

Botanicals, Biofilms, and Chronic Infections

PAUL BERGNER
TRADITIONAL ROOTS HERBAL CONFERENCE
NATIONAL COLLEGE OF NATURAL MEDICINE
MAY 2016
PORTLAND, OR

Paul Bergner
Director, North American Institute of Medical Herbalism
Editor, *Medical Herbalism Journal*
<http://naimh.com>
All material copyright Paul Bergner 2016. All Rights Reserved

Biofilms

Bacteria live in a biofilm state

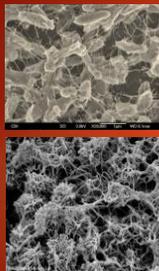


Planktonic form.
Free moving

Biofilm form.
Non-mobile, linked in a matrix

The biofilm form of bacteria is resistant to both antibiotic therapy and the immune system

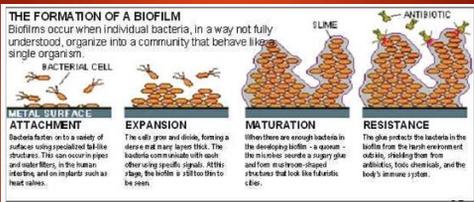
Most bacteria in an on the human body exist in biofilm form. Most are beneficial commensal bacteria and provide a barrier, immune, and metabolic functions



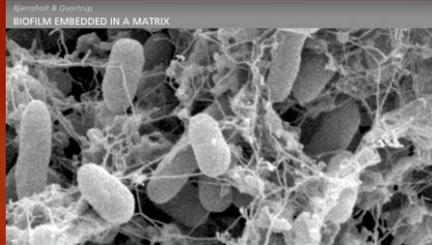
Biofilms are part of normal microbiome defense of the body but pathological biofilms are nearly universally present in:

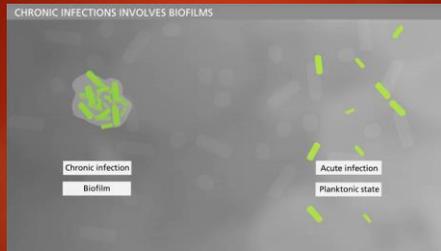
- Oral plaque, periodontal disease, abscess
- MRSA infections on skin
- Other skin infections
- Chronic wounds and ulcers
- Chronic sinus infection
- Upper GI disturbances
- Vaginal infection
- Bladder infection

Biofilms









CHRONIC INFECTIONS INVOLVES BIOFILMS

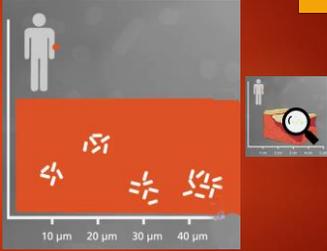
Borrelia biofilm on slide

- ▶ Biofilms are the normal life state for bacteria and some fungi
- ▶ Biofilms can be viewed as semi-independent multicellular organisms with specialized metabolism and immune defenses.
- ▶ They are interlinked by filaments of polysaccharide, protein, or strands of genetic material
- ▶ A gradient of metabolism from aerobic at the surface to anaerobic at the core develops, allowing resistance to substances which might attack the metabolism.
- ▶ In some species, an attached biofilm layer provides nutrients to a superficial layer, which may secrete antibiotics, reproduce, etc.
- ▶ Once aggregated, bacteria in biofilms can dramatically change their functions and secretions.

Biofilms have not been studied in the living organism.

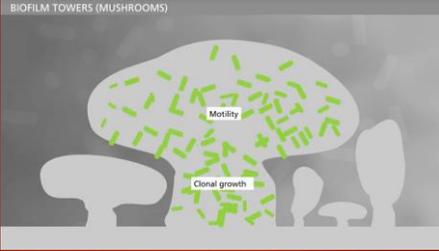
Biofilms in infected wounds are typically in the range of 5 to 10 micrometers, or 1/100 of a millimeter. Requires about 100x magnification to be visible.

One sample of *Borrelia* biofilm in tissue samples required 400X magnification.



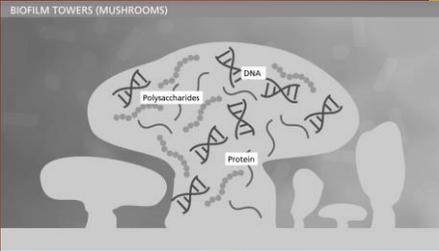
The diagram features a human silhouette on the left. Below it is a scale bar with markings at 10 μm, 20 μm, 30 μm, and 40 μm. To the right of the scale bar, several irregular, branching shapes represent biofilms. A small inset image shows a magnifying glass over a sample.

BIOFILM TOWERS (MUSHROOMS)



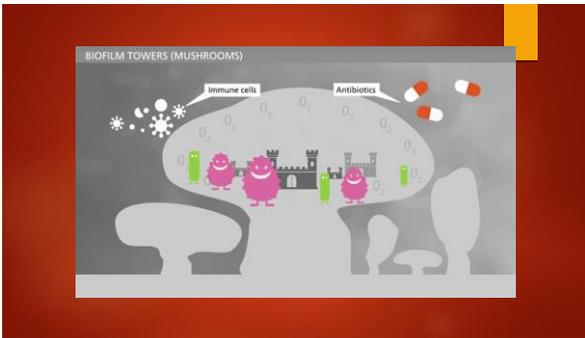
The diagram shows several mushroom-shaped biofilm towers. The stems are labeled "Clonal growth" and the caps are labeled "Motility".

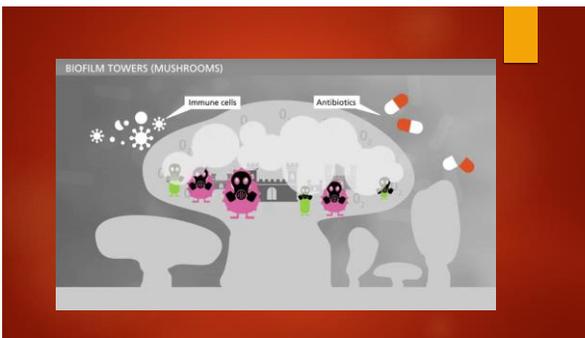
BIOFILM TOWERS (MUSHROOMS)

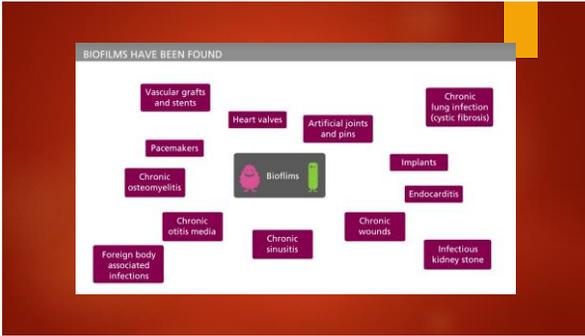


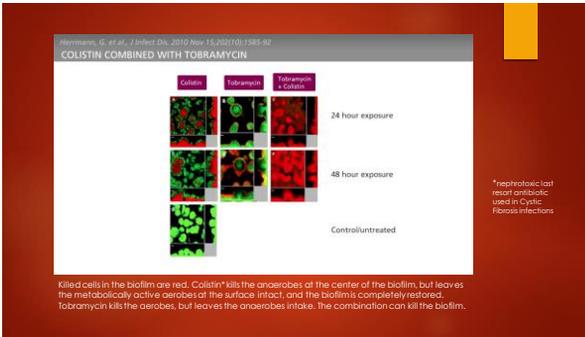
The diagram shows several mushroom-shaped biofilm towers. The stems are labeled "Polysaccharides" and "Protein". The caps are labeled "DNA".

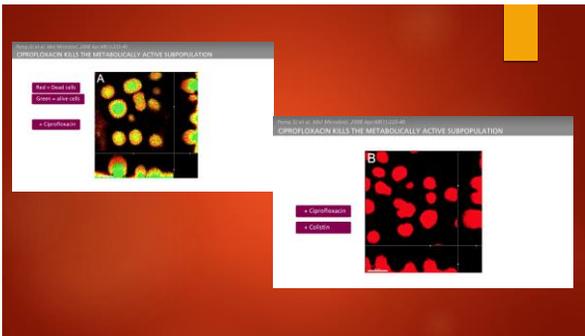


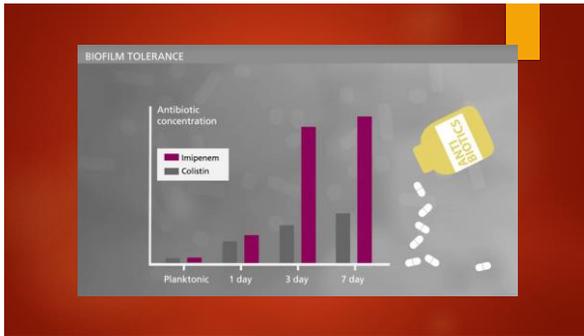












Multispecies biofilms

to separate microbe

to coaggregation

to leaching

Microorganism biofilms frequently form, which may also include fungi.

Below: Oral plaque is a multispecies biofilm with constantly changing and evolving components

Tolerance genes are most easily spread in multi-species biofilms. Multispecies biofilms evolve in their composition and their resistance with each dose of antibiotics

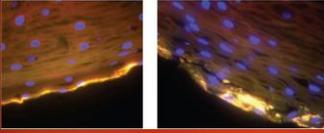
Barnette M, Webb JB, Rao D, Hansen LH, Serrano SA, Kjelleberg S. Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. *Appl Environ Microbiol*. 2005 Jun;71(6):3114-23.

Right: A 3 species biofilm grown in saliva

Below: a "corn cob" biofilm with cocci attached to bacilli

Bacterial vaginosis multispecies biofilm

"Currently, it is consensus that BV involves the presence of a dense, structured and polymicrobial biofilm, primarily constituted by *G. vaginalis* clusters, strongly adhered to the vaginal epithelium"



Red, yellow, and green hues show different species. Blue circles are nuclear DNA from the host cells.

Machêdo D, Castro J, Palmeira-de-Oliveira A, Martinez-de-Oliveira J, Cerca N. Bacterial Vaginosis Biofilms: Challenges to Current Therapies and Emerging Solutions. *Front Microbiol*. 2016 Jan 20;6:1528.

Berberine and companion alkaloids

MAY ACT AGAINST BIOFILMS BY ATTACKING BOTH AEROBES AND ANAEROBES

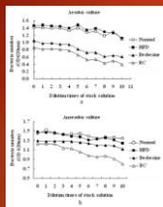


Figure 3. Effects of BB and berberine on growth of host bacteria *in vitro* under (a) aerobic and (b) anaerobic conditions. BB, berberine; BC, berberine chloride; OD600, optical density at 600 nm. *Significant difference (P < 0.05) between BB/BC and control. Error bars represent standard deviation. BB, berberine; BC, berberine chloride; OD600, optical density at 600 nm; P, probability.

In this *ex vivo* trial both *Coptis* root and its constituent berberine significantly inhibit the growth of gut bacteria under both aerobic and anaerobic conditions. *In vitro* trials, both RC and berberine significantly inhibit the growth of *Firmicutes* under anaerobic conditions.

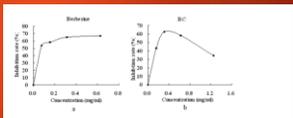


Figure 4. Effects of BB and berberine on the growth of *Lactobacillus sp.* under anaerobic conditions *in vitro*. BB, berberine; BC, berberine chloride; OD600, optical density at 600 nm.

Xia W, Gu D, Li J, Cai K, Zhang Y. Effects and action mechanisms of berberine and *Rhizoma coptidis* on gut microbes and obesity in high-fat diet-fed mice. *PLoS One*. 2011;6(2):e16200.

The Odwalla Juice E-coli epidemic

- ▶ Odwalla juice marketing unpasteurized juices during the 1990s.
- ▶ In 1996, a batch of their apple juice became infected with pathogenic E-coli bacteria. The apple juice is a component in most of their juices.
- ▶ An epidemic followed across the American West, with cases reported in Washington State, Colorado, and California. One child died in Colorado, and 13 more were hospitalized with kidney damage.
- ▶ A number of individuals in Boulder, CO became sick. None were ever recorded in the official statistics of the epidemic.
- ▶ A tincture formula of equal parts of *Hydrastis*, *Mahonia*, *Berberis* v., and *Coptis chinensis* proved rapidly effective against a case with fever and bloody diarrhea (blood resolved after two moderate doses)

Alkaloids in some berberine-containing plants.

Most of these alkaloids have anti-microbial or other pharmacological effects in scientific trials

Alkaloid	<i>Hydrastis</i>	<i>Mahonia</i>	<i>Berberis</i>	<i>Coptis</i>
Berberine	x	x	x	x
Berberamine		x	x	
Berberastine	x			x
Berbericine			x	x
Canadine	x			
Chondocurine			x	
Columbamine			x	x
Coptisine			x	x
Epiberberine				x
Hydrastine	x			
Hydrastinine	x			
Jatrorrhizine		x	x	x
Oxycanthine			x	
Oxyacanthine		x	x	
Palmitine		x	x	x
Tetrahydroberberastine	x			

Berberine compound formula

Potential synergistic alkaloids from *Hydrastis*, *Mahonia*, *Berberis*, and *Coptis* combination

New alkaloids with each addition are marked **bold italic**.

The possible synergistic auxiliary compounds in each plant may also be present.

<i>Hydrastis</i>	<i>H + M</i>	<i>H + M + B</i>	<i>H + M + B + C</i>
Berberine	Berberine	Berberine	Berberine
Berberastine	Berberastine	Berberastine	Berberastine
Canadine	Berberastine	Berberastine	Berberastine
Hydrastine	Canadine	Berberubine	Berberubine
Hydrastinine	Hydrastine	Canadine	Canadine
OH-4-berberastine	Hydrastinine	Chondocurine	Chondocurine
	Jatrorrhizine	Columbamine	Columbamine
	Oxyacanthine	Hydrastine	Coptisine
	Palmitine	Hydrastine	Epiberberine
	OH-4-berberastine	Jatrorrhizine	Hydrastine
		Oxycanthine	Hydrastine
		Oxyacanthine	Jatrorrhizine
		Palmitine	Oxycanthine
		OH-4-berberastine	Oxyacanthine
			Palmitine
			OH-4-berberastine

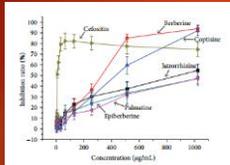


Figure 3. Inhibiting rates of different concentrations of colistin and five berberine alkaloids on MRSA.

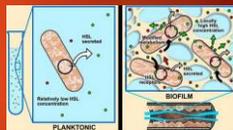
Berberine and its related alkaloids common in berberine-containing plants each inhibit bacteria individually

Luo J, Yan D, Yang M, Dong X, Xiao X. Multicomponent Therapeutics of Berberine Alkaloids. *Evid Based Complement Alternat Med*. 2013;2013:545698. doi: 10.1155/2013/545698. Epub 2013 Mar 24.

Disabling Microbial Defenses

This Biofilm will now come to order "Quorum sensing" by bacteria

- Planktonic bacteria secrete signaling molecules.
- As the population grows, the concentration of signaling molecules rises, and bind to surface receptors on the bacteria.
- This triggers bacterial DNA activation
- Increase production of the triggering molecule
- Expression of matrix materials to form a biofilm
- Production of antibiotics to protect the colony from other bacteria, fungi, etc.
- Production of adhesion molecules
- Production of proteases and other substances enabling invasion of tissues.



PUBMED search: [biofilm* OR quorum]

Some plants with anti-biofilm/quorum properties

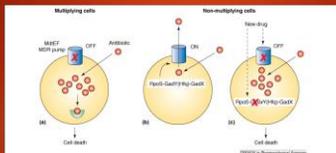
► The discovery of the quorum-sensing property essential to formation and functioning of a biofilm has led to a research quest for plant constituents with anti-quorum or anti-biofilm properties.

- | | |
|--|--|
| <p>Science + tradition</p> <ul style="list-style-type: none"> • Allium • Hydrastis (leaf) • Commiphora myrrha • Boswellia • Achillea • Aloe • Hypericum • Althaea • Arctostaphylos • Acalypha • Quercus and tannins | <p>Traditional use</p> <ul style="list-style-type: none"> • Anemopsis • Larrea • Baptisia • Thuja • Bursera |
|--|--|

Multiple Drug Resistant Efflux Pumps (MDR)

- Bacteria contain transporters in their membranes which actively pump harmful substances back out of the cell.
- The process is non-specific, ejecting a wide variety of substances. It can result in complete inactivation of antibiotic substances.
- MDR activity is responsible for bacterial resistance to both plant and pharmaceutical antibiotics.
- A bacterial population will evolve to contain robust MDR pump activity in response to plant or pharmaceutical antibiotics.
- Bacteria of unrelated species can acquire the MDR pump resistance genes from each other.
- The pharmaceutical quest for effects MDR pump inhibitors (MDRI) has led to a flurry of research into plant compounds in the last few years.

Efflux pumps



- Efflux pumps allow microorganisms to expel many kinds of substances harmful to them.
- Genes coding for more efficient efflux pumps are part of bacterial resistance.
- Efflux pump inhibition is a potential target for antimicrobial therapy with plants or drugs.

MDR pump inhibitors in plants

- ▶ Most isolated plant antimicrobial substances are not effective against gram negative bacteria, due to membrane functions and MDR pumps, but the plants themselves may be very effective due to synergistic constituents, including MDR inhibitors.
- ▶ Addition of MDRI constituents can multiply effectiveness dramatically 100-1000x.
- ▶ Many whole plants contain MDR pump inhibitors.
- ▶ Likewise, plant materia rich in MDR pump inhibitors may be added in formula to topical preparations or other herbs.

Some widely dispersed MDRI constituents

Luteolin	Apigenin	Kaempferol	Myricetin
Artemisia	Artemisia	Allium	Arctostaphylos spp.
Echinacea	Echinacea	Echinacea	Arbutus spp.
Plantago	Plantago	Althaea	Other Ericaceae
Baptisia		Berberis v	
		Calendula	

Some plants containing MDRI

- ▶ Hydrastis (leaf)
- ▶ Some Berberis species (leaf)
- ▶ Allium sativum
- ▶ Allium spp.
- ▶ Calendula
- ▶ Plantago
- ▶ Echinacea
- ▶ Artemisia spp.
- ▶ Hypericum
- ▶ Althaea
- ▶ Achillea
- ▶ Commiphora
- ▶ Boswellia
- ▶ Baptisia
- ▶ Arctostaphylos
- ▶ Arbutus

Plants and biofilms

Plants can do through multiple mechanisms what no drug can do

Many plants have developed mechanisms to kill bacteria, prevent or disrupt quorum-sensing in bacteria, or suppress efflux pumps. This is essential to their survival. Synergistic constituents in a single plant may:

- ▶ Attack microbial cell wall
- ▶ Attack microbial metabolism
- ▶ Disrupt bacterial resistance functions (MDR pumps for instance)
- ▶ Disrupt quorum sensing
- ▶ Disrupt the functions triggered by quorum sensing
- ▶ **In humans**, they may also stimulate local host resistance or circulation

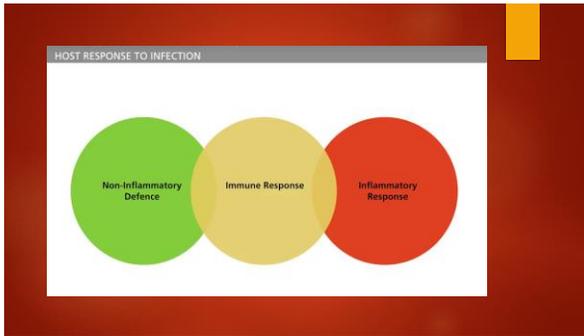
Hydrastis leaf

- ▶ Contains all the *Hydrastis* alkaloids but in lower concentration than the root
- ▶ Contains at least 2 MDRI which **effectively double the potency of berberine**
- ▶ Also contains anti-quorum **and** anti-biofilm properties unrelated to its alkaloids.
- ▶ Sustainably grown *Hydrastis* leaf may be added in formula to almost any topical antimicrobial to improve results



Czech MS, Jurek HA, Anderson LW, Kavanagh JS, Horvath AR. Quorum quenching and enhanced activity of gentamicin (Gent) against gentamicin-resistant *Sophylococcus aureus* (MRSA). *Phyto Med*. 2012 Sep;78(11):1550-61.

Host defense against biofilms



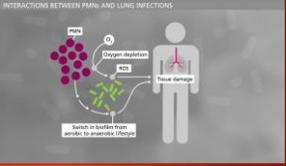
In the host response to chronic biofilms, the cellular components normally present only during the acute phase of the innate immune system are chronically activated, especially **Polymorphal nuclear leukocytes (PMN)**. This **chronic activation of an acute response** can result in tissue inflammation and damage.

PMN: Neutrophils, Eosinophils, Basophils, Mast Cells

Polymorphal nuclear leukocytes (PMN)

The biofilm protects bacteria from otherwise bactericidal PMNs.

Oxidative bursts from the PMN **damage the tissues** around the biofilm and produce inflammation.

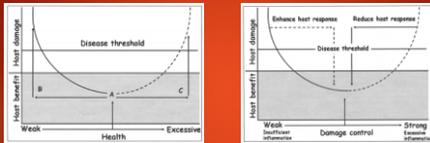





PMN stained in blue surround the biofilm. Their oxidative bursts can damage tissues.



Damage-response model of infection



Profissi LA, Casadevall A. The damage-response framework of microbial pathogenesis and infectious diseases. *Adv Exp Med Biol*. 2008;635:135-46.

Examples of host damage

- ▶ Non-healing wounds and ulcers. Immune response damages tissues.
- ▶ Tuberculosis. Damage to lung by immune response.
- ▶ Chronic viral hepatitis. Damage to liver by immune response.
- ▶ HIV infection triggering autoimmune response
- ▶ Chronic Lyme infection. Damage to connective tissues by response.
- ▶ Possible chronic-infection triggered autoimmunity
- ▶ Permanent presence of high volumes of antigenic food substances produce systemic inflammation.

The "Biofilm Complex"

- ▶ Planktonic microorganisms
- ▶ Microorganisms in a biofilm matrix
- ▶ Microorganisms actively resisting antimicrobial substances through efflux pumps.
- ▶ A continuous and ongoing evolution of resistance to host and antimicrobials.
- ▶ Damage to the tissues through invasion or toxins
- ▶ An ineffective active immune response which may further damage the tissues
- ▶ Non-resolving inflammation

A Plant Constituent-Synergy model of therapeutics for the chronic biofilm complex

Damage-response therapeutics

A synergy model for multi-constituent topical applications

Antimicrobial Effects	Enhance immunity	Reduce damage
Direct antimicrobial effects MDR pump inhibition Anti-quorum effects	Increase local circulation Enhance local immunity Support systemic immunity	Modify local inflammation Repair local tissue damage

These properties are all possessed by some single plants, and with some simple plant combinations

Direct applications

- ▶ The plant material or its extract comes in direct contact with the cell and its environment.
- ▶ All of the plant constituents can come directly into contact with tissue in high concentration, and can act synergistically. Significance for large molecules, essential oils.
- ▶ Plants may be combined for multiple effects
- ▶ Plants may be delivered in media with anti-biofilm effects
- ▶ May apply to external skin, throat, ear, sinus, stomach, vagina, and some constituents may be delivered through the urinary tract.

Potential synergistic actions against the biofilm complex

	Anti-inflammatory	Vulnerary	Antiseptic	Anti-biofilm	MRDI	Local Immunity
<i>Calendula</i>	x	x	x		x	x
<i>Plantago</i>	x	x	x	x	x	x
<i>Hypericum</i>	x	x	x	x	x	x
<i>Echinacea</i>	x	x	x		x	x
<i>Althaea</i>	x	x	x	x	x	x

Infused oils: Olive oil also has wound healing and anti-inflammatory effects
Echinacea wash from decoction of 1 ounce per liter for 40 minutes.
Echinacea wash from tincture 1 part Echinacea to 3-6 parts water.

Herbs with synergistic effects against biofilms

	Anti septic	Immune	Anti Biofilm	MRDI		Antiseptic	Immune	Biofilm	MRDI
Larrea	x	x	trad		Aloe	x		science	(-)
Thuja	x	x	trad		Commiphora	x	x	science	x
Anemopsis	x		trad		Boswellia	x	x	science	x
Baptisia	x	x	trad	x	Allium	x	x	science	x
Hypericum	x	x	science	x	Hydrastis	x		science	x
Althaea	x	x	science	x	Achillea	x		science	x

Stimulate local circulation

	Stimulant	Antiseptic	Immunity	Biofilm	MRDI
Thuja	x	x	x	trad	
Anemopsis	x	x		trad	
Myrica	x	x	x	trad	
Baptisia	x	x	x	trad	x
Commiphora	x	x	x	yes	x
Achillea	x	x		yes	x
Capsicum	x	x			
Anica	x				

Some historical combinations

Garden variety infused topical oil

	cool	Anti-inflammatory	Vulnerary	Antiseptic	Anti biofilm	MDRi	Local Immunity
Calendula	x	x	x	x		x	x
Plantago	x	x	x	x	x	x	x
Hypericum	x	x	x	x	x	x	x

Samuel Thomson's Number Six

	Stimulant	Anti-inflammatory	Antiseptic	Immunity	Biofilm	MDRi	Vulnerary
Commiphora	x	x	x	x	x	x	
Capsicum	xxx		x				
Echinacea		x	x	x		x	x

- ▶ "Rheumatic drops" taken internally, topical antiseptic, throat spray
- ▶ Externally: "The most powerful antiseptic known, and is on that account highly serviceable in all putrid affections whatever"
- ▶ Used as surgical disinfectant with simultaneous internal immune stimulation by the later Physiomedicalists (post germ-theory)
- ▶ RS Clymer later recommended substitution of Echinacea for Capsicum in the formula. Can use all three in suitable proportions

A classical pair

	Stimulant	Anti-inflammatory	Antiseptic	Immune	Biofilm	MDRi
Hydrastis			x		x	x
Myrrh	x	x	x	x	x	x

Traditionally used for oral infections and non-healing wounds

Hydrastis and Myrrh

- ▶ Topical wash for infection
- ▶ Antibacterial, antiviral, antifungal
- ▶ Spray for sore throat
- ▶ Gum disease
- ▶ Topical for gastric mucosa
- ▶ Powerful systemic effects (mucous membrane tonic, general alterative and tonic, antimicrobial through separate mechanisms, in low dose is balanced warm, cold, moist and dry.

Sinusitis spray

- ▶ Get a 2 ounce sinus spray bottle
- ▶ Add 1 teaspoon of glycerine. Not more.
- ▶ Add 15 drops each of *Hydrastis* and *Myrrh**. Not more.
- ▶ Fill to 2 oz with water.
- ▶ Spray into sinuses up to 4 times per day.
- ▶ Frequently will clear chronic sinusitis within 4 days.

*Original recipe called for 30 drops of *Anemopsis*

Possible combination

		Stimulant	Anti-inflammatory	Antiseptic	Immune	biofilm	MDRi
<i>Commiphora</i> (sub: <i>Bursera</i> ?)	warm	x	x	x	x	science	x
<i>Larrea</i>	cool		x	x	x	trad	

Esberitox

		Stimulant	Anti-inflammatory	Vulnerary	Antiseptic	Immune	Biofilm	MDRi
Echinacea	cool		x	x	x	x	x	x
Baptisia	cold	x			x	x	trad	x
Thuja	warm	x	x		x	x	trad	

- Developed in Europe for internal use as an immune stimulant.
- A very potent potential topical treatment. Prepare as decoction.
- Note traditional use of Baptisia was primarily external application of the tea

Roberts formula for ulcers

- ▶ *Helicobacter pylori* is a normal component of the gastric microbiome. In some cases it is the dominant species.
- ▶ It normally grows in a biofilm separated from the mucosa by a mucous layer. Pathology may be due to loss of the mucous layer.
- ▶ For a complete discussion of H Pylori, the history of its discovery, and subsequent discovery of systemic harms that can result from it eradication, see *Missing Microbes* by Glaser.
- ▶ Roberts formula for ulcers was developed mid 20th century, long before the possible infectious basis of gastric ulcers was known, and before the discovery of H pylori. Most of the herbs are those that would traditionally be used on topical ulcers or poorly healing wounds. Later in the 20th century, J. Bastyr added Baptisia and several other components to the formula.

Roberts Formula for Ulcers

		Antiseptic	Anti biofilm	MDRi	Local Immunity	Anti-inflammatory	Vulnerary
Althaea	cool	x	x	x	x	x	x
Geranium maculatum	cool	x*	x	x			
Hydrastis (leaf)	cold	x*	(x)	(x)			
Echinacea	cool	x		x	x	x	x
Phytolacca	cold	x			x		
(Baptisia)	cold	x	x	x	x		

*Specific strong activity against *H. pylori* in vitro

Acalypha spp. Yerba del Cancer.

A universal folk remedy for wounds in Mexico



A. californica

Michael Moore: "For chronic infections when nothing else has worked."

Acalypha phleoides (syn: *lindeheimeri*)

Acalypha and Arctostaphylos

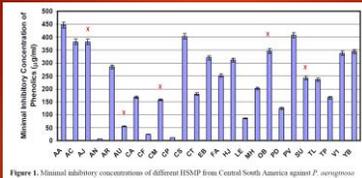


Figure 1. Minimal inhibitory concentrations of different HSMF from Central South America against *P. aeruginosa*

Acalypha (AJ) is a relatively **poor antimicrobial**.
Arctostaphylos u. (AU) is **very strong**

Huerta V, Mihalek K, Crowl SH, and Valtem, DA*
Herbs, Spices and Medicinal Plants Used in Hispanic
Traditional Medicine Can Decrease Quorum Sensing
Dependent Virulence in *Pseudomonas aeruginosa*.
International Journal of Applied Research in Natural
Products
Vol. 1 (2), pp. 9-15, June/July 2009

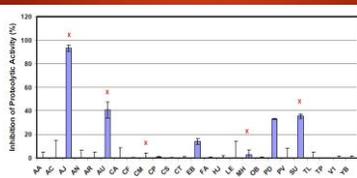


Figure 3. Effect of sub-lethal concentrations of HSMF from Central-South America on quorum sensing dependent total proteolytic enzyme activity in *P. aeruginosa*.

Of 25 Mexican plants tested, Acalypha and Uva ursi were #1 and #2 in one measurement of anti-quorum activity. Most had no activity.

Allium sativum

- ▶ Raw fresh cut garlic contains high amounts of allicin, which has broad spectrum antimicrobial and anti-biofilm effects
- ▶ Allicin breaks down rapidly once garlic is cut or crushed. Breakdown products have anti-biofilm and antimicrobial effects.
- ▶ The constituent ajoene, which is abundant in oil-infused garlic preparations, has a potent anti-biofilm effect.
- ▶ Some of these non-allicin constituents may be delivered to a biofilm after oral ingestion.
- ▶ *Fresh garlic can produce second and third degree in burns.*

Allium sativum applications

- ▶ Two cloves (not whole bulbs) in liter of water, blended and strained through cheesecloth.
- ▶ Poultice
- ▶ Foot bath or handbath.
- ▶ Mouthwash for thrush
- ▶ Douche
- ▶ Infused oil to ear

Media

Vinegar and biofilms

- ▶ Acetic acid has an anti-microbial effect against established biofilms both in-vitro and in open wounds.
- ▶ It is effective at 100% eradication of established *P. aeruginosa* and *S. aureus* at a concentration of 1% acetic acid.
- ▶ The anti-biofilm effect is not due to pH value of the bacteria, because HCl at the same pH has no effect.
- ▶ The effect is due to the acetic acid molecule itself.
- ▶ Application six times a day for twenty minutes on non-healing diabetic ulcers. (See following slides)



Day 0 vs Day 11 of antibiotic resistant diabetic foot ulcer treated with vinegar. Note complete lack of suppuration.



Days 0, 3, and 6 of vinegar treated antibiotic resistant diabetic foot ulcers. Note disappearance of suppuration and appearance of circulation by day 3.



Treatment of a year-long antibiotic resistance diabetic foot ulcer with vinegar. Days 0 and 6. See method of application in middle slide.

Stages of chronic ulcers

MICROORGANISMS

When a wound occurs
 - If contaminated with microorganisms
 ...normally cleared by the immune and adaptive immune defense system

- If vivacious microorganisms
 - a skin with ulcers
 - if diabetic foot ulcers
 - Bacteria cannot be eradicated

NON-SUSCEPTIBLE MICROORGANISMS

Antibiotic treatment results in resistance, evolution of the biofilm, and ultimately to co-infection by additional species and yeasts (purple circles) in multispecies biofilms

Honey

- ▶ Honey in a dilution of 1/4 was tested against planktonic and biofilm forms of antibiotic resistant *P. aeruginosa* and *S. aureus*
- ▶ Tested honey was Manuka honey, which may contain antimicrobial volatile substances. Some Canadian honey samples were ineffective.
- ▶ The honey completely eradicated planktonic forms and reduced biofilm forms of both bacteria by 63-91%

Alandjani T, Marsan J, Ferris W, Singer R, Chan F. Effectiveness of honey on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. *Otolaryngol Head Neck Surg*. 2009 Jul;141(1):114-8.

Oral biofilms

- ▶ A healthy microbiome may exist in the biofilm on the teeth.
- ▶ Sugars drive evolution of the biofilm on the teeth toward acid producing bacteria and caries.
- ▶ Poor hygiene results in evolution of the a multispecies biofilm of anaerobes which can live under the gum line. Subsequent inflammation is destructive to the tissues.
- ▶ An entirely new biofilm of anaerobes evolves in a tooth abscess.
- ▶ Anaerobes in severely infected gum pockets or abscesses may spread through virulent planktonic bacteria to other areas of the body, to medical implants, kidney stones, atherosclerotic plaque, etc.

Treatments for oral infection

- ▶ Combinations of *Hydrastis* and *Myrrh*, applied generously, diligently, and persistently have saved teeth that were due to be pulled because of severe gum disease. Consider *Hydrastis* leaf.
- ▶ May also work with powdered *Myrrh* and sea salt.
- ▶ Will not work without first mechanical clearing of the teeth.
- ▶ Abscesses or infected root canals cannot be addressed with herbs.
- ▶ Strong *Echinacea* teas internally, and also held as a mouth wash, have effectively prevented or treated oral infections following gum surgery when antibiotics were refused.
- ▶ Also effective internally in a case study of facial cellulitis following root canal, when antibiotics were refused.

Tooth powder

For treatment or maintenance after cleaning

	Parts	Stimulant	Anti-inflammatory	Antiseptic	Immune	Biofilm	MDRi
<i>Quercus alba</i>	4		x	x		x	
<i>Myrrh</i>	4	x	x	x	x	x	x
<i>Myrica</i>	2	x		x		x	
<i>Hydrastis</i>	1			x		x	x
<i>Cinnamomum cassia</i>	1	x	x	x			
<i>Eugenia</i>	1	x		x			

This is a formula from Candis Cantin Kiriagis

Bacterial vaginosis

- ▶ The normal biome of the vagina is dominated by one of several vagina-specific *Lactobacillus* species.
- ▶ BV is characterized by strongly tissue-adherent multi species biofilms constructed on a dominant *Gardnerella* matrix.
- ▶ Antibiotics are ineffective because of the biofilm, and because restoration of the vaginal specific *Lactobacillus* is necessary.
- ▶ The general pattern of therapy is:
 - ▶ Keep the environment acidic
 - ▶ Apply probiotics of vaginal-specific lactobacillus.
 - ▶ Apply topical therapeutics with antimicrobial and anti-biofilm effects

Some traditional treatments

- ▶ Vinegar douches. May have anti biofilm effects independent of pH effects
- ▶ Boric acid capsules. BID. Boron may have anti-biofilm effects independent of pH.
- ▶ Boric acid mixed with powder of *Hydrastis*, *Mahonia*, or *Berberis*. Might be enhanced by the use of leaf of *Hydrastis* or *Mahonia*.
- ▶ Douche of *Hydrastis* tea. Consider adding the leaf, with the entire Berberine compound formula.
- ▶ Douche of *Allium sativum*. Must strain the blended preparation through cheesecloth (allicin from cut garlic can cause burns)

Boric acid and biofilm formation

Bacteria	Biofilm production					
	In the absence of either of the B compounds		In the presence of H_2O_2		In the presence of $Na_2B_4O_7 \cdot 10H_2O$	
	OD	Bif	OD	Bif	OD	Bif
<i>S. aureus</i> ATCC 29223	0.075 ± 0.002	+	0.02 ± 0.003	-	0.013 ± 0.002	-
<i>S. anguillarum</i> No. 218	0.079 ± 0.003	+++	0.031 ± 0.003	-	0.019 ± 0.001	-
<i>A. hydrophila</i> ATCC 19770	0.08 ± 0.006	++	0.03 ± 0.002	-	0.02 ± 0.001	-
<i>A. hydrophila</i> No. 219	0.079 ± 0.005	++	0.025 ± 0.001	-	0.021 ± 0.001	-
<i>F. macleodii</i> No. 217	0.076 ± 0.011	++	0.03 ± 0.001	-	0.022 ± 0.002	-
<i>F. aeruginosa</i> ATCC 27833	0.216 ± 0.014	+++	0.04 ± 0.002	+	0.033 ± 0.001	-
<i>F. aeruginosa</i> No. 266	0.09 ± 0.017	++	0.06 ± 0.001	+	0.011 ± 0.001	-
<i>A. gossypii</i> NCH-8280	0.380 ± 0.012	+++	0.014 ± 0.002	-	0.04 ± 0.001	+
<i>B. melanosus</i> Rev-1	0.05 ± 0.003	+	0.02 ± 0.002	-	0.019 ± 0.001	-
<i>B. abortus</i> No. 31	0.075 ± 0.004	++	0.024 ± 0.002	-	0.022 ± 0.001	-

Beneficial effects in BV may be due to the effect of the Boron molecule on biofilm formation rather than to the acidity.

Sayin Z, Ucan US, Sakmanoglu A. Antibacterial and Antibiofilm Effects of Boron on Different Bacteria. Biol Trace Elem Res. 2016 Feb 11.

Garlic vs Flagyl for Bacterial Vaginosis

- ▶ 500 mg powder of *Allium sativum*
- ▶ 250 mg Metronidazole
- ▶ Two tablets with meals orally each 12 hrs.
- ▶ Successful oral application with reduction of the biofilm implies that the anti-microbial and possibly the anti-biofilm constituents are delivered systemically to the vaginal mucosa

Mohammadzadeh F, Dolatian M, Jorjani M, Alavi Majd H, Boumardina N. Comparing the Therapeutic effects of garlic tablet and oral metronidazole on bacterial vaginosis: a randomized controlled clinical trial. Iran Red Crescent Med J. 2014; Jul; 15(7):e19115.

Table 4. Comparison of Laboratory Improvement in Women With Bacterial Vaginosis.⁴³

Group	Garlic	Metronidazole	Total
Lab Improvement	40 (94.3)	40 (93)	74 (94.1)
Lack of Lab Improvement	2 (2.3)	2 (4.7)	4 (2.8)
Total	42 (46.6)	42 (46.6)	84 (94.9)

^a Data are presented as the (%).
^b Chi square = 0.004 and P = 0.93.

Table 5. Comparison of Treatment Success in Women With Bacterial Vaginosis.⁴³

Group	Garlic	Metronidazole	Total
Successful Treatment	38 (91.3)	29 (66.5)	67 (75.4)
Failure to Treat	22 (28.7)	11 (25.3)	33 (34.2)
Total	60 (90)	40 (100)	100 (100)

^a Data are presented as the (%).
^b Chi square = 0.004 and P = 0.93.

Table 6. Comparison of Medication Side Effects in Women With Bacterial Vaginosis.⁴³

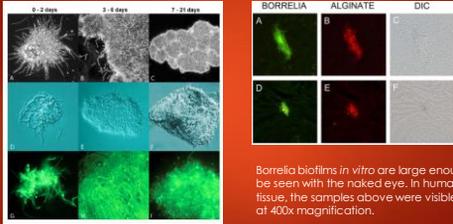
Group	Garlic	Metronidazole	Total
With Side Effect	0 (0)	40 (93)	40 (45.2)
Without Side Effect	5 (12)	40 (94.7)	45 (51.4)
Total	5 (12)	80 (90)	85 (96)

^a Data are presented as the (%).
^b Chi square = 3.002 and P = 0.082.

Internal biofilms

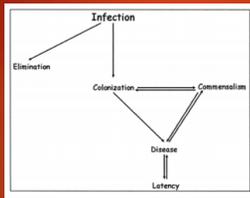
- ▶ These usually require mechanical assistance to remove.
- ▶ High doses of single antibiotics are ineffective
- ▶ High dose antibiotic combinations may be effective.
- ▶ Tooth abscess
- ▶ Medical devices and implants
- ▶ Tissue fillers
- ▶ Chronic tissue infection (Borreliia)

Borrelia biofilms *in vitro* and *in vivo*



Borrelia biofilms *in vitro* are large enough to be seen with the naked eye. In human tissue, the samples above were visible only at 400x magnification.

Possible outcomes of chronic infection



Profokl LA, Casadevall A. The damage-response framework of microbial pathogenesis and infectious diseases. *Adv Exp Med Biol.* 2008;635:135-46.

Paul Bergner
Director, North American Institute of Medical Herbalism
Editor, *Medical Herbalism Journal*

<http://noimh.com>

All material copyright Paul Bergner 2/1/2014. All Rights Reserved
