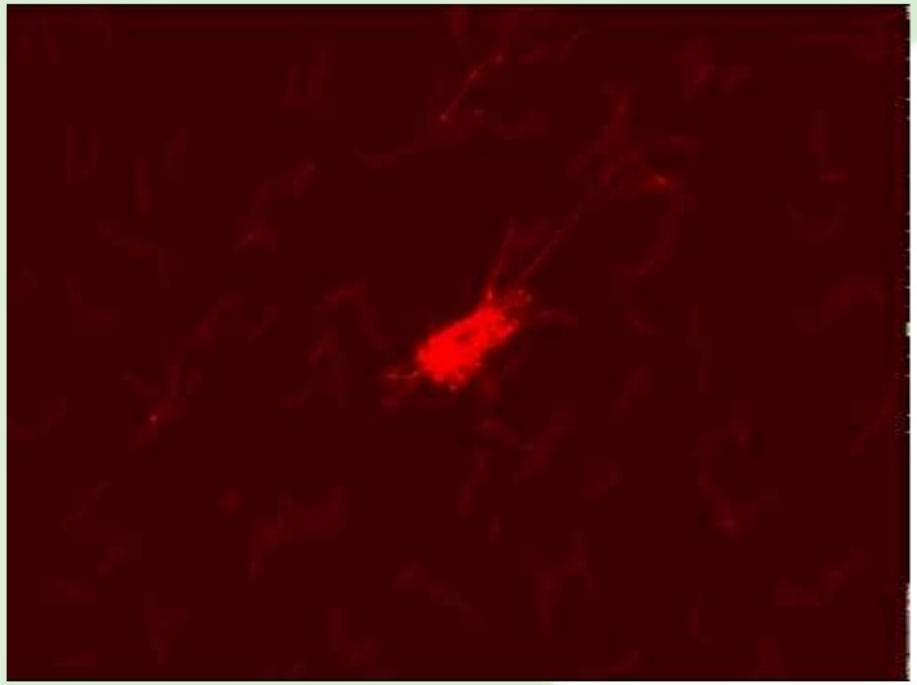


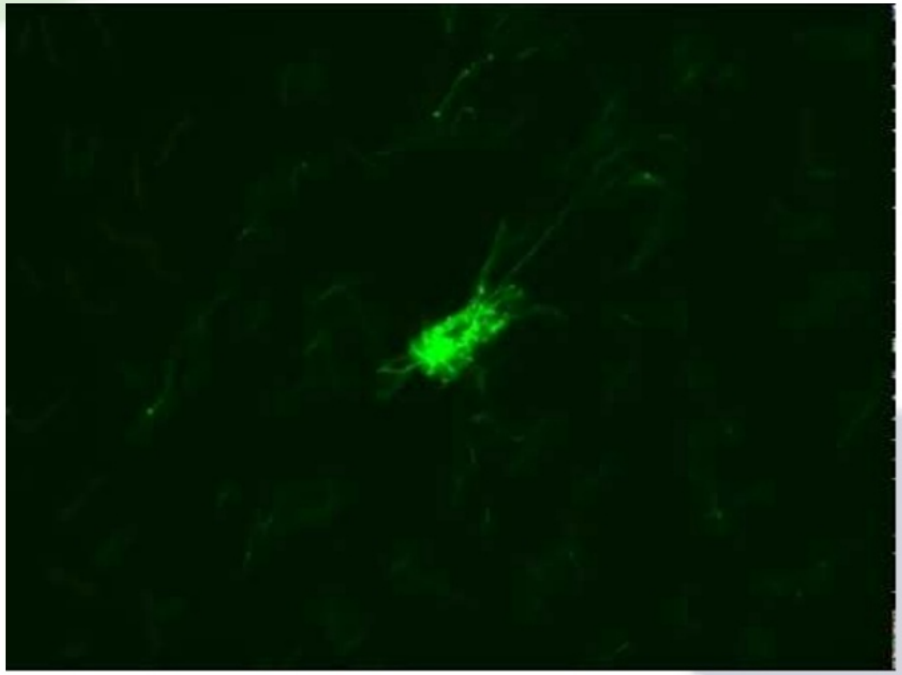





4. Phytochemicals and Borreliosis Research

BacLive dead stain with PI (propidium iodide), Syber Green / PI, or Live/dead BacLight Bacterial Viability Assay





grapefruit seed extract	2007	Brorson & Brorson	grapefruit seed extract	BacLive dead stain wth PI
Cistus creticus EO & terpenes	2010	Hutschenreuther et al	Cistus creticus oil	Counting chamber and counted not PI
Uncaria tomentosa. Samento and Otaba Parvifolia Banderal	2010	Datar et al	Uncaria tomentosa. Samento and Otaba Parvifolia Banderal	Live/dead BacLight Bacterial Viability Assay with PI
Dipsacus sylvestris teasel root extract	2011	Liebold et al	Dipsacus sylvestris teasel root extract	Counting chamber and counted not PI
Stevia Rebaudiana	2015	Theophilus et al	Stevia Rebaudiana	Syper Green and PI
Artemisia annua	2015	Feng et al	Artemisin	Syber Green and PI
Scutellaria baicalensis Baicalein compound	2015	Goc et al	cis-2-decenoic acid, baicalein, monolaurin and kelp (iodine); whereas, only baicalein and monolaurin revealed significant activity against the biofilm. Grape seed, wild cherry, black walnut green hull, apricot seed, oregano, anise	Syber Green and PI
Artemisia annua	2016	Feng et al	Artemisinin agains round bodies, cinnemon bark	SYBR Green / propidium iodide (PI) assay
Juglans nigra Green hull extract	2016	Goc & Rath Review and	From Goc et al 2015, Grape seed, wild cherry, black walnut green hull, apricot seed, oregano, anise	Syber Green and PI
baicalein and luteoline	2016	Goc et al	doxycycline with flavones such as baicalein and luteoline	LIVE/DEAD® BacLightTM Bacterial Viability
	2016	Yarnell Review	"So while the theoretical basis for their use may be as sound as any other extrapolations for other diseases, they remain theoretical"	

20 Peer Reviewed Publications

20 Peer Reviewed Publications

Oregano, cinnamon bark, and clove	2017	Feng et al	with top five essential oils (oregano, cinnamon bark, clove bud, citronella, and wintergreen) at a low concentration of 0.25%. **oregano, cinnamon bark, and clove** bud completely eradicated all viable cells without any regrowth in subculture in fresh medium, whereas but not citronella and wintergreen	SYBR Green I/PI viability assay AND SUBCULTURING
baicalein with luteolin as well as monolaurin with cis-2-decenoic acid, baicalein and luteolin, when combined with rosmarinic acid or iodine on biofilm	2017	Goc et al	baicalein with luteolin as well as monolaurin with cis-2-decenoic acid on spriochetes and rb, baicalein and luteolin, when combined with rosmarinic acid or iodine on biofilm	SYBER Green I/IP
bilberry extract, milk thistle, echinacea, goldenseal, shiitake, white willow, garlic, grape seed extract, black walnut, raspberry, fumitory, gentian, tea tree oil, galbanum oil, lavender oil, oregano oil	2018	Karvonen et al	Biocidin LSF composed of bilberry extract (25% anthocyanosides), noni, milk thistle, echinacea (purpurea and angustifolia), goldenseal, shiitake, white willow (bark), garlic, grape seed extract (minimum 90 percent polyphenols), black walnut (hull and leaf), raspberry, fumitory, gentian, tea tree oil, galbanum oil, lavender oil (plant and flower), oregano oil (plant and flower).	Green fluorescent bacteria and spectroscopy
Allium sativum L. bulbs essential oil and cinnamon bark cinnamaldehyde sterilized the *B. burgdorferi* stationary phase culture, as shown by no regrowth during subculture,	2018	Feng et al	In subculture studies, the top five essential oil hits Allium sativum L. bulbs, Pimenta officinalis Lindl. berries, Commiphora myrrha (T. Nees) Engl. resin, Hedychium spicatum Buch.-Ham. ex Sm. flowers, and Litsea cubeba (Lour.) Pers.	SYBR Green I/PI
Bay leaf oil, Birch oil, Cassia oil, Chamomile oil German, and Thyme oil	2019	Goc et al	5 oils (Bay leaf oil, Birch oil, Cassia oil, Chamomile oil German, and Thyme oil) AGAINST SPIROCHETE AND Round Body, Bay leaf oil and Cassia oil, including their major constituents, eugenol and cinnamaldehyde ON BIOFILMS	Syber Green /PI
Labdanum and Labdanes of *Cistus creticus and C. ladanifer*	2019	RAWALDK ET AL	LADDANUM AND LABDANES CISTUS CRETICUS	COUNTING
UNCARIA TOMENTOSA SAMENTO, OTOBA PARVIFOLIA BANDERAL	2019	WEISS	UNCARIA TOMENTOSA SAMENTO, OTOBA PARVIFOLIA BANDERAL	on human cell lines. Pharmocokenetics.
Cryptolepis sanguinolenta, Polygonum cuspidatum, Juglans nigra, Artemisia annua, Uncaria tomentosa, Cistus incanus, and Scutellaria baicalensis.	2020	Feng et al	Cryptolepis sanguinolenta, Polygonum cuspidatum, Juglans nigra, Artemisia annua, Uncaria tomentosa, Cistus incanus, and Scutellaria baicalensis.	SYBR Green I and PI (propidium iodide) with subculturing. But viability with subculturing
Dipsacus fullonum L., also known as wild teasel	2022	Saar-Reismaa et al	*Dipsacus fullonum L.*, also known as wild teasel	Viability of B. burgdorferi, the SYBR Green/propidium iodide (PI) assay was performed as described by Feng et al. [35]. BUT viability of cells was determined using the cell viability assay WST-1. WST-1 allows colorimetric measurement of cell viability due to reduction of tetrazolium salts to water-soluble formazan by viable cells.

20 Peer Reviewed Publications

17 Anecdotal Accounts

1999	Bock	L-Carnitine, Ginkgo biloba Astragallus
2006	Schreibert et al	α-Lipoic
2006	Ha et al	α-Lipoic
2006 a, b, c,	Vojdani & Erde	Boswellia, Parsley extract, Echinacea, Grapeseed Extract, Boswellia, Royal Jelly(
2007	Arthur	Cumada, Burbur, Parsley extract,, Nettle, Devil's claw, Samento, Cumada, Burbur and Dr. Lee Cowden's protocol
2007	Duke	Oregano tea, Allicin, Echinacea Dragon's blood, Cat's Claw
2007	Vojani et al	Review, Parsley extract,
2007	Beltran et al	Capsaicin
2007	Nicolson	Ginkgo biloba
2007	Rauwald	Cistus creticus
2008	Rauwald et al	Cistus cretius
2009	Hückel and Rauwald	Cistus creticus
2009	Voljani et al	Review, Astragallus, Parsley extract, astragallus, Curcumin, thyme, Red chili pepper, Quercetin, grape seek, wild cherry, black walnut, green hull, white peony, apricot seed, olive leaf, oregano, anis, sage
2012	Wolf-Dieter	nettle yarrow horsetail birch leaves

- According to some, the Cowden treatment improved acute and chronic Lyme symptoms in over 70 percent of the patients in whom he prescribed the full protocol.

Based on this statement it was recommended by some societies.

Cistus creticus EO & terpenes	2010	Hutschenreuther et al
Dipsacus sylvestris teasel root extract	2011	Liebold et al
Oregano, cinnamon bark, and clove	2017	Feng et al
bilberry extract, milk thistle, echinacea, goldenseal, shiitake, white willow, garlic, grape seed extract), black walnut, raspberry, fumitory, gentian, tea tree oil, galbanum oil, lavender oil, oregano oil	2018	Karvonen et al
Allium sativum L. bulbs essential oil and cinnamon bark cinnamaldehyde sterilized the *B. burgdorferi* stationary phase culture, as shown by no regrowth during subculture,	2018	Feng et al
Labdanum and Labdanes of *Cistus creticus and C. ladanifer*	2019	Rauwald et al
Cryptolepis sanguinolenta, Polygonum cuspidatum, Juglans nigra, Artemisia annua, Uncaria tomentosa, Cistus incanus, and Scutellaria baicalensis.	2020	Feng et al
Dipsacus fullonum L., also known as wild teasel	2022	Saar-Reismaa et al

***In vitro* studies that use other methods than PI (propidium iodide) such as subculturing**

4. Phytochemicals and Borreliosis *In vitro* Research Summary (8)

Dipsacus sylvestris, teasel root extract Liebold et al 2011, Saar-Reismaa et al 2022

oregano, cinnamon bark and clove essential oils Feng et al 2017

Labdanum and Labdanes of Cistus creticus, rockrose. Hutschenreuther et al 2010, Rawald et al 2019

Biocidin, Karvonen & Gilbert 2018

bilberry extract, milk thistle, echinacea , goldenseal, shiitake, white willow, garlic, grape seed extract, black walnut, raspberry, fumitory, gentian, tea tree oil, galbanum oil, lavender oil, oregano oil

Garlic, All spice, Myrrh gum, spiked ginger lily, and mountain pepper. Feng et al 2018

Scutellaria baicalensis
Chinese skullcap

Feng et al
2020

Juglans nigra
Eastern Black Walnut

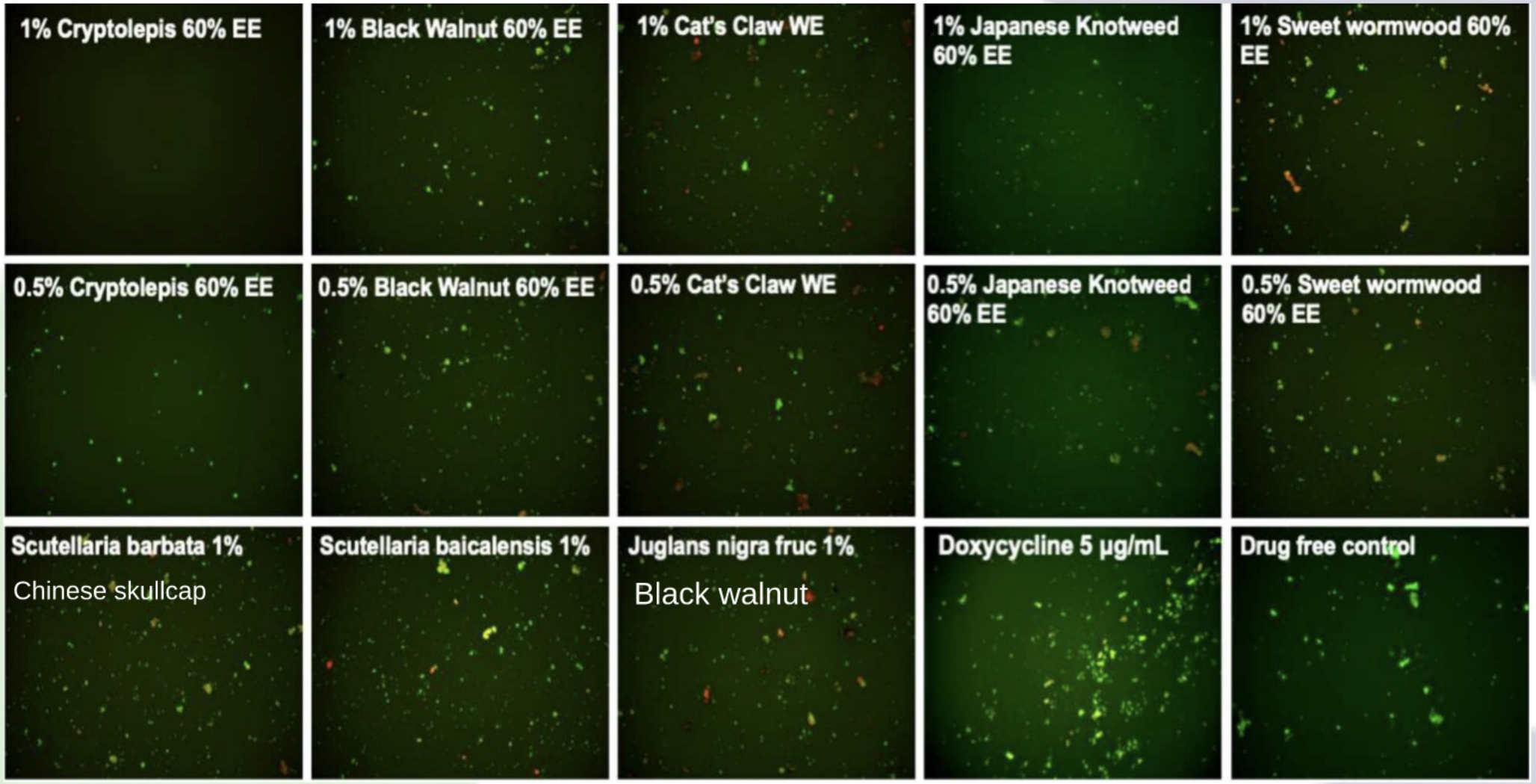
Cryptolepis sanguinolenta
root known as Ghanaian quinine

Polygonum cuspidatum
Japanese Knotwood

Artemisia annua
Sweet wormwood

Uncaria tomentosa
Cat's Claw Samento

Cistus incanus
Hairy Rock-rose



Subculture of *Borrelia burgdorferi*

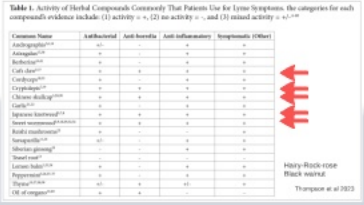
7 day stationary phase, 7 day exposure to natural product extracts, 21 day sub-culture

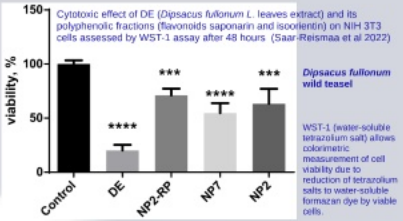
Viability of the subculture was examined by SYBR Green /PI (propidium iodide) stain and fluorescence microscopy.

Feng et al 2020

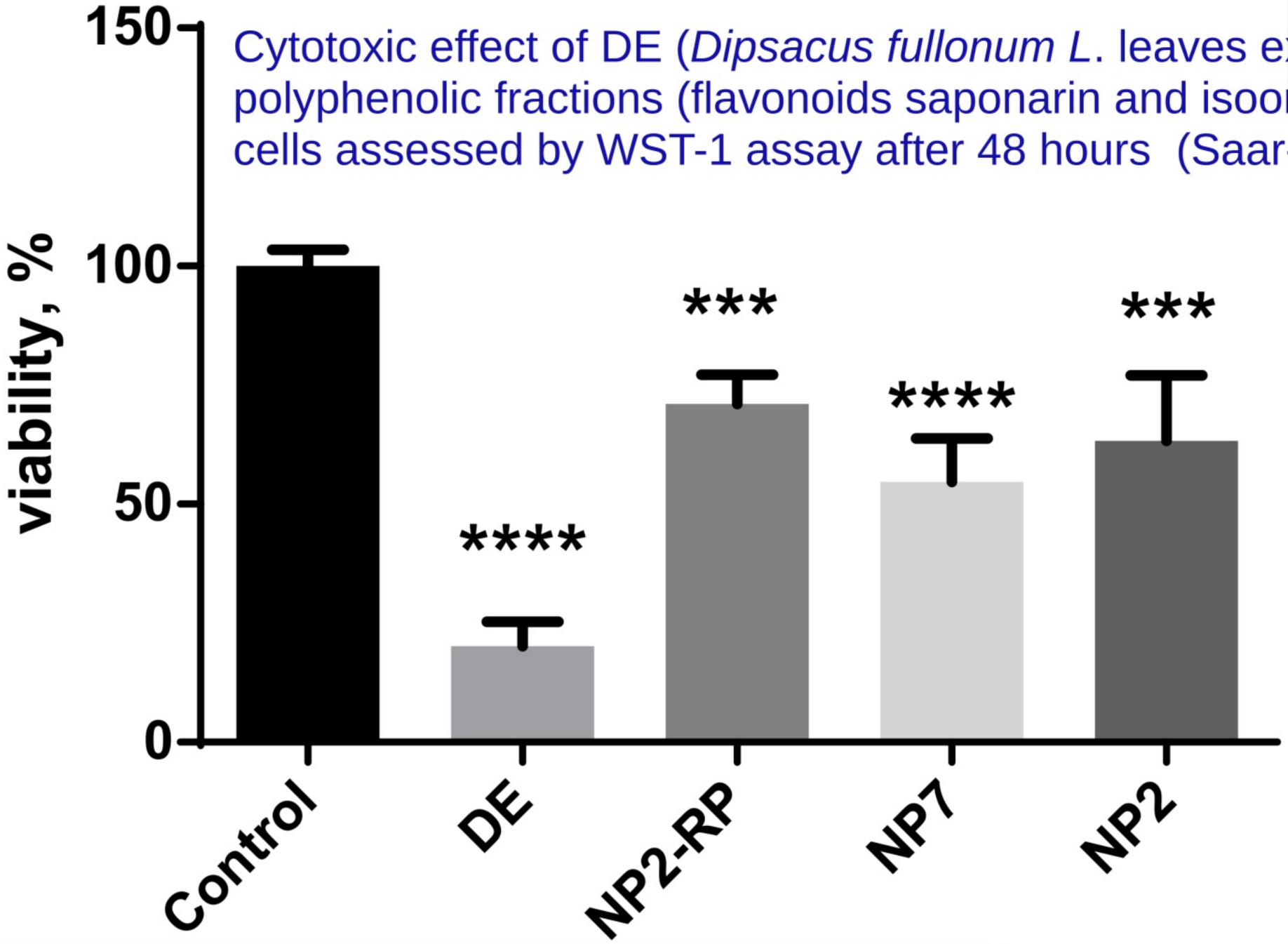
Andrographis paniculata / Green chiretta
Stevia rebaudiana (Theophilus et al 2015)
Colloidal silver (Argentyn 23TM)
Monolaurin (LauricidinTM) / coconut
Dipsacus spp. / teasel
Withania somnifera / winter cherry

Did not show significant activity against either stationary phase or growing *B. burgdorferi* in this study. Feng et al 2020





Cytotoxic effect of DE (*Dipsacus fullonum L*. leaves extract) and its polyphenolic fractions (flavonoids saponarin and isoorientin) on NIH 3T3 cells assessed by WST-1 assay after 48 hours (Saar-Reismaa et al 2022)



Dipsacus fullonum
wild teasel

WST-1 (water-soluble tetrazolium salt) allows colorimetric measurement of cell viability due to reduction of tetrazolium salts to water-soluble formazan dye by viable cells.

Table 1. Activity of Herbal Compounds Commonly That Patients Use for Lyme Symptoms. the categories for each compound's evidence include: (1) activity = +, (2) no activity = -, and (3) mixed activity = +/-[4-40]

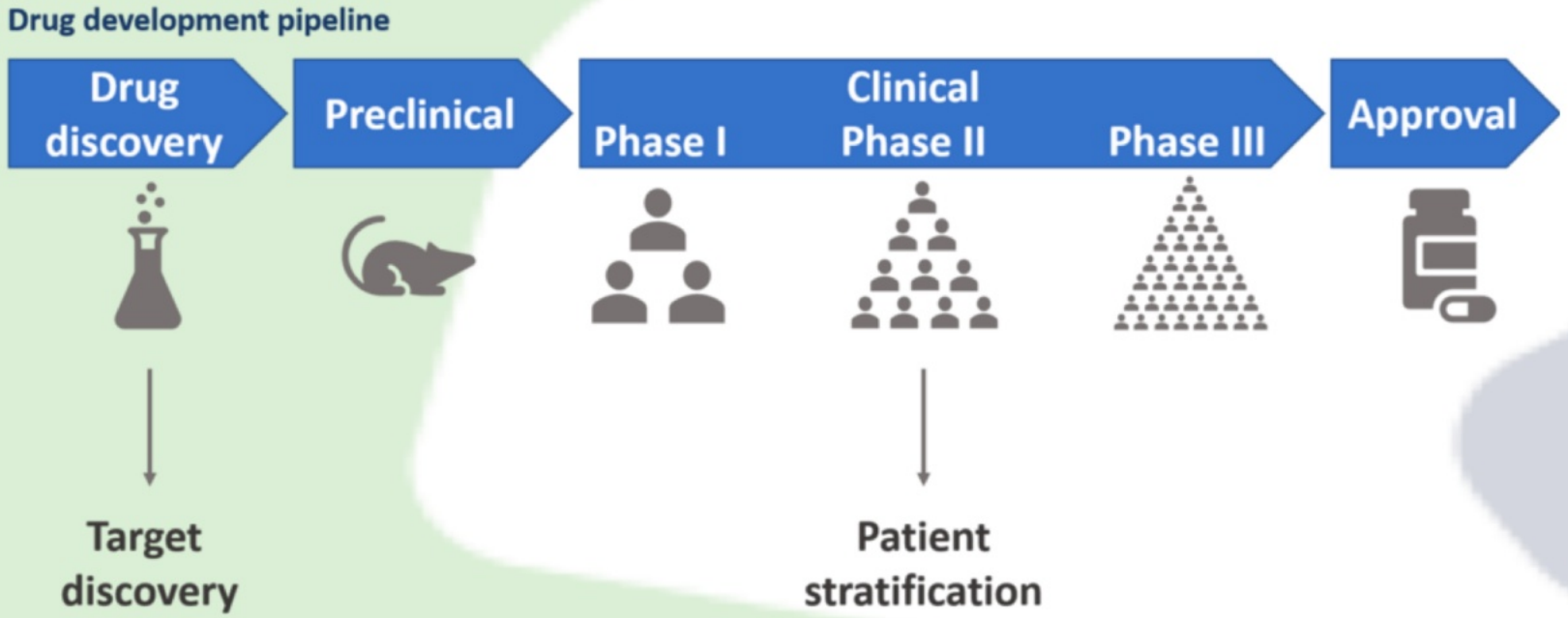
Common Name	Antibacterial	Anti-borrelia	Anti-inflammatory	Symptomatic (Other)
Andrographis[5,6,14]	+/-	-	+	+
Astragalus[15,30]	+	-	+	+
Berberine[16,41]	+	-	+	+
Cat's claw[5,17]	+	+	+	+
Cordyceps[18,31]	+	-	+	+
Cryptolepis[5,19]	+	+	+	+
Chinese skullcap[5,20,28]	+	+	+	+
Garlic[21,22]	+	-	+	+
Japanese knotweed[5,7,8]	+	+	+	+
Sweet wormwood[5,9,10,29,32,54]	+	+	+	+
Reishi mushrooms[23]	+	-	-	+
Sarsaparilla[11,24]	+/-	-	+	+
Siberian ginseng[25]	-	-	+	+
Teasel root[13]	-	-	-	-
Lemon balm[5,33,34]	+	-	+	+
Peppermint[5,26,35-37]	+	-	+	+
Thyme[13,27,38,39]	+/-	+	+/-	+
Oil of oregano[12,40]	+	+	-	-

Hairy-Rock-rose
Black walnut

Thompson et al 2023

4. Phytochemicals and Borreliosis Research Summary

There are no human clinical studies on the usage of phytochemicals and Lyme patients. We need more studies and clinical studies.



5. Our Current Research: Karvonen & Gilbert 2018

AIM: To investigate the effects of the phytochemical antimicrobial compounds on the life cycle and morphology of the *B. burgdorferi* cultured *in vitro* at +37°C.

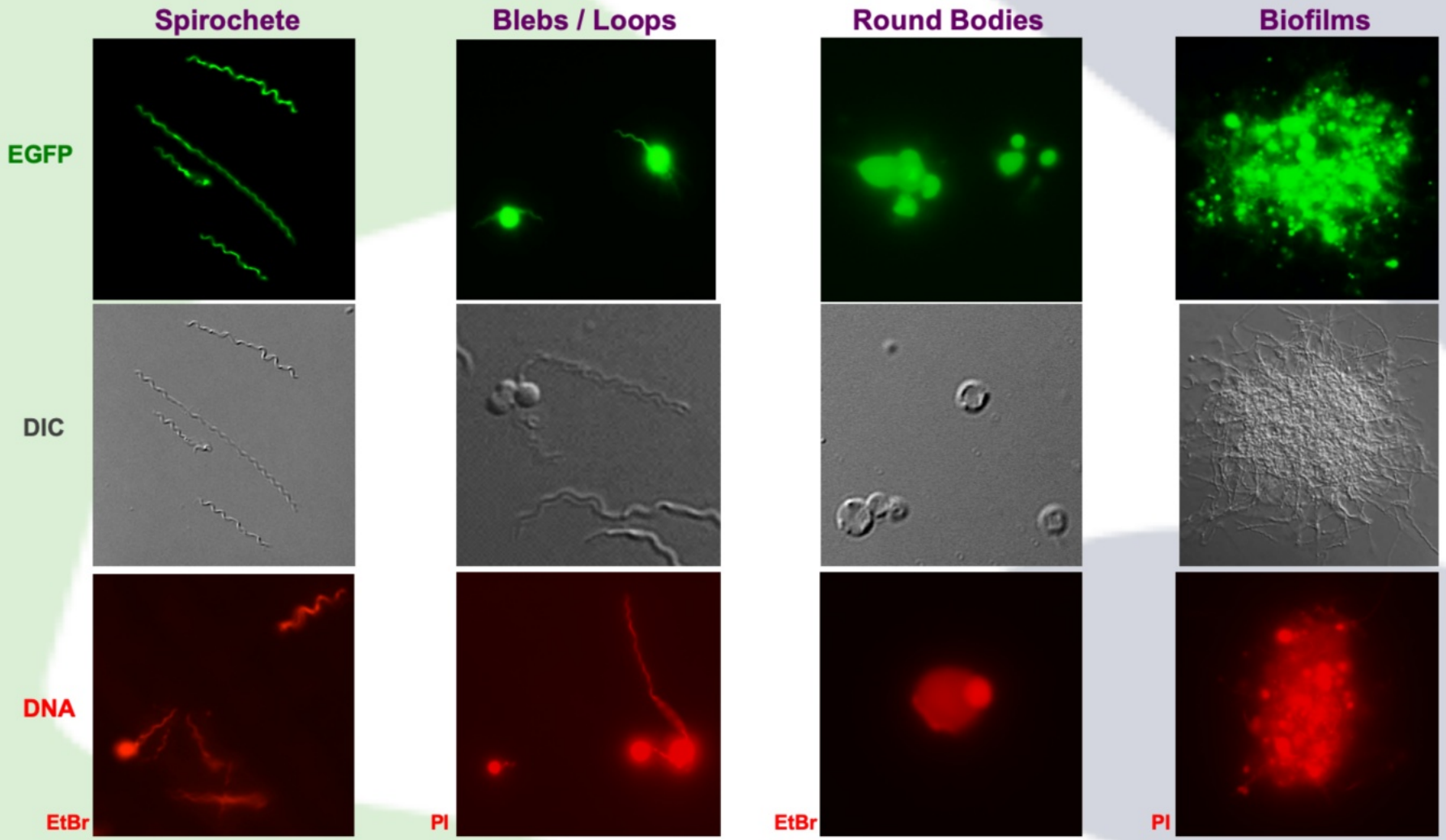
HOW:

1. MIC - minimum initial concentration assay; lowest concentrations that will inhibit visible growth (Ates et al. 2010)

2. MBC - minimum bactericidal death assay; minimum concentration beyond which no bacteria can be sub-cultured (Ates et al. 2010)

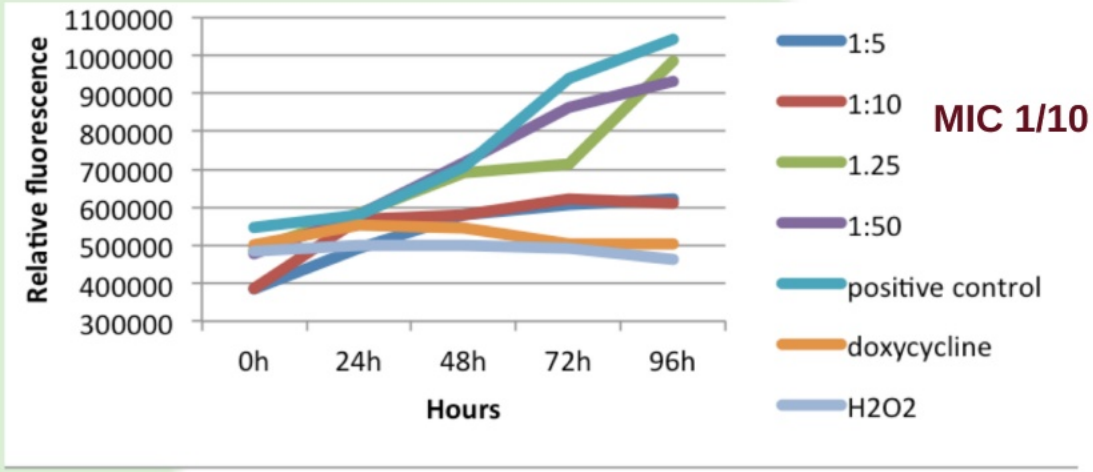
3. Death Curve - effectiveness / quickness of death

4. Checkerboard Method - possible synergetic effect with antibiotics.

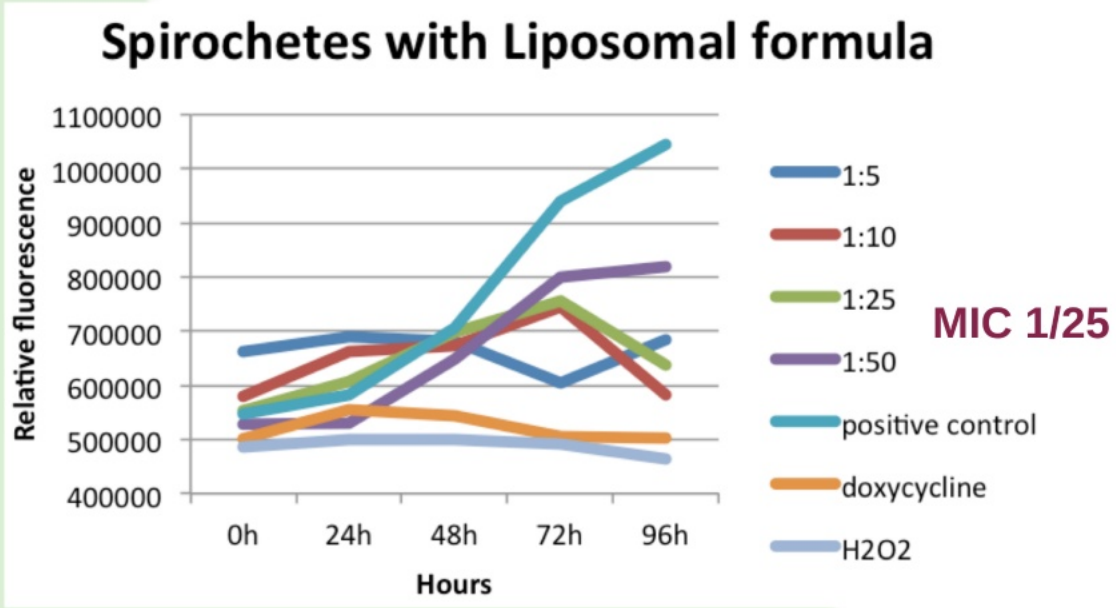


EGFP: enhanced green fluorescent protein
EtBr: Ethidium bromide

DIC: Differential Interference Contrast
PI: Propidium iodine



MIC 1/10

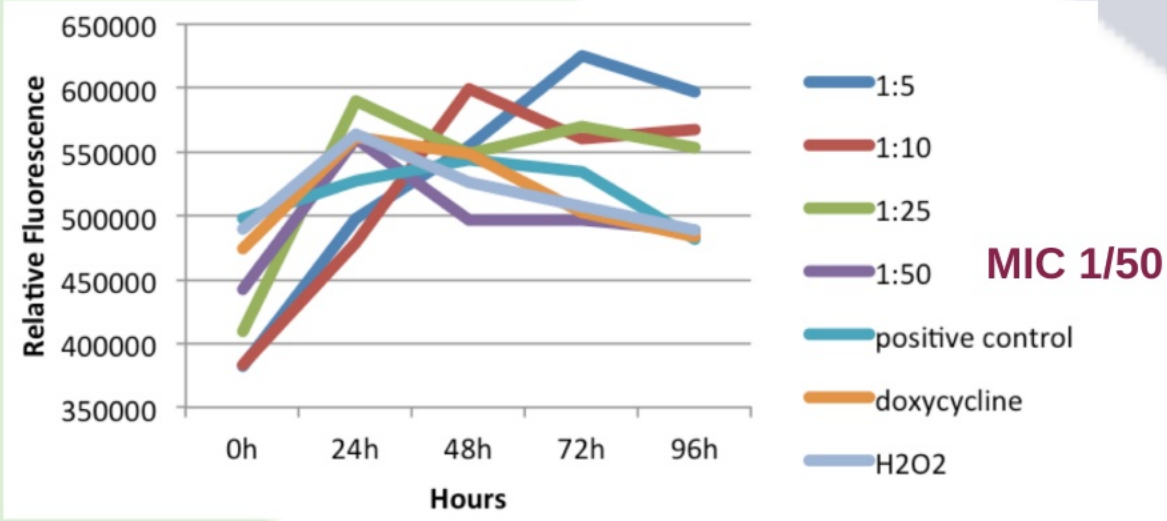


MIC 1/25

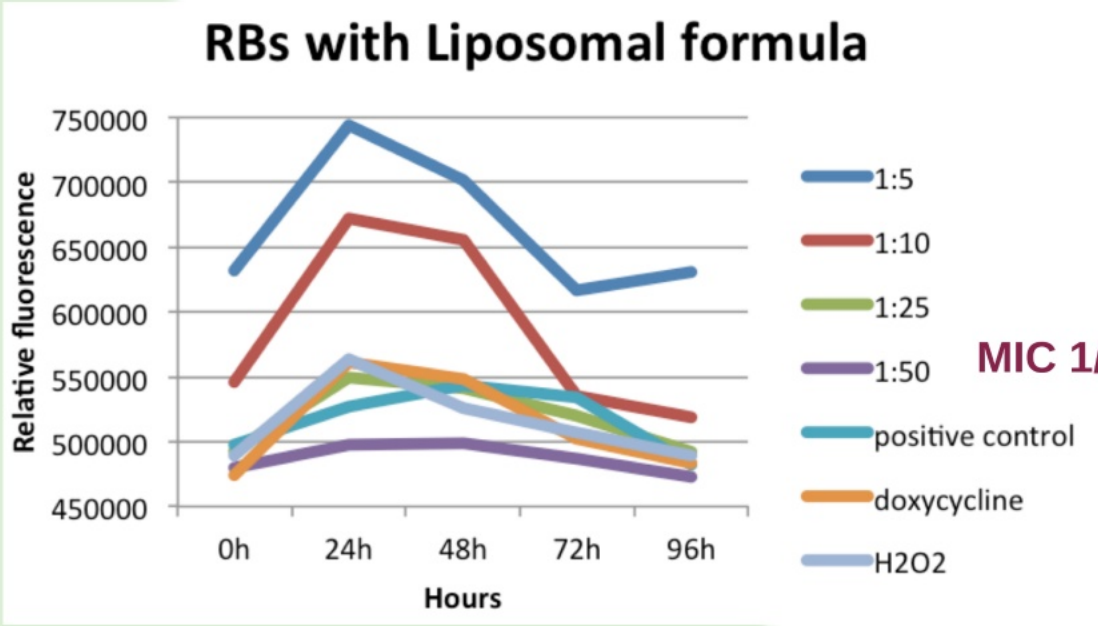
MBD (minimum bactericidal death assay) confirmed MIC (minimum initial concentration assay) results

Karvonen & Gilbert 2018

RB: Round Bodies



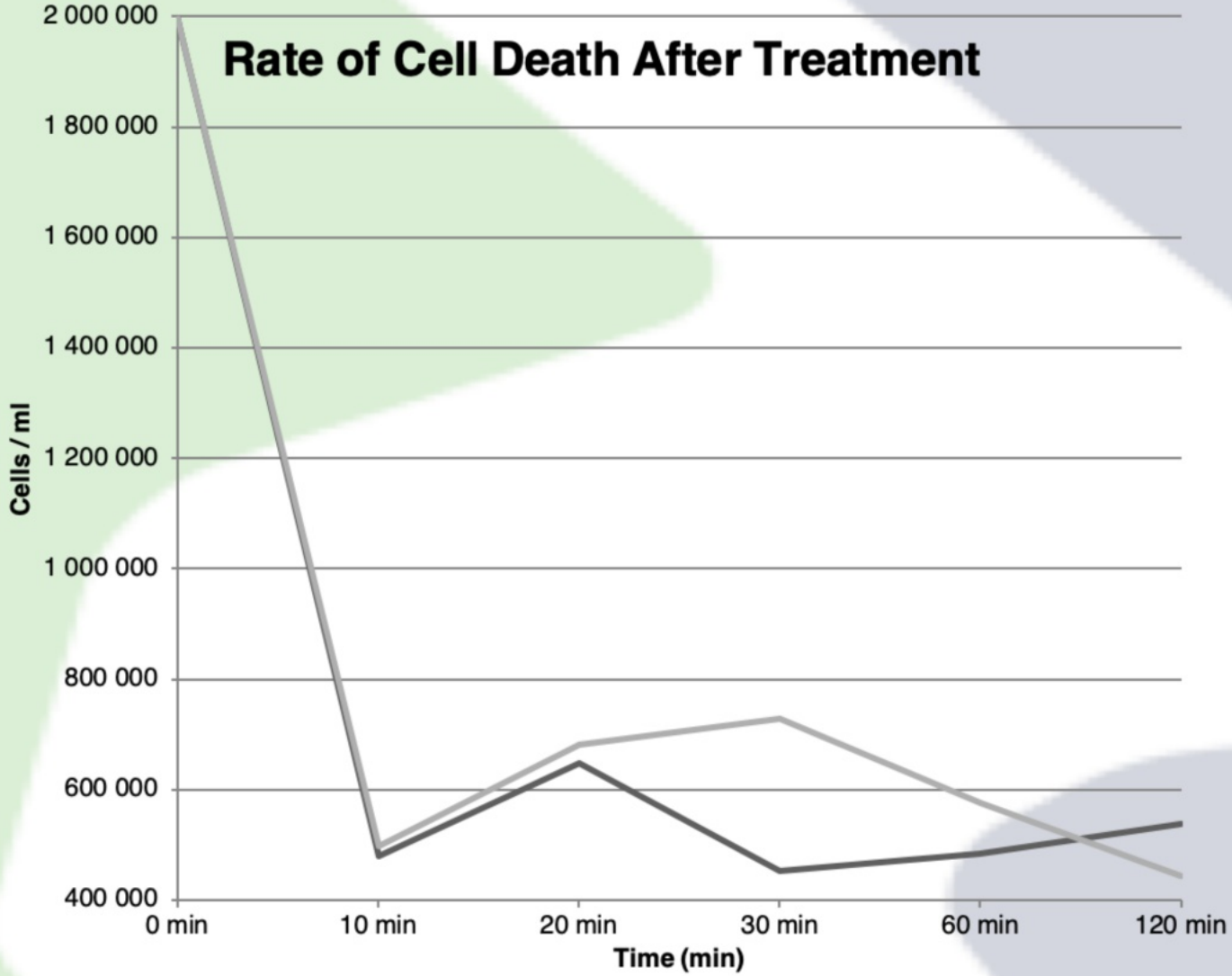
MIC 1/50



MIC 1/50

MBD confirmed MICs

Karvonen & Gilbert 2018

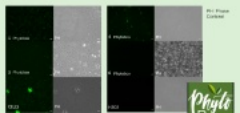


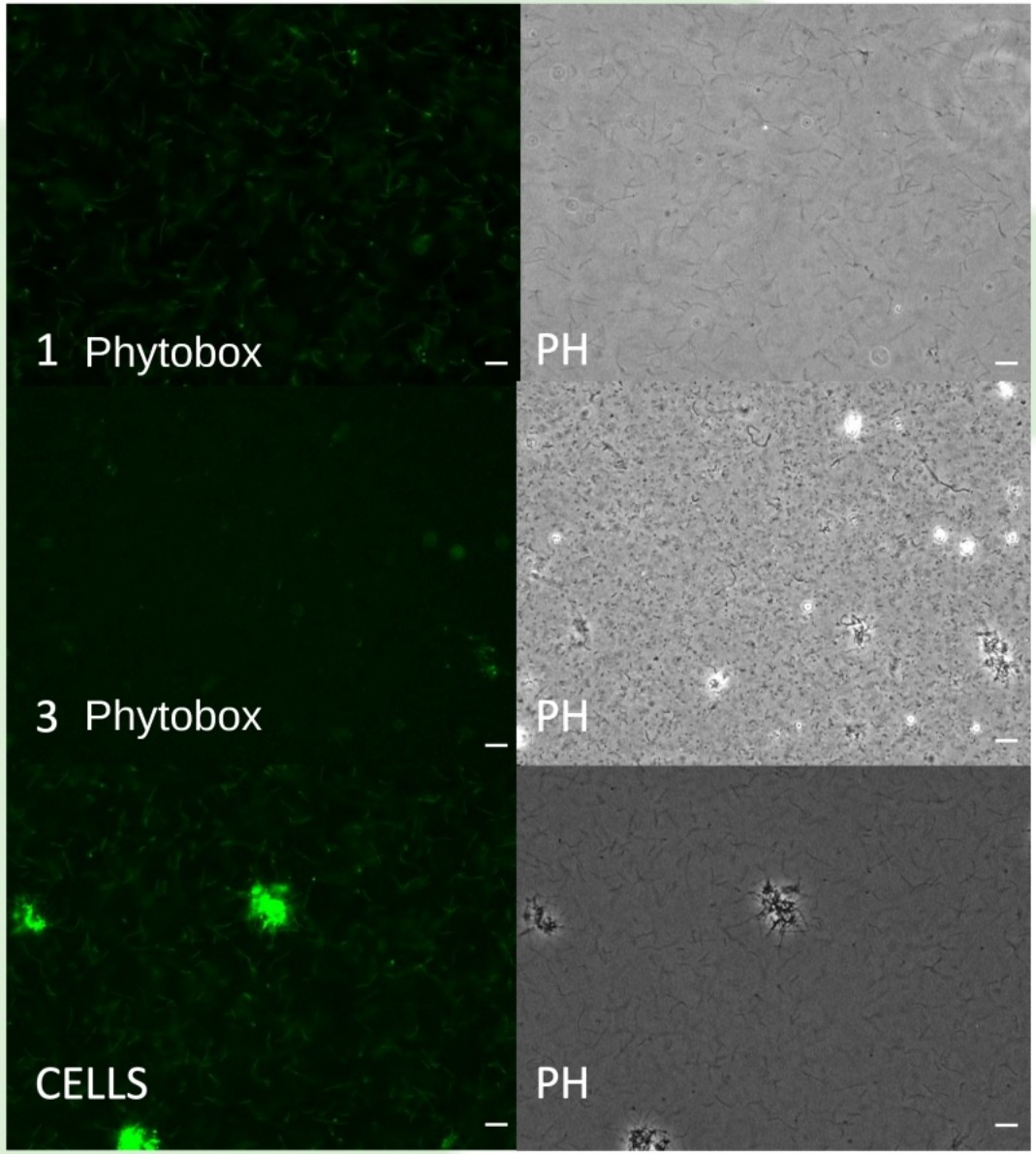
Karvonen & Gilbert 2018

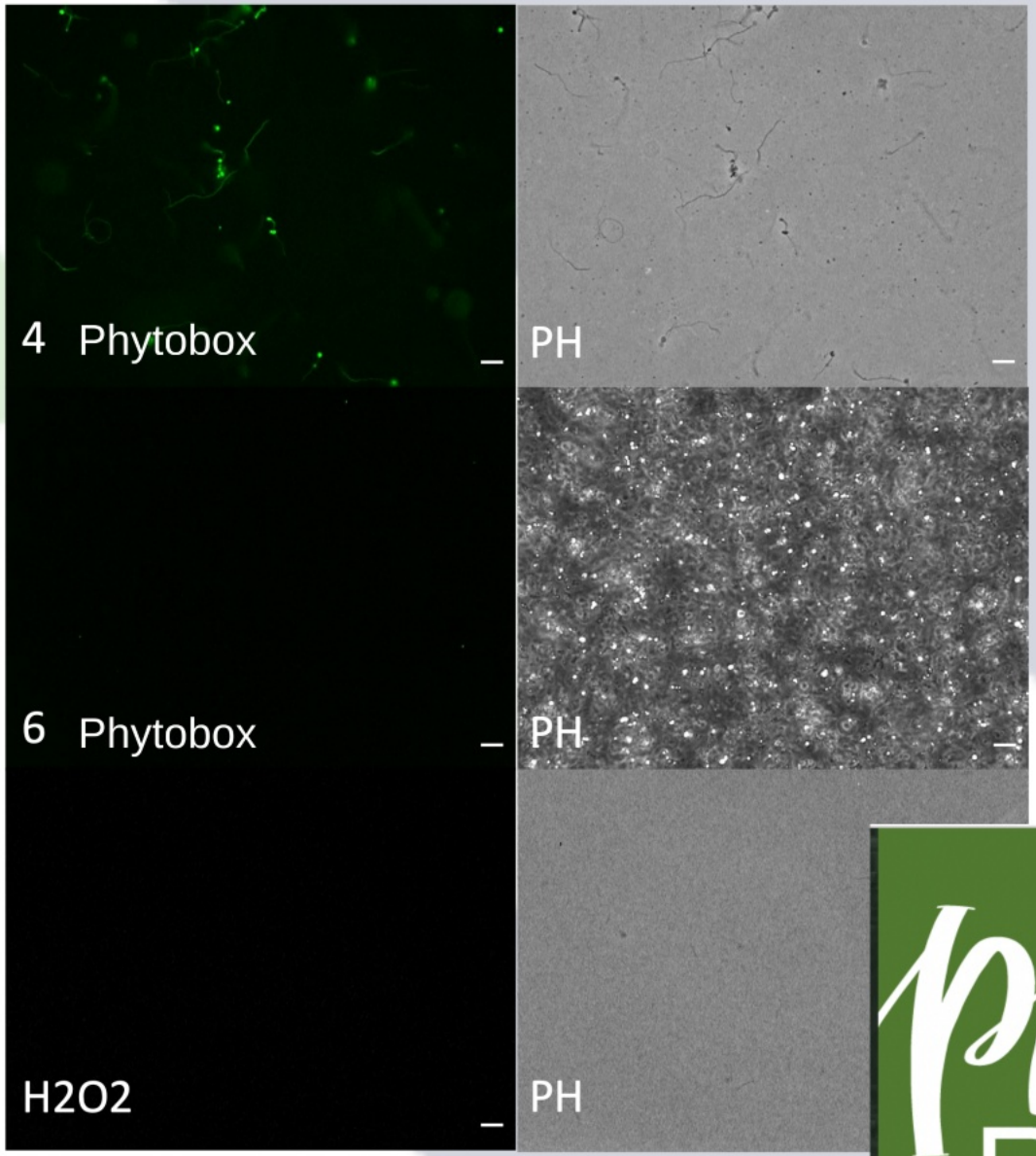
Synergetic Effect of Doxycycline and Phytochemical on Borrelia.

Doxycycline	0	1:5	1:10	1:25	1:50
0 µg/ml	yes	yes	yes	yes	yes
50 µg/ml	no	yes	yes	yes	yes
100 µg/ml	no	yes	yes	yes	no
150 µg/ml	no	yes	yes	yes	no
200 µg/ml	no	yes	yes	yes	no

Horowitz & Freeman 2020, 2022 Used in patients with antibiotics and other phytochemicals, such as Biocidin, Stevia, and oregano oil.





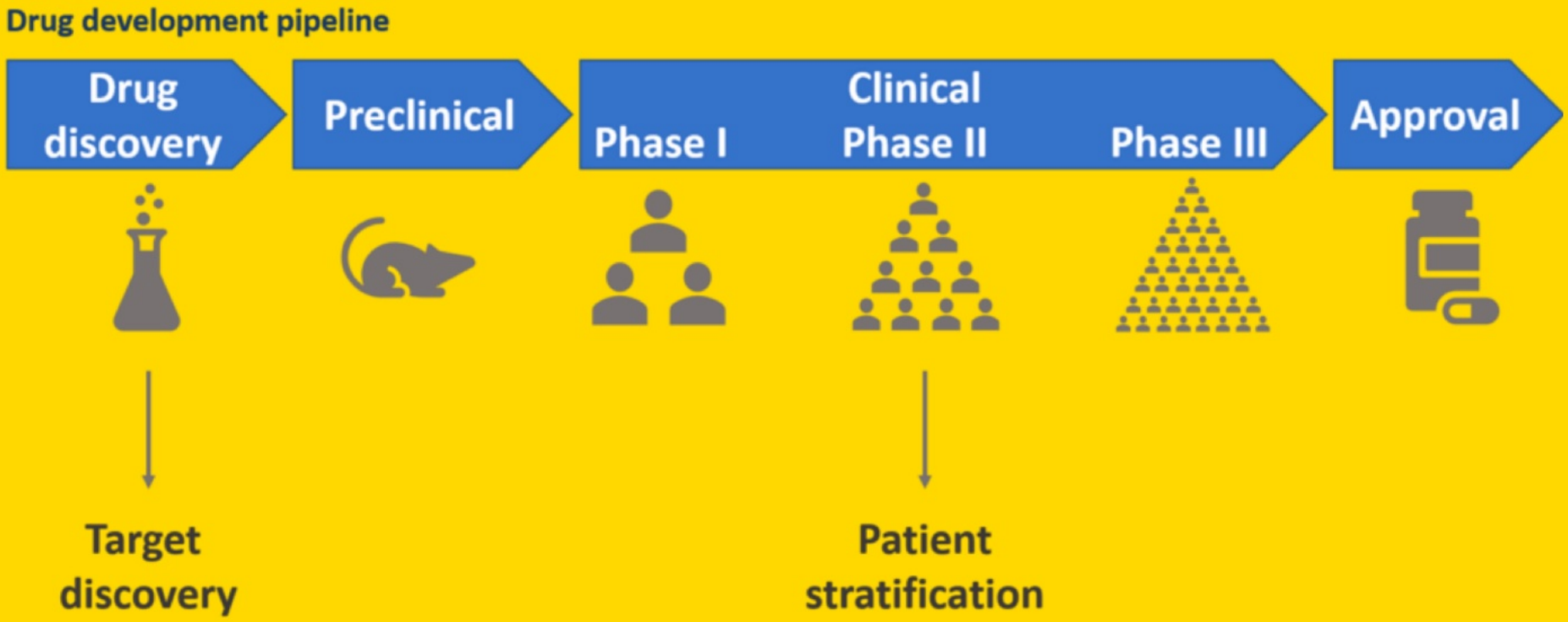


PH: Phase Contrast

Quick studies to see if compounds kill bacteria

Phyto Box

6. Future Studies



Thank you!

Questions?

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