In vitro evaluation of antibacterial activity of phytochemicals and micronutrients against Borrelia burgdorferi and Borrelia garinii

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What is Lyme Disease (Borreliosis)

- Bacterial infection disease
- Transmitted by ticks of the genus *Ixodes*, which harbor a bacterium of genus Borrelia

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Lyme disease causing bacteria: Borrelia

- Most common species causing this disease:
  - Borrelia Burgdorferi (predominant in US, also present in Europe)
  - Borrelia Afzelii (predominant in Europe and Asia)
  - Borrelia Garinii (predominant in Europe and Asia)
- All species are host-dependent, invasive and slow-growing

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How Borrelia is transmitted

- This type of bacteria infect humans and a wide range of animals, including small mammals, lizards, and birds
- Bacteria replicate in the midgut, migrate into the salivary glands of the ticks and with a tick bite are released into the bloodstream of the host
- After infection it can be found in many tissues/organs, including the skin, joints, heart, brain, bladder

Lyme disease Bacteria, Borrelia Burgdorferi

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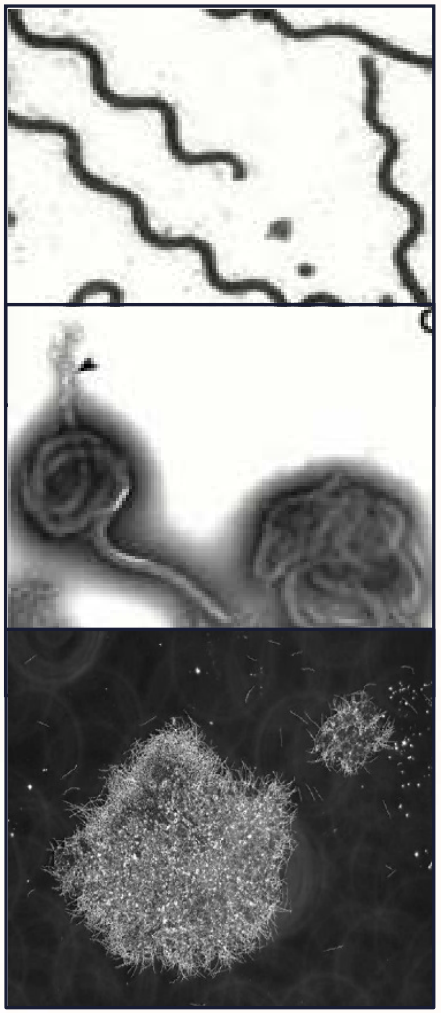
Borrelia converts to different forms



Spirochetes (active form)

- extracellular and intracellular pathogen
- rapidly converts to latent forms

Rounded forms (latent)

- resistant to antibiotics, starvation, etc.
- converts back to spirochetes
- the cause of patient's relapse

Biofilm (latent, complex)

- contains all morphological forms of *Borrelia*
- even 1000x more resistant to antibiotics
- the cause of patient's relapse

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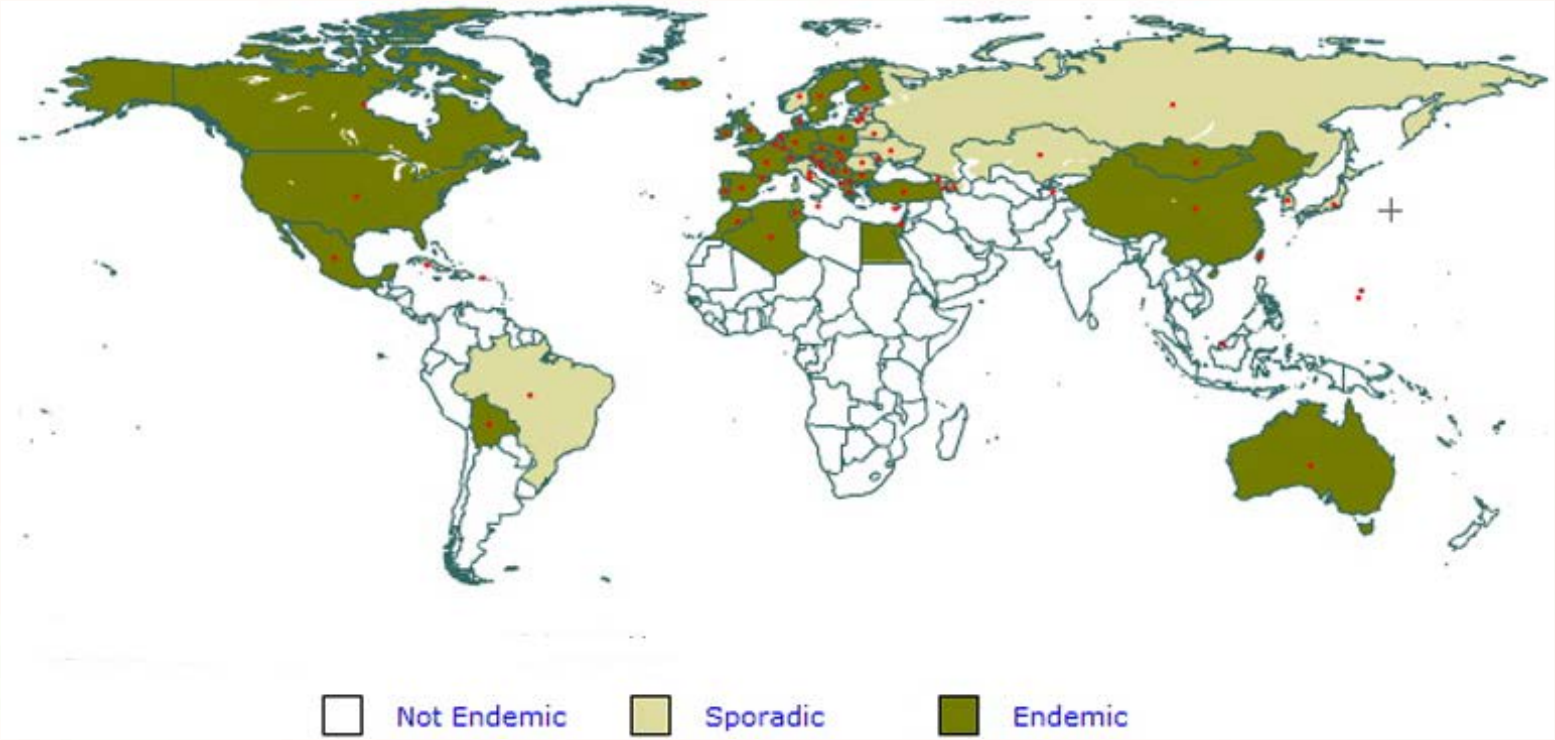
US: mainly Borrelia burgdorferi
Europe: mainly Borrelia afzelli and garinii

Countries with reported Lyme Disease Cases



Source: Gideon-online. Helping doctors make better decisions

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Disease symptoms - stage 1

Early localized stage (3-30 days after tick bite)*

Flue like symptoms

- Fever, chills, fatigue
- Sweats
- Joint & Muscle aches
- Nausea
- Headache

Skin lesions

- Red spot at the site of the tick bite
- Expands over time

*Sometimes different types or no symptoms are detected

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Disease symptoms - stage 2

Early disseminated stage (days/weeks after tick bite)*

- Rashes appearing in other places on the body
- Fatigue, nausea, diarrhea
- Depression, anxiety, mood swings
- Severe headaches
- Pain/swelling in large joints
- Heart palpitations and dizziness due to changes in heartbeat
- Cognitive impairment, light/sound sensitivity

*Sometimes different types or no symptoms are detected

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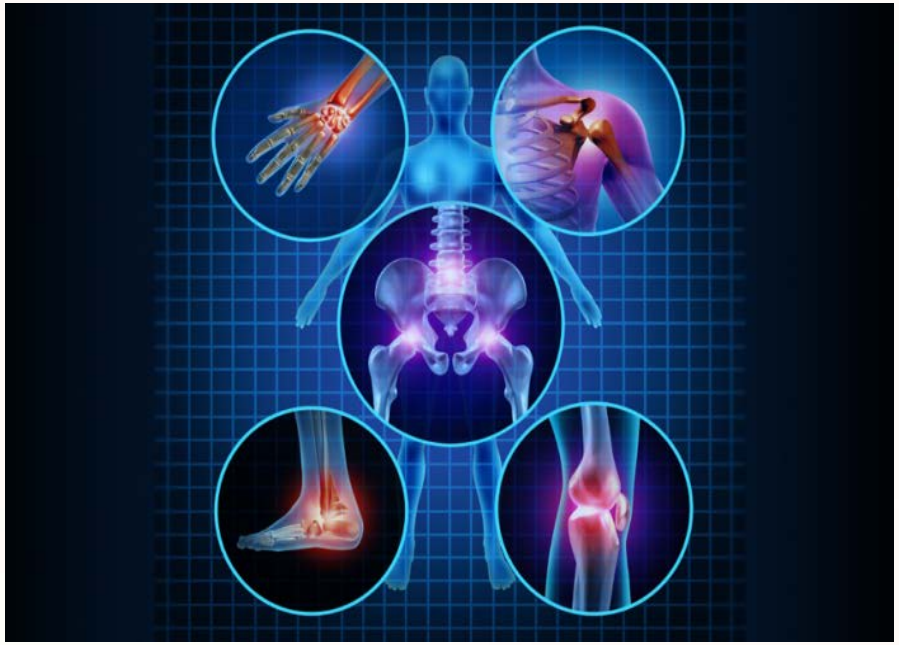
Disease symptoms- stage 3

Late disseminated stage (months/years after tick bite)*



Arthritis

- About 60% patients with untreated disease develop arthritis
- Severe joint pain with swelling

Neurological complications

- 5% patients with untreated disease develop neurological symptoms
- Symptoms include shooting pains, weakness or itching/tingling in hands/feet, impaired short-term memory.

*Sometimes different types or no symptoms are detected

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Disease symptoms- stage 4

Persistent (chronic) stage

Lyme Disease (Borreliosis) Syndrome

- Muscle/ joints
- Cognitive impairments
- Sleep disturbance
- Fatigue
- Neurological abnormalities
- Micronutrient deficiencies
- Changes in vitamin D metabolites
- Mild elevation of auto- immune disease markers

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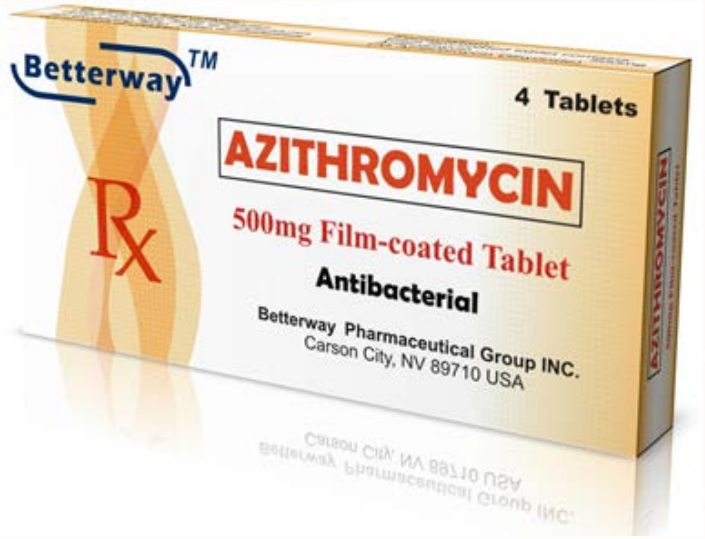
Therapy

CONVENTIONAL TREATMENTS

- Oral and intravenous antibiotics
- Lack of efficacy when applied at later stages of a disease/ in the long term



ALTERNATIVE TREATMENTS

- Plant extracts, oils, enzymes, vitamins are applied to alleviate some symptoms of the disease
- Largely used as individual components mimicking the pharmaceutical drugs approach
- Clinical efficacy varies. No well defined or uniform treatment protocol is present

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New approach tested in this study

- **Evaluation of the efficacy of**

15 phytochemicals and micronutrients against two species of Borrelia: B. Burgdorferi and B. Garinii

- **The compounds**

selected from naturally occurring (nonsynthetic) and plant-derived substances with potential anti-bacterial properties that have not yet been scientifically evaluated against Borrelia sp

- **The selection was limited to**

compounds with validated safety in *in vivo* studies and with chemical structure suggesting effectiveness against all morphological forms of Borrelia sp

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Natural compounds tested in the study

Tested nutrients included the following:

- Hydroxytyrosol
- Baicalein
- Cis-2-decenoic acid
- Morin
- Oenin
- Vitamin D3
- Vitamin C
- Rosmarinic acid
- Kaemferol
- Piceatannol
- Rottlerin
- Luteolin
- Fisetin
- Monolaurin
- (Kelp) Iodine

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Micronutrients were evaluated against all morphological forms of Borrelia

Three morphological forms of Borrelia species: B Burgdorferi and B. Garinii were tested:

- **Vegetative (active) form - *Spirochetes*:** this spiral-shaped form of Borrelia allows to survive viscous conditions (blood), enter into tissue or cells and is the cause of intracellular infection. When these bacteria feel threatened, they adapt by transforming into latent forms
- **Latent (atypical) forms- *Rounded forms & Biofilm:*** these forms allow the bacteria to hide and survive any hostile condition for a long time and become active again after the conditions cease. Biofilm is the most difficult to eliminate.



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RESULTS

- Efficacy of tested compounds against spirochete forms of Borrelia
- Efficacy of tested compounds against rounded forms of Borrelia
- Efficacy of tested compounds against Biofilm formed by Borrelia

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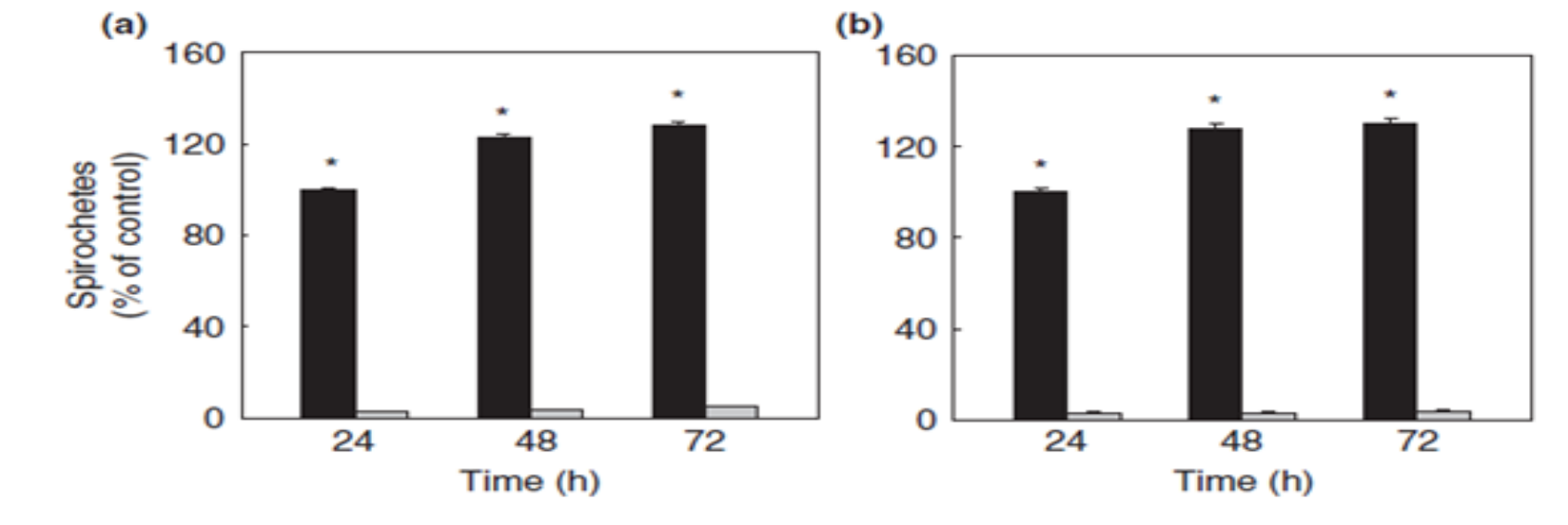
Normal growth and viability of spirochetes of Borrelia sp

Growth kinetic and proportion of alive and dead untreated spirochetes of Borrelia burdorferi (a) and Borrelia garinii (b) without any treatment was monitored up to 72 h

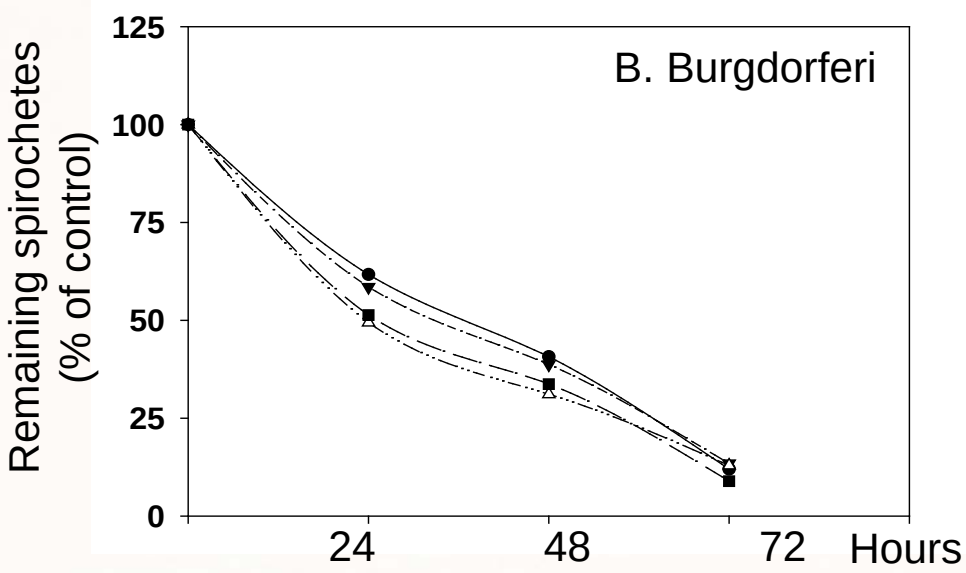


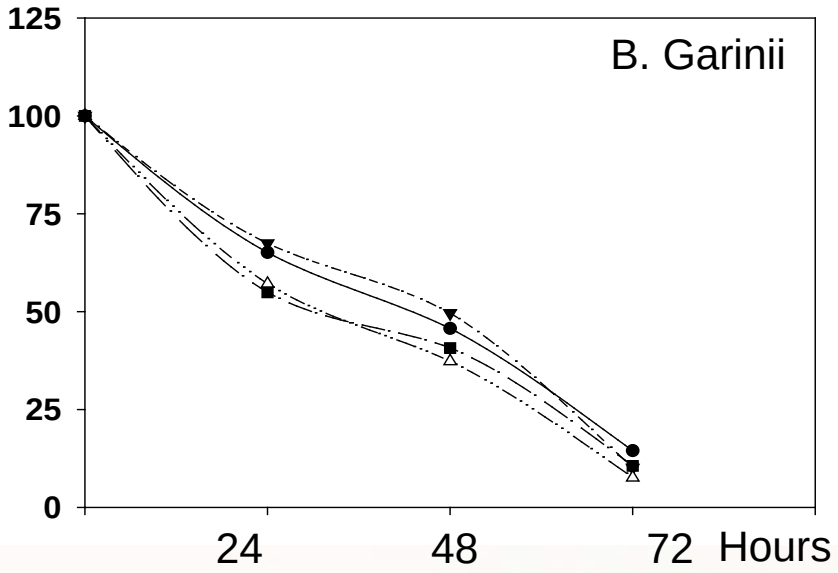
Live spirochetes

Dead spirochetes

* = $P \leq 0.001$ means a significant difference

Effects of tested compounds on growth inhibition of spirochetes





Growth inhibition of spirochetes in two strains of Borrelia incubated in the presence of Baicalein (▼), Monolaurin (Δ), Cis-2-decenoic acid (●), and Kelp (Iodine) (□). After 72 hours of exposure to these compounds growth of spirochetes in both bacteria strains was brought almost to a halt.

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Efficacy of tested compounds in growth inhibition and elimination of spirochetes

- **Growth inhibition was achieved at the following lowest concentrations of tested compounds (MIC value)**
  - **Phytochemicals:** range between 50 - 250 $\mu g\ ml^{-1}$
  - **Micronutrients:** range between 0.0005 and 35 $\mu g\ ml^{-1}$

- **Elimination of spirochetes occurred at the following lowest concentrations of tested compounds (MCB values)**
  - **Phytochemicals:** range between 200 - 500 $\mu g\ ml^{-1}$
  - **Micronutrients:** range between 0.002 - 88 $\mu g\ ml^{-1}$

The effect of 17 selected natural compounds on the growth of spirochette forms of B. Burgdorferi and B. Garinii was examined as

(MIC)= The minimal inhibitory concentration - the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation.

(MBC)= The minimal bactericidal concentration - the lowest concentration of an antibacterial agent required to kill a particular bacterium.

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Effects of natural compounds against spirochetes in different Borrelia species

Growth inhibition:

- **Fisetin, Kaemferol, Rosmarinic acid, Baicalein, Monolaurin, Morin, Piceatannol, Rottlering, Vitamin D3 and Kelp** were **equally effective** at their respective concentrations against spirochetes in **both Borrelia** species tested

- **Hydroxytyrosol, Oenin, Cis-2-decenoic acid, Luteolin and Vitamin C** were **more effective against B. burgdoerferi** (growth inhibition at lower concentrations than against B. garinii)

Elimination of spirochetes:

- **All tested compounds** were **equally effective** in killing spirochetes from **both Borrelia species** (the MBC values of all tested compounds corresponded to each other in both tested Borrelia sp)

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Elimination of rounded forms of Borrelia by tested compounds

- Select doses of tested natural compounds and antibiotic doxycycline applied at non-toxic concentration were not able to completely eliminate (90-99%) rounded forms

- Kelp, Hydroxytyrosol, Cis-2-decenoic, Baicalein and Monolaurin were most effective in killing rounded bodies

- Their concentrations (LD50 values) needed to eliminate 50% of rounded bodies of Borrelia were:
  - 20 $\mu g\ ml^{-1}$ - Kelp (iodine)
  - 200 $\mu g\ ml^{-1}$ - Luteolin),
  - *300-350* - Hydroxytyrosol, Monolaurin
  - 500 $\mu g\ ml^{-1}$ - Cis-2-decenoic acid).

The bactericidal effects were examined and presented as LD50 values = the amount of a substance that kills 50% of a test sample

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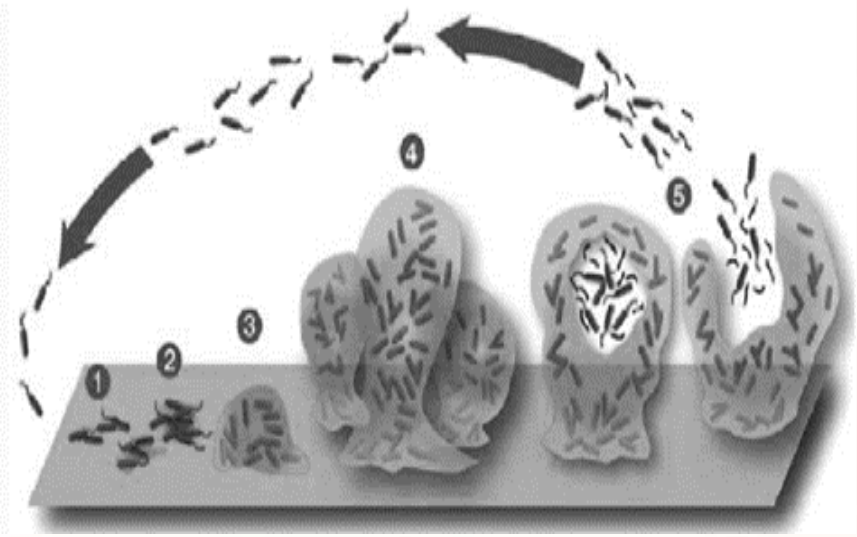
Biofilm: Its elimination is important in fighting Borrelia infection

Biofilms: Biological complex structures formed by bacteria adhering to natural surfaces and excreting a slimy, glue-like substance composed of complex carbohydrates and many other molecules. Biofilm can harbor both active and latent forms of bacteria.

Function: serves as very effective protective mechanism against the immune cells and other compounds, such as antibiotics aiming at eliminating the bacteria



How Biofilm is formed:

1: Individual cells adhere to the surface
2: Extracellular polymeric substance (EPS) is produced and attachment becomes irreversible
3 & 4: Biofilm architecture develops and matures
5: Single cells are released from the biofilm to spread the infection

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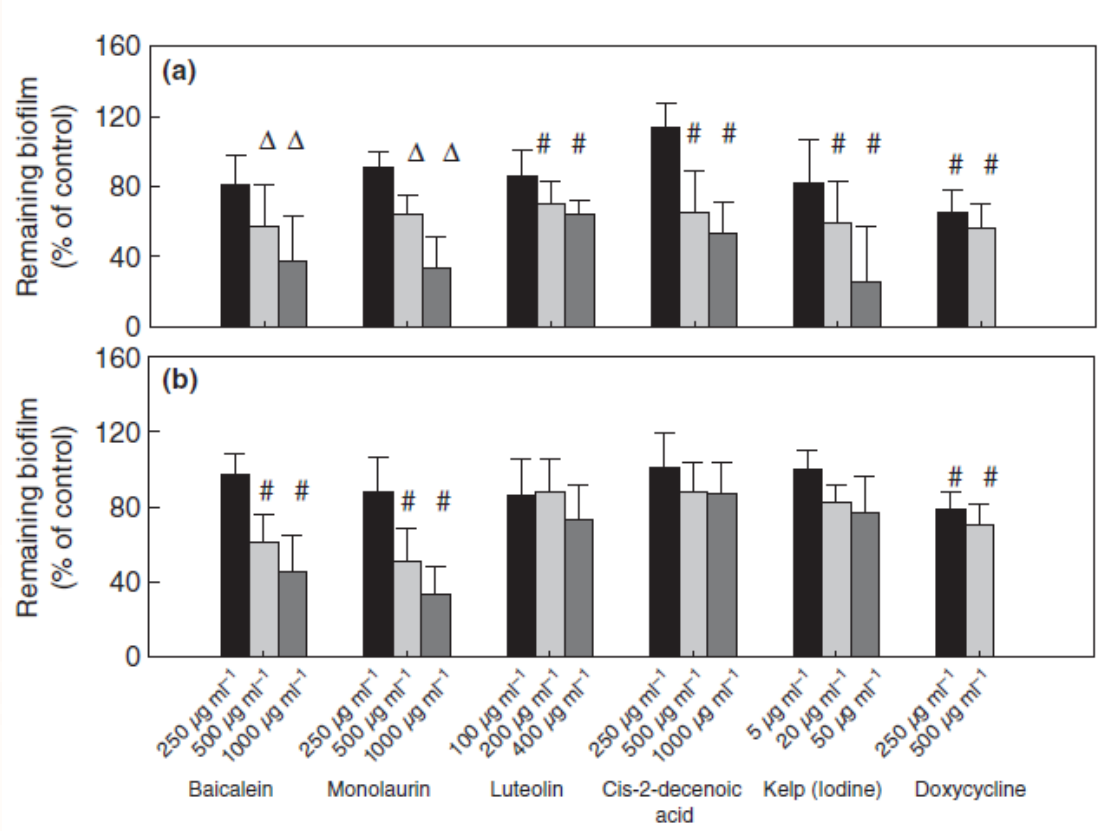
Natural compounds can destroy biofilm produced by Borrelia

Efficacy of select natural compounds in elimination of biofilm formed by **B. Burdorferi (a)** and **B. Garinii (b)** after 72h of exposure



Marks above columns show:
= statitically significant effect (P≤ 0.05) Δ = highly significant effect (P≤0.01)

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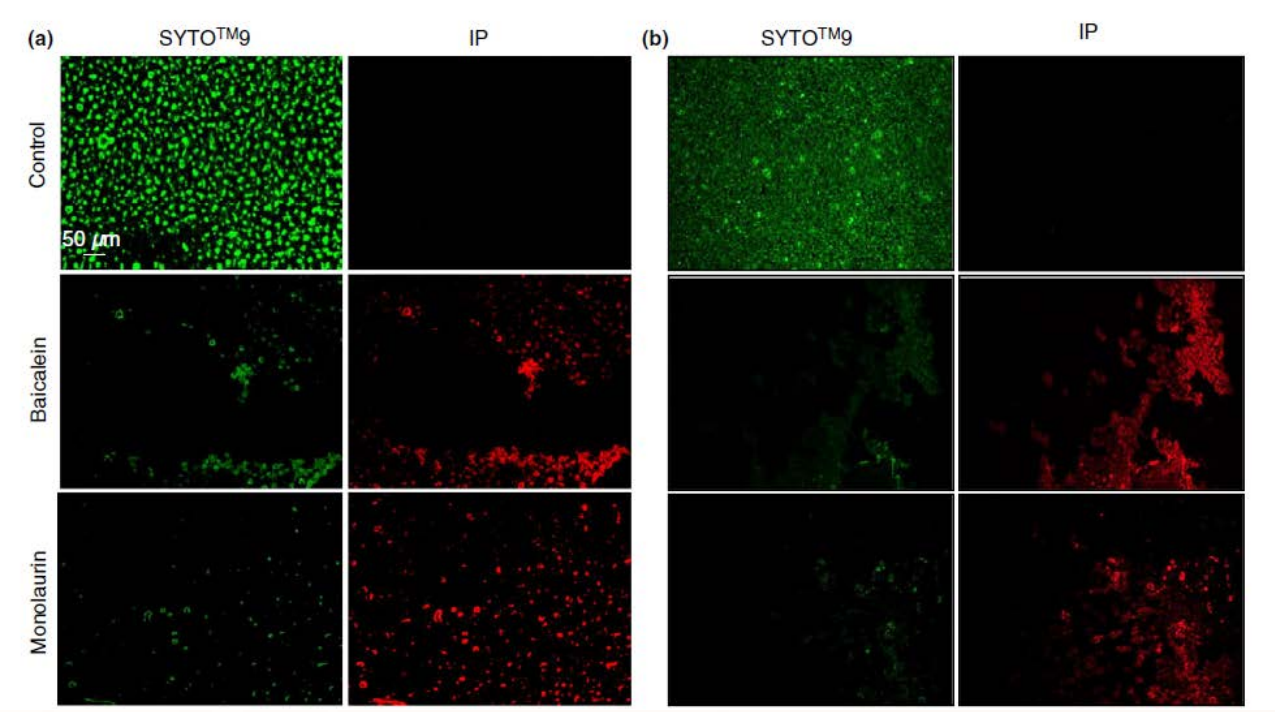
Microscopic evaluation of natural compounds against biofilm: an example

Microscope pictures of biofilm formed by Borrelia Burdoreri (a) and Garinii (b) strain untreated (control) and treated for 72h with Baicalein (500 *µg ml*$^{-1}$ and Monolaurin (500 *µg ml*$^{-1}$)

Green= live biofilm
Red= destroyed biofilm



4 x magnification

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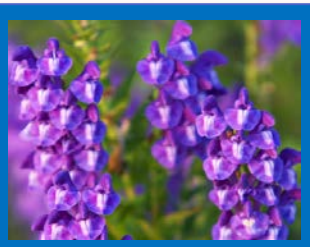
Summary of anti-biofilm potential of tested compounds in two Borrelia species

- **Biofilm-like colonies formed by B. Burgdorferi were reduced by:**
  - Baicalein, Luteolin, Monolaurin, Cis-2-decenoic acid and Kelp (iodine)
- **Biofilm formed by B. Garinii was reduced by:**
  - Baicalein and Monolaurin


Baicalein


Luteolin


Monolaurin


Cis-2-decenoic acid


Kelp

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Most effective components working in synergy against Borrelia

- **Vitamins:**
  - Vitamin B-complex
  - Vitamin C
  - Vitamin D3
- **Specific natural phytobiologicals from:**
  - Kelp
  - Royal Jelly
  - Coconut oil
  - *Saphora japonica*
  - *Rosmarinus officinalis*
  - *Scutellaria baicalensis*

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Summary

- The most potent substances against spirochete and rounded forms of B. burgdorferi and B. garinii were **Cis-2-decenoic acid, Baicalein, Monolaurin** and **Kelp (iodine)**

- **Baicalein** and **Monolaurin** demonstrated the highest potency against Borrelia biofilm.

Baicalein = has shown anti-inflammatory and anti-microbial activities. It is an active compound in Chinese and American Skullcap.

Monolaurin = a component of coconut oil.

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Conclusions

- The results show that phytochemical compounds can be an important tool in the fight against Borrelia species causing Lyme disease (Borreliosis) by affecting both active and latent forms of the bacteria

- It appears that the presence of fatty acid and phenyl groups in these natural molecules is important for this pleiotropic antibacterial efficacy.

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