Section Three Slides

Botanicals, Microbiome, Biofilms, and Chronic Infections

PAUL BERGNER

NORTH AMERICAN INSTITUTE OF MEDICAL HERBALISM

OCTOBER 2019

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

http://naimh.com
Notes and readings http://naimh.com/csch-biofilms

Seminar Overview

SECTION One

- The New Microbiology
- The Human Microbiome

SECtION TWO

- Infection
- Biofilms
- Berberine and related alkaloids
- Microbial defenses

SECTION THREE

- Host defenses
- Constituent synergy herbal therapeutics

SECTION FOUR

- Antifungal therapeutics

SECTION FIVE

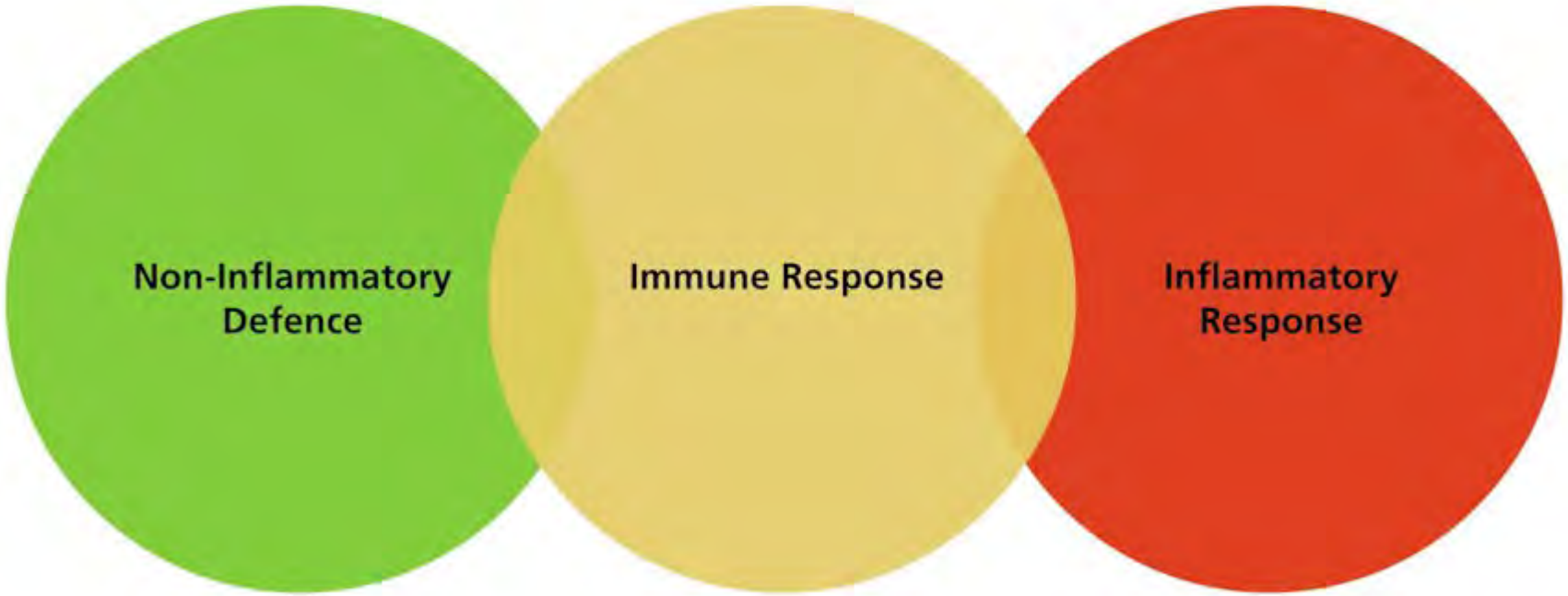
- Biofilms in the gut
- Internal Biofilms

Host defense against biofilms

Themes for the weekend

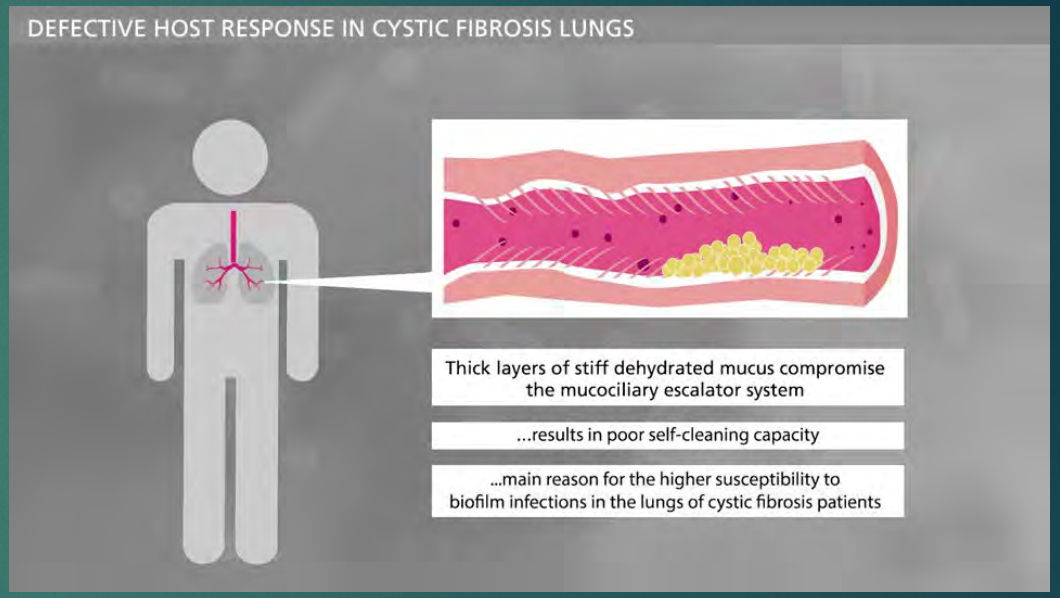
- Germs are not the enemy, and attempts to eradicate them have led to serious unintended consequences, collectively and individually
- Biofilms are the natural base state of bacteria, archaea, and some fungi. Biofilms are not the enemy, and attempts to eradicate them may also produce unexpected and unintended adverse consequences.
- The microbiomes in the various regions of the body perform essential functions, and, if damaged, can allow increased pathogenic infections
- A **single** course of antibiotics ***can*** cause lasting damage to the microbiome. **Repeated** courses ***will*** cause lasting and irreversible damage

HOST RESPONSE TO INFECTION



Non-inflammatory defense

- Mechanisms which “self-clean” the body, especially in the skin, respiratory, and urinary tracts.
- Action of cilia
- Urinary flow
- Eustachian tube function
- Circulation to skin.



Small intestine

Bacteria count and biofilm formation is kept low by non-immune factors

- Gastric acidity that kills ingested organisms
- Antimicrobial peptides secreted by epithelial cells
- Propulsive intestinal motility producing a fast transit time of the small intestine (about 2 hours).
- Long-chain fatty acids in conjugated bile are antimicrobial.
- Preview: overgrowth of bacteria and/or biofilms in the upper GI (SIBO) may be due to loss of these factors in a hypochlorhydria/amotility syndrome, and they might be restored/reinforced with herbal medicines.

The Lymphatic Immune-Cell Triad

MACROPHAGE
or other antigen-presenting cell

B-LYMPHOCYTE B-LYMPHOCYTE

- Where all three elements are present in close proximity, a systemic immune reaction can be launched
- This occurs in the lymph nodes, in the lining of the gut, and in the skin.

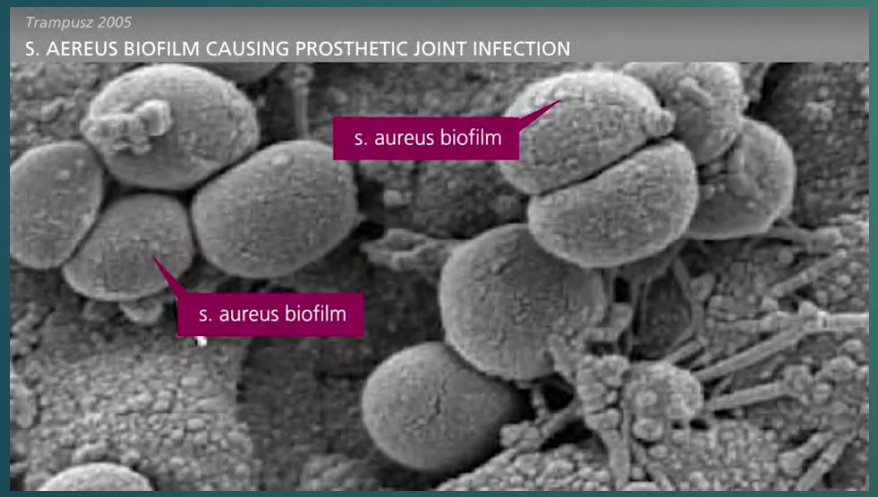
Polymorphal nuclear leukocytes response and host tissue damage

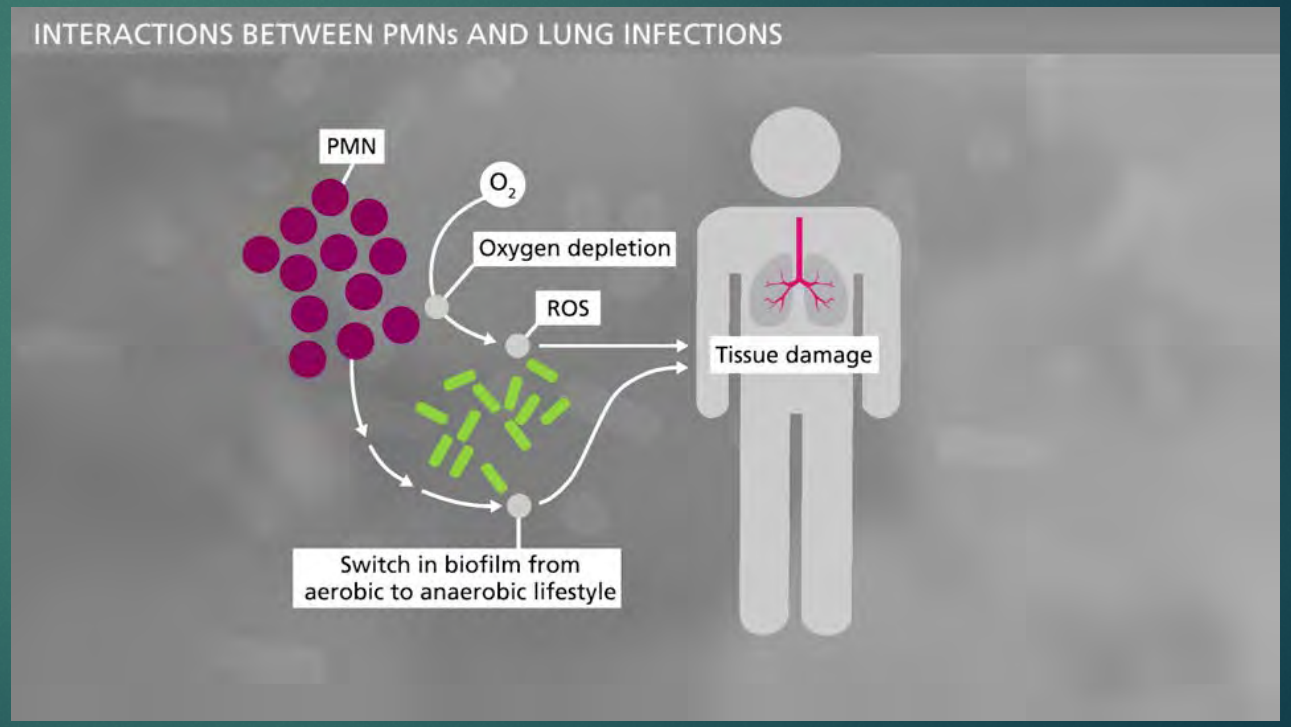
- White blood cells typically involved in acute infection: neutrophils, eosinophils, basophils, mast cells secrete substances to destroy microbes.
- Because the microbe-triggers in biofilms cannot be eliminated, these cells can become chronically activated.
- Chronic activation can result in host damage through oxidative destruction of host cells by the elements used to kill the bacteria.

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.



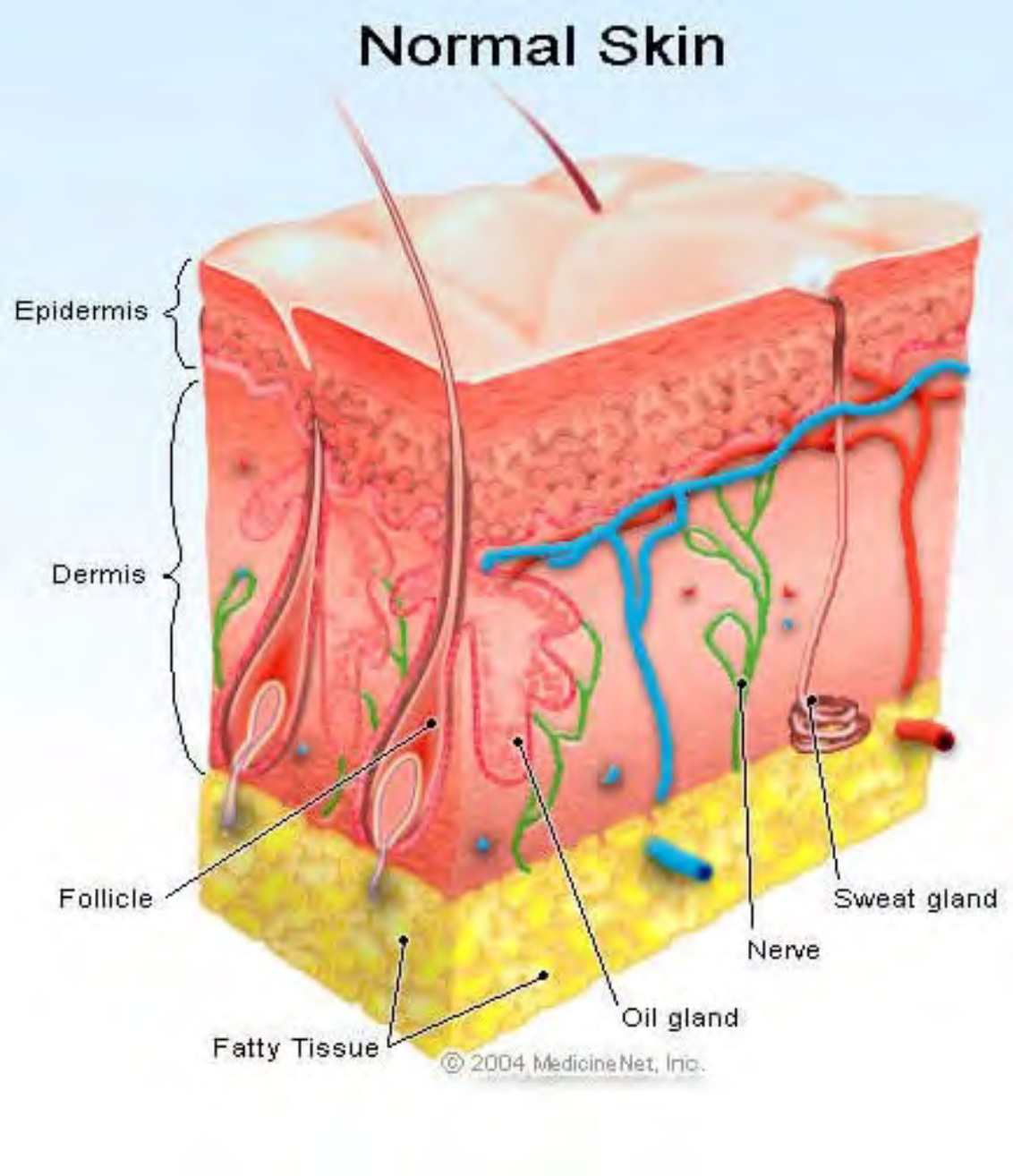


PMN stained in blue surround the biofilm. Their oxidative bursts can damage tissues.

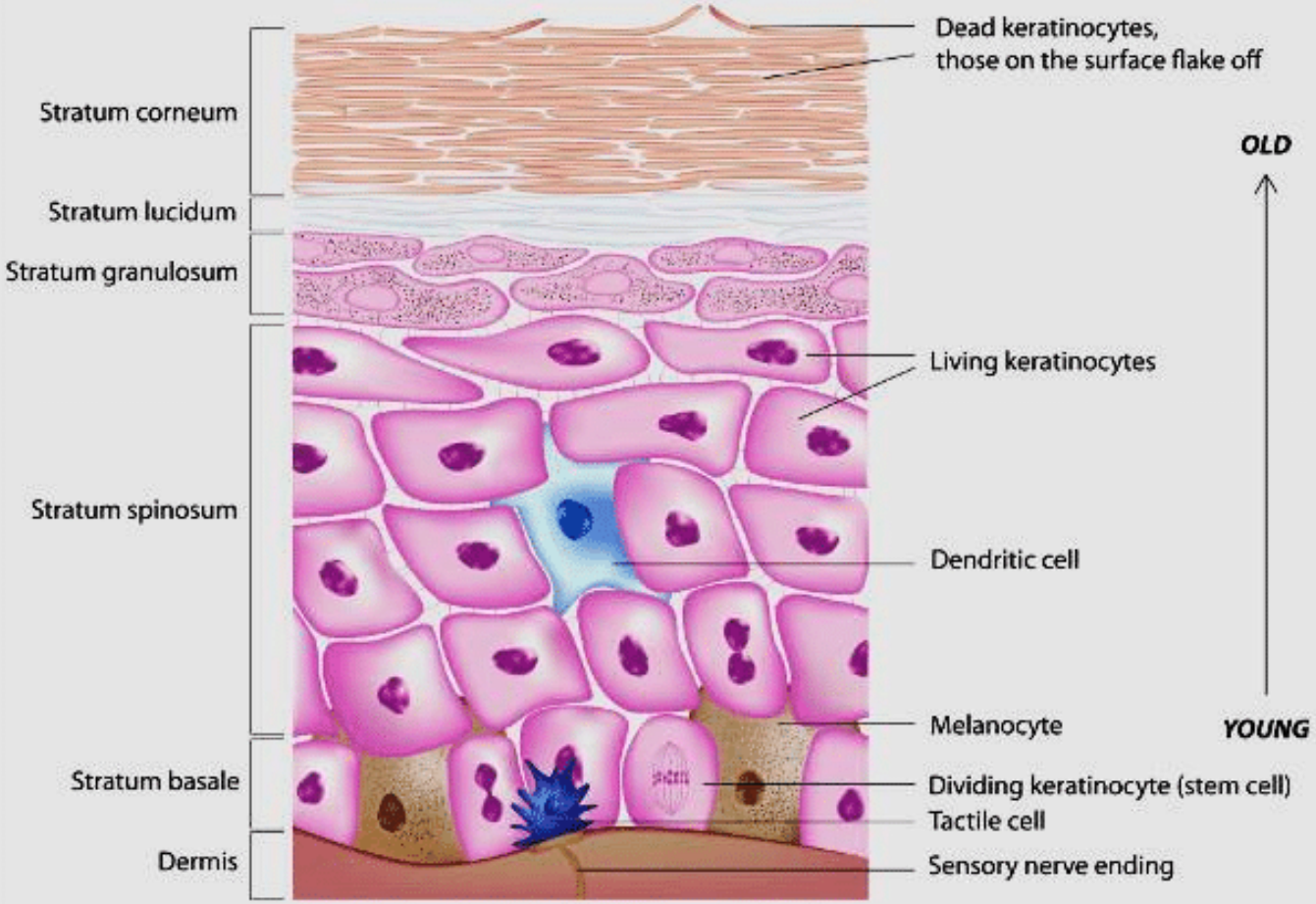
The Skin as immunological organ

THE SKIN POSSESSES A SEMI-AUTONOMOUS IMMUNE SYSTEM

THIS CAN BE DYNAMICALLY ACTIVATED BY HERBAL MEDICINES.

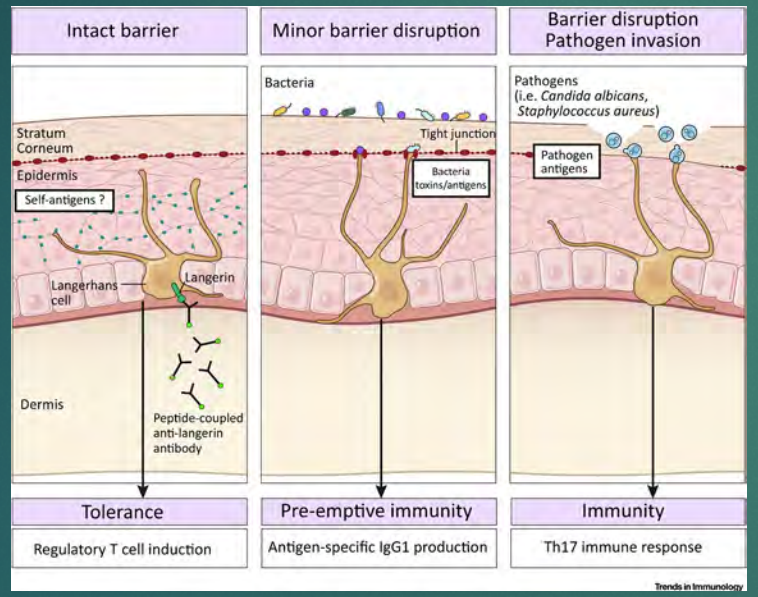

Normal Skin
Epidermis
Dermis
Follicle
Fatty Tissue
Oil gland
Nerve
Sweat gland
© 2004 MedicineNet, Inc.

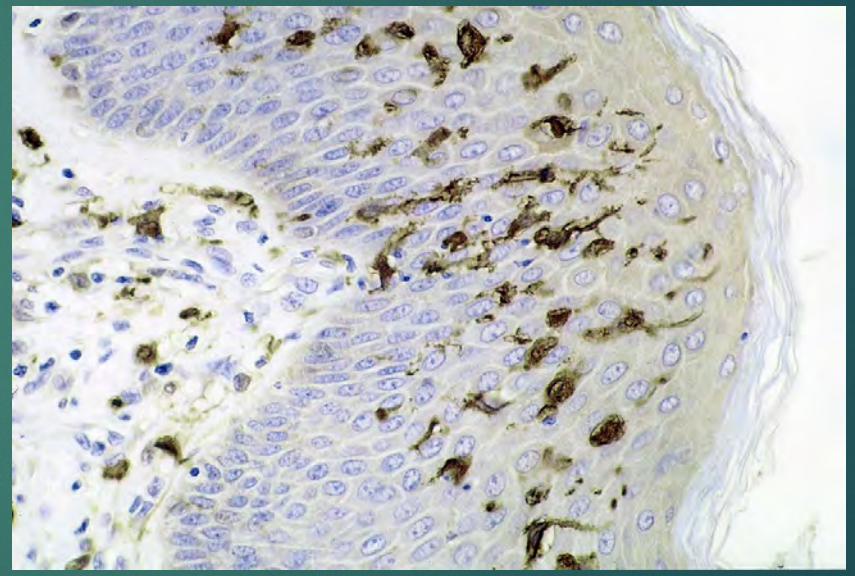
Structure of the Epidermis



Epidermal immunity

Langerhans cells. The major epidermal phagocyte. Dendritic antigen-presenting cells. About ½ the phagocytic capacity of tissue macrophages. Activate epidermal and dermal T-Cells





Salmon JK, Armstrong CA, Ansel JC. The skin as an immune organ. West J Med. 1994 Feb;160(2):146-52. Review.

The major skin cell types all possess immunological properties

- **Keratinocytes.** Immune competent epithelial cells. Make up 90% of epidermal cells. Phagocytes which secrete T-cell activating cytokines.
- http://www.jidonline.org/article/S0022-202X(15)47825-X/pdf)
- https://www.ncbi.nlm.nih.gov/pubmed/11549105

- **Melanocytes.** Pigmented cells. 3-5% of epidermal cells. Like keratinocytes, can secrete cytokines.

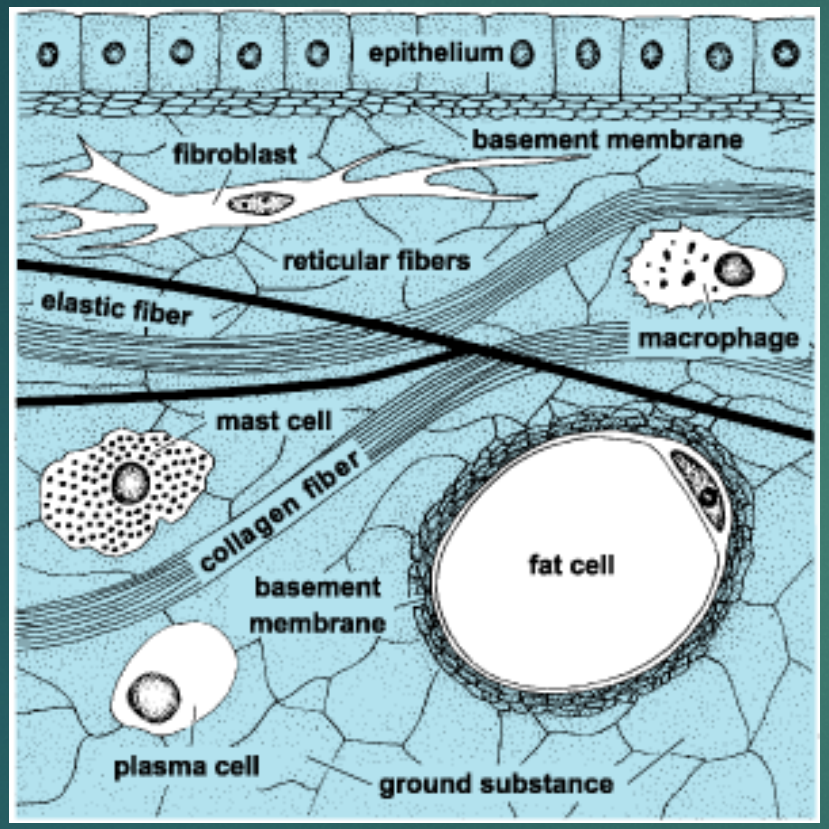
Lymphocytes in the skin

The skin is the largest reservoir of T-Lymphocytes in the body.

- **Resident CD8+ T cells.** Most of the CD8 (cytotoxic) cells in the skin reside in the epidermis
- **Epidermotropic T-Lymphocytes**. Skin-homing lymphocytes from the dermis or system.
- **Dermal T4-lymphotyces** (most abundant in dermis)
- **B-Lymphocytes** present in low numbers but home to the dermis from cutaneous lymphatic material in response to infection or inflammation

Egbuniwe IU, Karagiannis SN, Nestle FO, Lacy KE. Revisiting the role of B cells in skin immune surveillance. Trends Immunol. 2015 Feb;36(2):102-11.

The dynamic dermis



- Circulation can increase or decrease from external or internal (or herbal) stimuli
- The immune-cell-rich dermis is semi-independent of the larger immune system, and can be regulated or stimulated by local factors. Including herbal applications.
- Collagen and elastin forming fibrocytes circulate in the system in the same manner as white blood cells, and can migrate into an injured or inflamed dermis to produce healing and **scarring.**
- More than 90% of T-lymphocytes in the dermis may be “mature” cells already alert for their designated antigen.

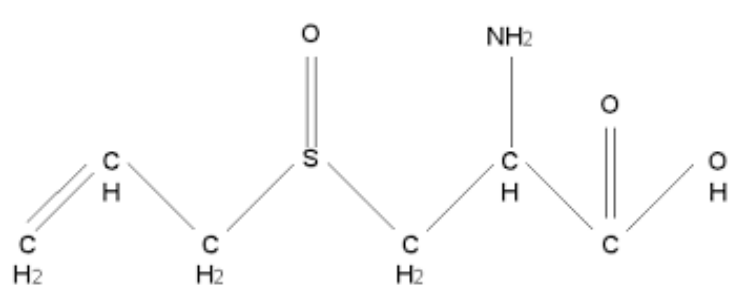
Immune stimulant herbs are more potent locally than systemically

- The skin contains all the essential elements of the immune system
- The skin cells themselves are immune cells and form both a mechanical and a biological layer against invaders.
- Most of our “immune herbs” which we know affect systemic immunity, have an exponentially more potent effect on local immunity when applied topically because of the greater concentration at the site of infection that can be achieved with internal use.
- Echinacea wash from decoction of 1 ounce per liter decocted for 40 minutes.
- Echinacea wash from tincture 1 part Echinacea to 3-6 parts water.

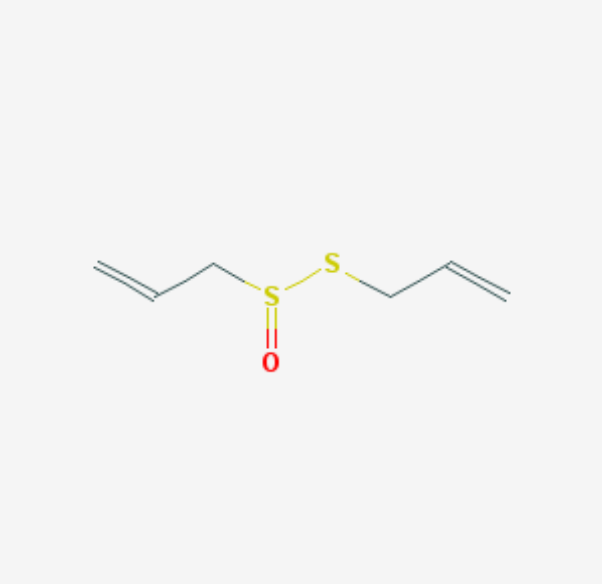
Cutaneous absorption of plant constituents

Molecular size

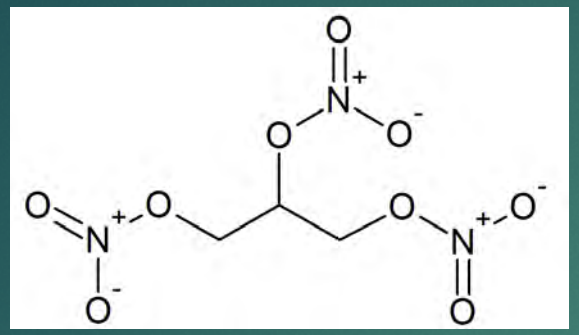
- Aromatic/volatile constituents are readily absorbed in the skin resulting in ***systemic circulation*** and pharmacological effects
- An average of 8% of a concentrated essential oil applied to the skin is absorbed.
- This may be greatly increased by the application of heat.
- Allicin and other sulfur compounds from garlic are rapidly absorbed across the skin and some metabolites are excreted in the breath.
- Molecules of less than 500 molecular weight can pass the corneal outer layer of the skin.



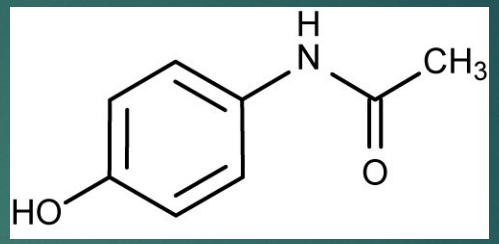
Aliin



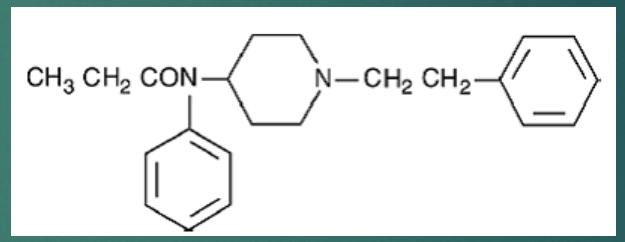
Allicin



Nitroglycerine



Acetaminophen



Fentanyl

Most medicinal plant constituents will pass through the skin, enhanced by soaking the body part in warm water. Relevant to foot bath, sitz bath, hand bath, and compress.

Molecular weights of some Plant Constituents

- Azuline 128
- Achillene 136
- Pentose (Althaea) 150
- Methyl salicylate 152
- *Thujone* 152
- *Pulegone* 152
- Menthol 156
- Carvacrol 158
- Allantoin 158
- *Saffrole* 162
- *Nicotine* 162
- Harman (passiflora) 182
- Chamazuline 184
- Pectin 194
- Apigenin 270
- Baicalein 270
- Luteolin 286
- Capsaicin 305
- Tetrahydrocannibinol 314
- Berberine 336
- Canadine 338
- *Lobeline* 337
- *Symphytine* 381
- Verbenalin 338
- Hydrastine 389
- Scutellarin 462

Almost any plant constituent, when applied locally, will penetrate to all levels of the local area as well as into the general circulation

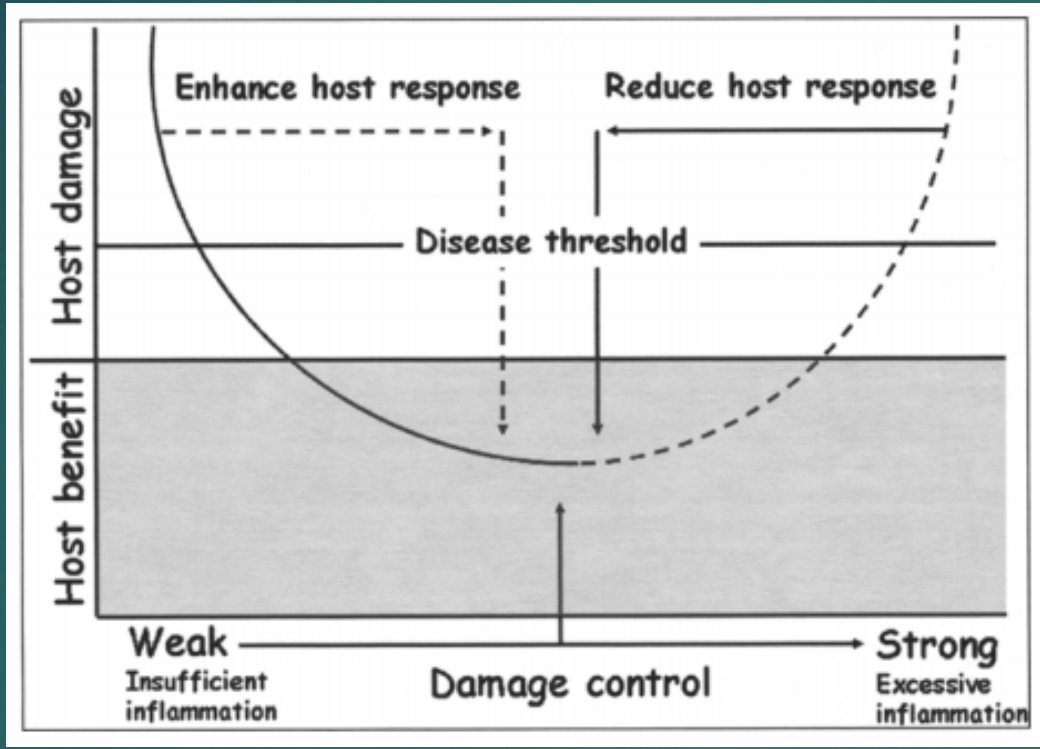
Case: MRSA cellulitis

- Patient was elder in her 70s
- Chronic MRSA cellulitis on both legs extending from the knees to the ankles.
- Treatment: persistent applications of a strong decoction of *Echinacea angustifolia*, plus a tincture of Hydrastis.
- The condition was completely cleared.
- The patient's skin was stained yellow, which she complained about.
- Consider that evidence that a berberine-rich environment was created throughout the tissues.

MRSA cellulitis.
(not this patient)

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

Damage-response model of infection



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

The “Biofilm Complex”

- Planktonic micro-organisms.
- A biofilm matrix with evolved or enhanced quorum-dependent functions
- Active resistance to antimicrobial substances through efflux pumps.
- A continuous, ongoing and rapid evolution of resistance to host and antimicrobials through robust exchange of resistance genes
- Damage to the tissues through invasion, toxins, or other quorum-dependent activities
- A strong but ineffective active immune response which may further damage the tissues through non-resolving inflammation

A synergy model for multi-constituent topical applications

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

These properties are all possessed by some single plants, and with some simple plant combinations. Formulas combining "specialist" plants in each category may be extremely potent for topical use.

Herbal/Constituent actions for synergy

- Direct antimicrobial (bacteria/fungi)
- Efflux pump inhibition
- Anti-quorum/biofilm effects
- Immune stimulating effects
- Modify local inflammation
- Promote tissue repair
- Increase local circulation

Topical applications

- The constituent synergy model requires that all constituents come into contact with the biofilm simultaneously in a manner similar to plant defense.
- May be applied in different topical contexts: skin, ear, mouth, throat, sinus, stomach, (duodenum?), vagina.
- Plants may be combined for multiple effects
- Plants may be delivered in **media** with anti-biofilm effects
- Application below the duodenum is questionable due to digestion/degradation of constituents.
- Application for possible internal biofilms is unlikely, due to pass through gut and liver and unlikely parallel pharmacokinetics of all the constituents at the same time.

Potential synergistic plant actions against the biofilm complex

	Anti-inflammatory	Vulnerary	Antiseptic	Anti-biofilm	EPI	Local Immunity
Calendula	x	x	x		x	x
Plantago	x	x	x	x	x	x
Hypericum	x	x	x	x	x	x
Echinacea	x	x	x		x	x
Althaea	x	x	x	x		x

Infused oils: Olive oil also has wound healing and anti-inflammatory effects

Some cautions

- Caution in applying herbs with strong wound healing effects to suppurating wounds. Potential to “seal in” a biofilm and produce septicemia.
- Case: A man applied comfrey poultices to an extensive burn on his hand. This resulted in severe infection of the hands, swollen nodes in the armpit, and fever from septicemia.
- Caution in applying topical herbs in salve form (with wax) to an infection, even if the herbs may be antimicrobial. May create anaerobic environment.
- Case: A young man with fungal infection in pubic hair region shaved the hair and applied a salve. The bacteria flourished in the anaerobic environment and entered the body through the micro-tears. Result: nearly a week in the hospital on IV antibiotics. Systemic infection with both staph and strep.

Herbs with synergistic effects against biofilms

	Anti septic	Immune	Anti Biofilm	EPI
Larrea	x	x	trad	
Thuja	x	x	trad	
Anemopsis	x		x	x
Baptisia	x	x	trad	
Hypericum	x	x	science	x
Althaea	x	x	science	

	Antiseptic	Immune	Biofilm	MRDi
Aloe	x		science	X
Commiphora	x	x	science	x
Boswellia	x	x	science	x
Allium	x	x	science	x
Hydrastis	x		science	x
Achillea	x		science	x

Stimulate local circulation

	Stimulant	Antiseptic	Immunity	Biofilm	MDRi
Thuja	x	x	x	trad	
Anemopsis	x	x		x	
Myrica	x	x	x	trad	
Baptisia		x	x	trad	
Commiphora	x	x	x	yes	x
Achillea	x	x		yes	x
Capsicum	x	x			
Arnica	x	x	x	yes	

Some historical combinations

Garden variety infused topical oil

	cool	Anti-inflammatory	Vulnerary	Antiseptic	Anti biofilm	EPI	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

Table 1
Antibacterial activity of different *Calendula officinalis* extracts against clinical bacterial pathogens.

Bacteria	Antibacterial activity of *Calendula officinalis* extracts in term of inhibition zone (IZ) in mm[a]		
	Methanol extract	Ethanol extract	Ciprofloxacin
Bacillus subtilis NCTC 10400	22 ± 1^{b}	18 ± 2^{a}	47 ± 3^{c}
B. subtilis [JEM7]	14 ± 1^{b}	10 ± 1^{a}	39 ± 3^{c}
Pseudomonas aeruginosa NCTC 27853	19 ± 2^{b}	13 ± 1^{a}	42 ± 2^{c}
P. aeruginosa [JEM16]	12 ± 1^{a}	10 ± 1^{a}	40 ± 2^{b}
Bacillus cereus NCTC 7464	14 ± 1^{a}	16 ± 2^{a}	38 ± 3^{b}
B. cereus [JEM8]	15 ± 2^{b}	10 ± 1^{a}	36 ± 3^{c}
Escherichia coli (UUC collection)	10 ± 1^{a}	9 ± 1^{a}	44 ± 2^{b}
E. coli (Ampicillin-resistant) (UUC collection)	13 ± 1^{b}	10 ± 1^{a}	44 ± 1^{c}
E. coli [JEM1]	21 ± 2^{b}	14 ± 1^{a}	36 ± 2^{c}
E. coli [JEM4]	14 ± 2^{a}	14 ± 1^{a}	35 ± 2^{b}
E. coli [JEM17]	22 ± 1^{b}	18 ± 1^{a}	35 ± 3^{c}
Staphylococcus aureus MSSA 25923	18 ± 2^{a}	28 ± 2^{b}	42 ± 1^{c}
S. aureus [JEM18]	22 ± 2^{a}	19 ± 2^{a}	30 ± 2^{b}
Klebsiella aerogenes NCTC 9528	19 ± 1^{b}	13 ± 1^{a}	39 ± 2^{c}
K. aerogenes [JEM2]	14 ± 1^{a}	12 ± 1^{a}	40 ± 3^{b}
Enterococcus faecalis NCTC 775	14 ± 1^{a}	18 ± 2^{b}	42 ± 3^{c}
E. faecalis [JEM10]	13 ± 1^{a}	15 ± 1^{a}	36 ± 2^{b}
Bacillus pumilis [JEM15]	14 ± 1^{a}	13 ± 1^{a}	41 ± 1^{b}
Klebsiella pneumoniae [JEM19]	16 ± 1^{a}	14 ± 1^{a}	45 ± 3^{b}

[a] Inhibition zones including the disc diameter of 6 mm.
[b] Values are mean ± standard deviation (SD) of three replicates. Difference letter in superscript represent significant ($p < 0.05$) difference in activity between extracts.

Methanol and ethanol extracts of Calendula vs Ciprofloxacin on various microorganisms.

All show broad spectrum antimicrobial activity.

Efstratiou, E., Hussain, A. I., Nigam, P. S., Moore, J. E., Ayub, M. A., & Rao, J. R. (2012). *Antimicrobial activity of Calendula officinalis petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens. Complementary Therapies in Clinical Practice, 18(3), 173–176.*

Samuel Thomson's Number Six

	Stimulant	Anti inflammatory	Antiseptic	Immunity	Biofilm	EPI	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	EPI
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.)

Chronic sinusitis

- Despite location, presence of anaerobic bacteria common
- MRSA common in 60% or more
- Eosinophilic fungal sinusitis in CRS patients.
- Patients with bacteria present typically had 2-3 species.
- 83% had fungus present, and typically 3-5 species.
- The condition may be characterized by a multi-species, multi-kingdom biofilm.
- In allergic fungal sinusitis, the condition is complicated by an allergic reaction to the fungus.

Dósa E, Dóczi I, Mojzes L, Molnár EG, Varga J, Nagy E. Identification and incidence of fungal strains in chronic rhinosinusitis patients. Acta Microbiol Immunol Hung. 2002;49(2-3):337-46.

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*

Esberitox

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

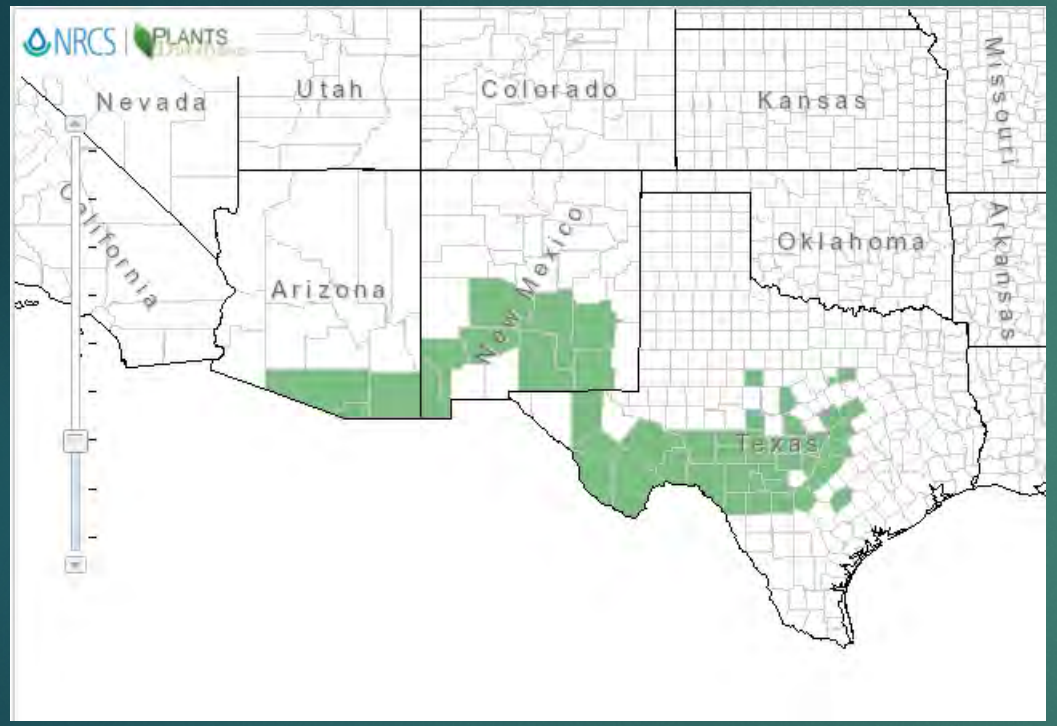
- Developed in Europe for ***internal*** use as an immune stimulant.
- A very potent topical treatment. Prepare as ***decoction***.
- Note traditional use of Baptisia was primarily external application of the tea

Possible combinations

		Stimulant	Anti inflammatory	Antiseptic	Immune	biofilm	EPI
Bursera	warm	x	x	x	x	trad	?
Larrea	cool		x	x	x	trad	?
Anemopsis	warm	x	x	x	trad	x	x

Acalypha spp. Yerba del Cancer.

A universal folk remedy for wounds in Mexico



Acalypha phleoides (syn: *lindheimeri)*

A. californica *"Yerba del Cancer"*

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

Acalypha and Arctostaphylos

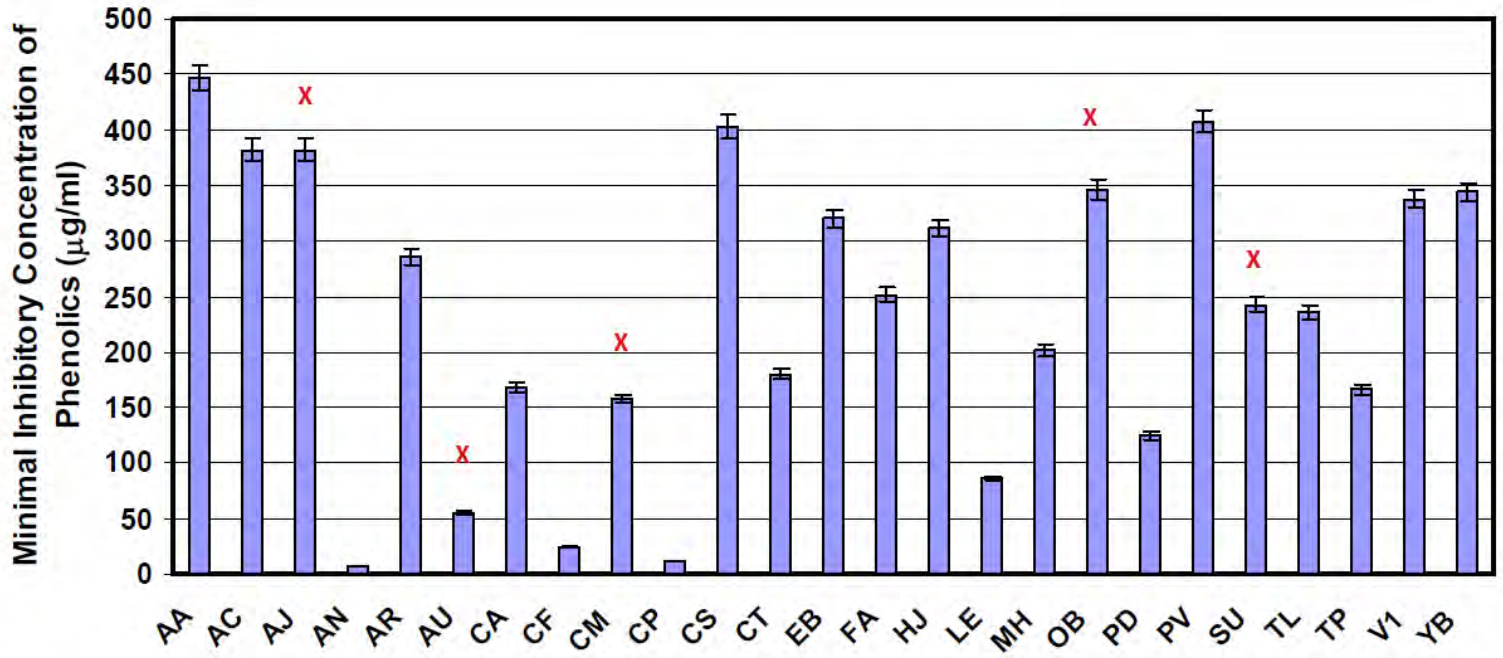


Figure 1. Minimal inhibitory concentrations of different HSMP from Central/South America against *P. aeruginosa*

Acalypha (AJ) is a relatively ***poor antimicrobial***. (higher number means higher dose is necessary to inhibit bacteria.) Arctostaphylos u. (AU) is ***very strong***

Huerta V, Mihalik K, Crixell SH, and Vattem, DA*
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 2008

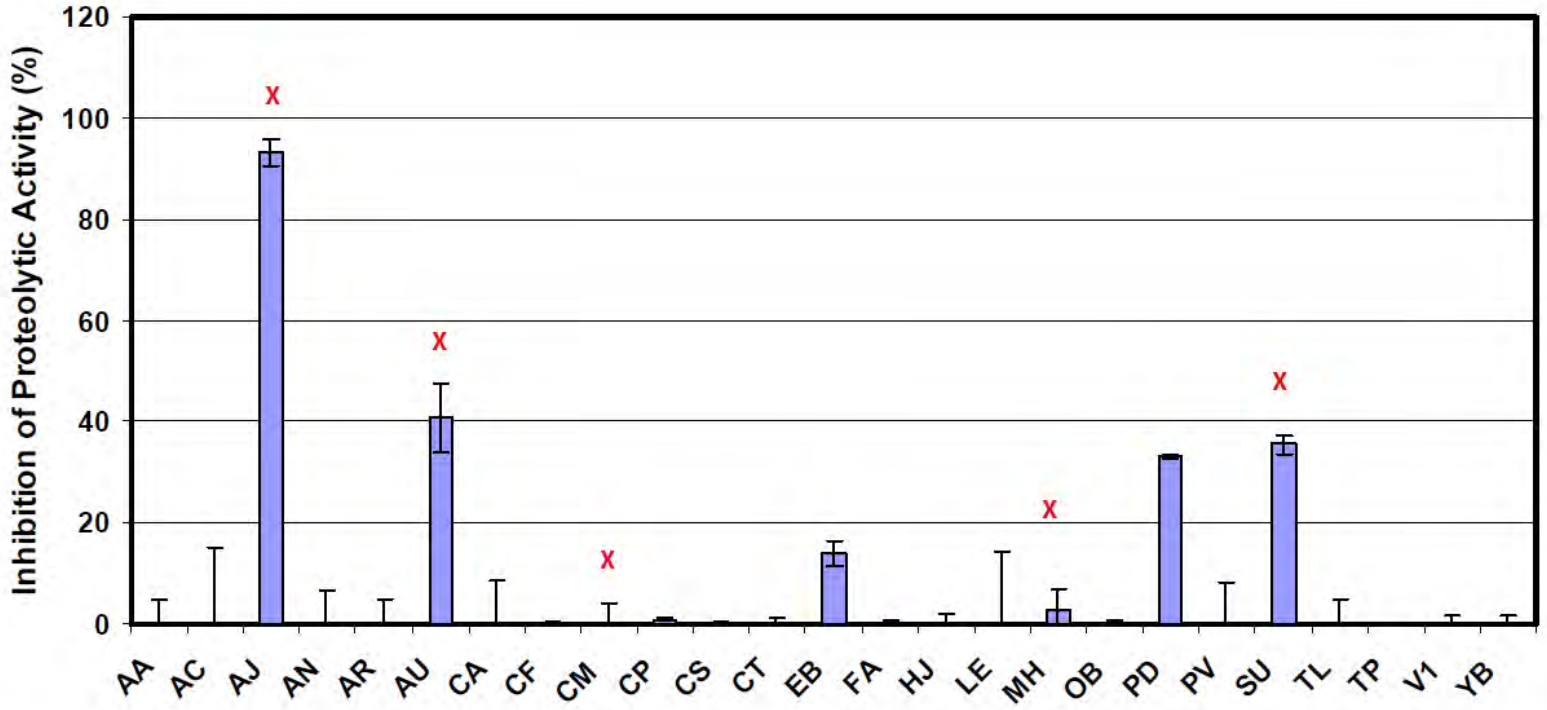


Figure 3. Effect of sub-lethal concentrations of HSMP from Central/South America on quorum sensing dependent total proteolytic enzyme acticity in *P. aeruginosa.*

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

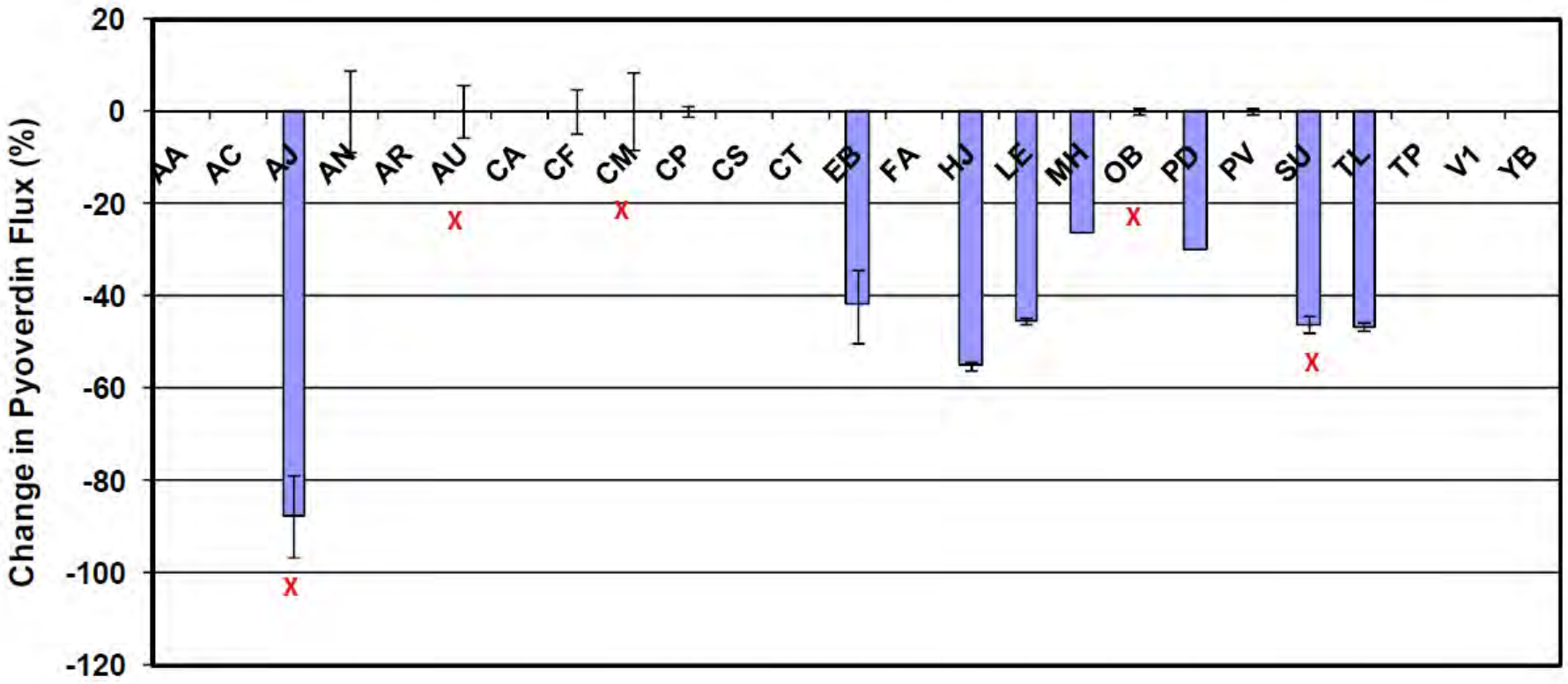
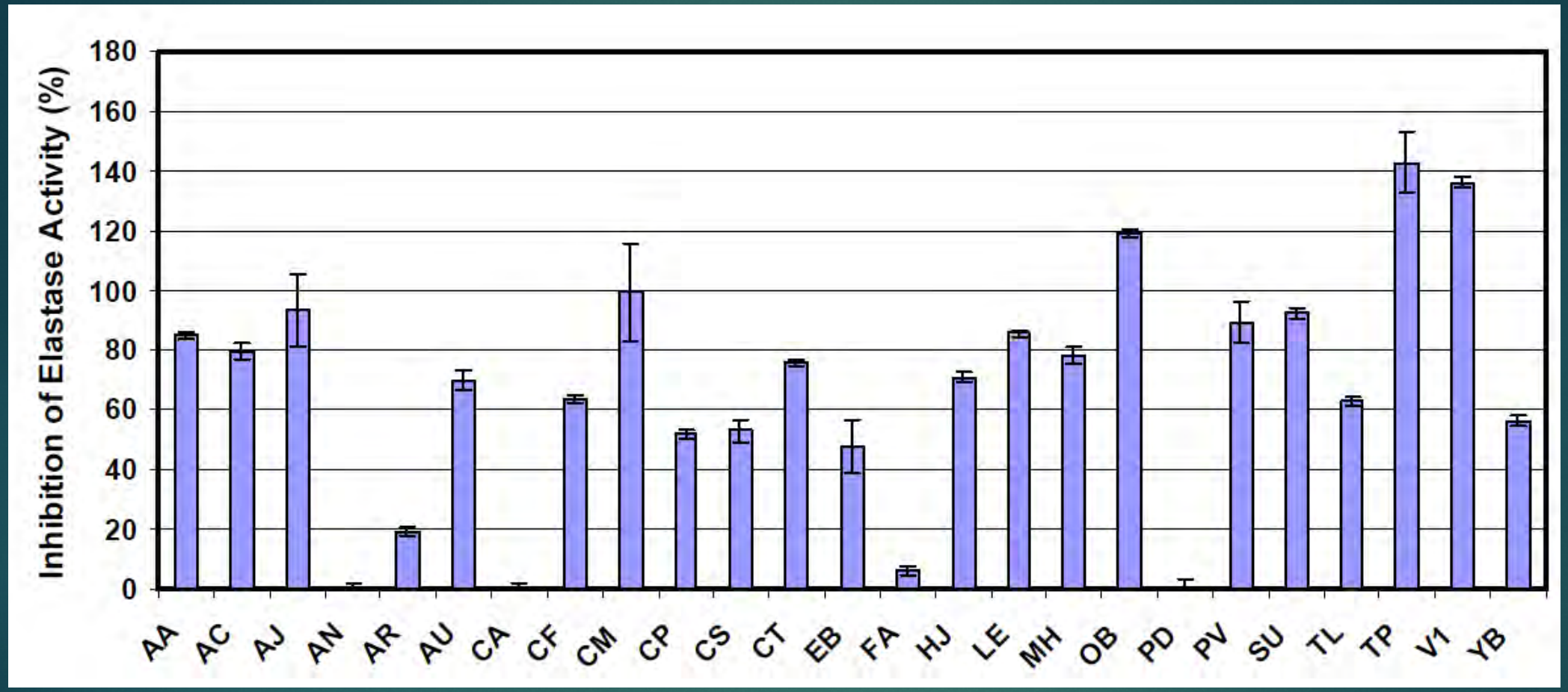


Figure 5. Effect of sub-lethal concentrations of HSMP from Central/South America on formation of pyoverdin pigment formation in *P. aeruginosa*.

In another measure of quorum sensing activity, Acalypha was #1 of the 25.



In a third measure (qs-dependent elastase activity) both Acalypha and Arctostaphylos were higher than average in inhibition

Anti-microbial and anti-biofilm effects of Uva Ursi

Table II. Anti-microbial and anti-QS activity of the screened aqueous and ethanolic plant extracts against wild-type C. violaceum *ATCC 31532 and reporter* C. violaceum *NCTC 13274 strain*

Plant species	Inhibition zone area (mm^2) against wild-type *C. violaceum* ATCC 31532 strain				Inhibition zone areas (mm^2) against reporter *C. violaceum* ACTC 13274 strain[a]			
	Growth inhibition		Pigment inhibition		Growth inhibition		Pigment inhibition	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
Arctostaphylos uva-ursi	235	207	126	189	207	235	153	126
Betula verrucosa	0	19	89	105	0	9	113	105
Calendula officinalis	0	0	0	19	0	0	0	31
Chelidonium majus	9	19	0	0	9	14	0	0
Comarum palustre	0	0	0	19	0	0	0	75
Eucalyptus viminalis	19	31	75	127	19	31	276	204
Inula helenium	0	0	0	35	0	0	0	85
Juniperus communis	0	0	31	31	0	0	0	19
Ledum palustre	0	31	0	19	0	19	19	25
Quercus robur	0	0	75	94	0	0	94	94
Rosa majalis	0	0	0	9	0	0	0	14
Salvia officinalis	0	31	0	0	0	25	0	0
Vaccinium vitis-idaea	31	44	0	69	31	59	83	54

[a] Supplemented with C_6-AHL (5×10^{-7} mol L^{-1}).

Achillea millefolium, Bidens tripartita, Matricaria chamomilla, Plantago major, Taraxacum fficinale, Tussilago farfara and *Viola tricolor* did not show any activity.

Tolmacheva AA, Rogozhin EA, Deryabin DG. Antibacterial and quorum sensing regulatory activities of some traditional Eastern-European medicinal plants. Acta Pharm. 2014 Jun;64(2):173-86.

Ocimum basilicum

Musthafa KS, Ravi AV, Annapoorani A, Packiavathy IS, Pandian SK. Evaluation of anti-quorum-sensing activity of edible plants and fruits through inhibition of the N-acyl-homoserine lactone system in Chromobacterium violaceum and Pseudomonas aeruginosa. Chemotherapy. 2010;56(4):333-9.

- In the study of Hispanic use of plants above, *Ocimum basilicum* also showed potential effects against biofilms
- Although not a comparatively strong antimicrobial, it was the most potent of the plants in suppression of quorum sensing in one measure (pyocyanin pigment formation).
- In another measure, it ranked 3rd among the plants (quorum-sensing dependent elastase activity)
- A separate study likewise found strong anti-quorum-sensing but low antimicrobial potential
- This plant might be added to a topical formula to increase antibiofilm effects

Tannic acid

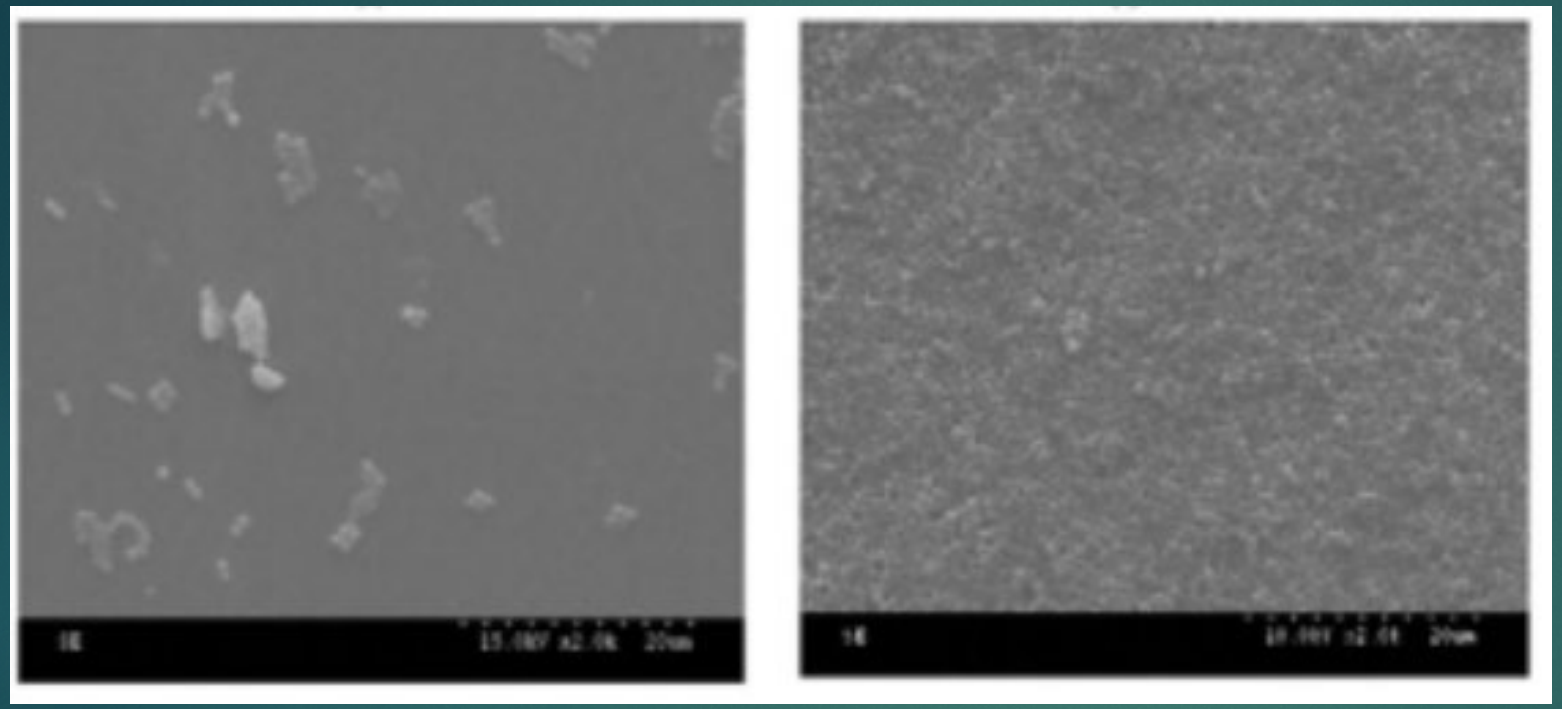


Plate cultures of Staphylococcus aureus With (left) and without (right) tannic acid at 40 micrograms per ml concentration.

Tannic acid binds to and destroys the Staphyloccus species cell walls, and inhibits biofilm formation

Tannic acid was equally effective against both antibiotic-sensitive and antibiotic-resistant bacteria.

Dong G, Liu H, Yu X, Zhang X, Lu H, Zhou T, Cao J. Antimicrobial and anti-biofilm activity of tannic acid against Staphylococcus aureus. Nat Prod Res. 2018 Sep;32(18):2225-2228.

Quercus species
Constituent synergy for anti-quorum properties

- Dried then rehydrated *Quercus* bark
- The whole plant had mild anti-microbial but very strong anti-quorum sensing activity.
- Ten constituents tested individually
- Two of ten showed anti-microbial and anti-quorum activity
- Five more showed anti-quorum activity without anti-microbial activity
- Only a recombination of all constituents together showed activity equal to the whole plant.

Deryabin DG, Tolmacheva AA. Antibacterial and Anti-Quorum Sensing Molecular Composition Derived from Quercus cortex (Oak bark) Extract. Molecules. 2015 Sep 17;20(9):17093-108.

Quercus
Hamamelis
Magnifera
Punica
Arctostaphylos
Capsella

We have the traditional use of tannin-rich oak barrels to ferment wine. The Oak may prevent the formation of contaminant biofilms during the fermentation process. A useful addition to an antiseptic formula to affect biofilms.

Quercus dose and form

- The MIC and MBC for staphylococcus aureus were 10 and 12.5 µg/ml and for pseudomonas aeruginosa they were 10 and 17.5 µg/m consecutively.
- This was water extract, evaporated to powder.

Study of the inhibitory effect of Quercus Coccifera's aqueous extract on Staphylococcus aureus and Pseudomonas aeruginosa In vitro Arezo Judaki, Jafar Panahi, Mohamad Reza Havasian, Parnian Tajbakhsh, Mohamad Ali Roozegar Bioinformation. 2014; 10(11): 689–692.

Quercus tincture

- Ethanol extraction of acorn herb were evaluated against Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Salmonella typhi and Pseudomonas aeroginosa in in vitro and in vivo conditions.
- Minimal Inhibitory Concentration (MIC) was 10 microg/ml, 10 microg/ml, 5 microg/ml, 15 microg/ml and 15 microg/ml for K. pneumoniae, E. coli, S. typhi, S. aureus and P. aeroginosa, respectively.
- The in vivo results showed that experimental infection produced by K. pneumoniae, E. coli, S. typhi and P. aeroginosa was totally inhibited in rats treated by the acorn extraction, while positive control rats died after five days.e

Mohebi R, Ghafourian S, Sekawi Z, Khosravi A, Galehdari EA, Hushmandfar R, Ranjbar R, Maleki A, Mohammadzadeh M, Rahbar M, Sadeghifard N. In vitro and in vivo antibacterial activity of acorn herbal extract against some Gram-negative and Gram-positive bacteria. Roum Arch Microbiol Immunol. 2011 Oct-Dec;70(4):149-52. PubMed PMID: 22568261.

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.
- Half life of allicin is about 18 hours.
- Some of these non-allicin constituents may be delivered to a biofilm systemically after oral ingestion.
- ***Fresh garlic can produce second and third degree in burns***.

Allium sativum applications

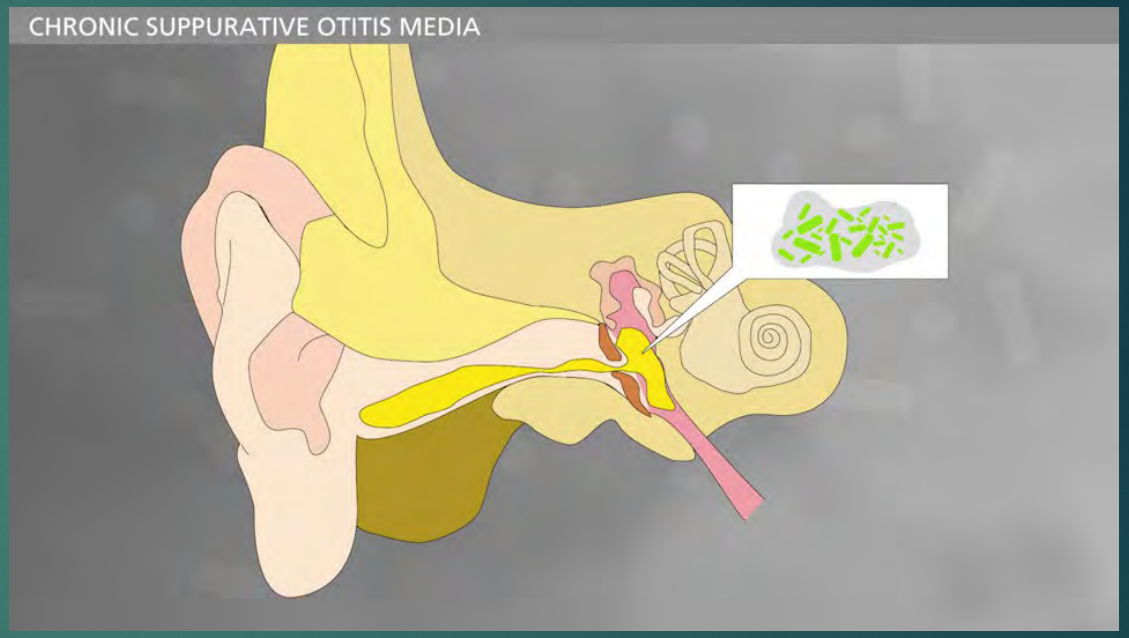
- Two cloves (not whole bulbs) in liter of water, blended and strained through cheesecloth.
- Poultice
- Foot bath or handbath.
- Mouthwash for thrush
- Douche
- Infused oil to ear

Galen's treatment for arterial wounds in gladiators

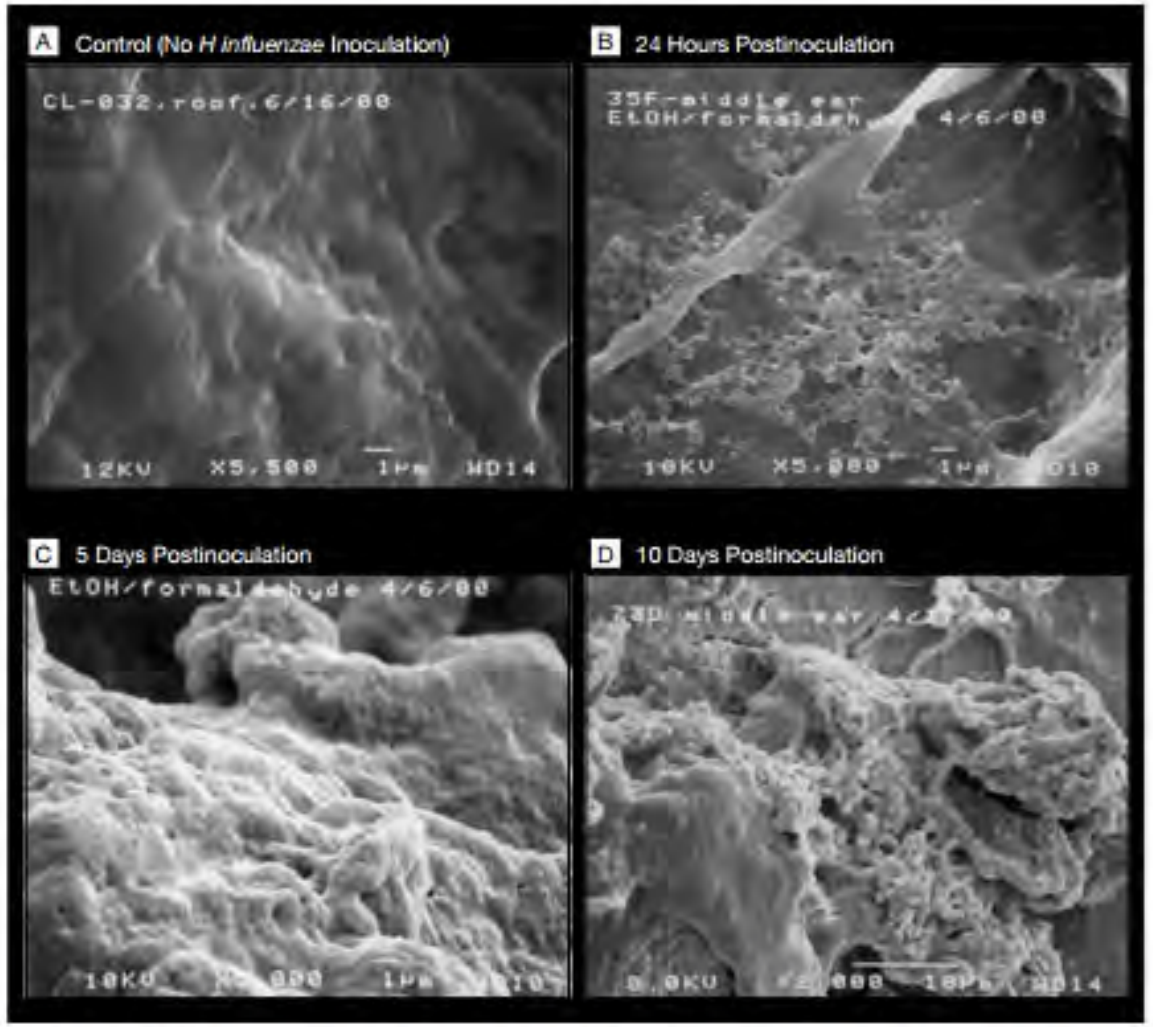
- Crush and soak garlic in wine overnight. Retains a reduced level of allicin, and adds the allicin breakdown products.
- Make a wet poultice of flour with this and pack into the wound to stop bleeding.
- Cover with a cloth, and keep moist with the garlic/wine tincture.
- Stops bleeding with the flour matrix, and prevents infection and formation of biofilm with the garlic/wine mixture.

Chronic otitis media

- Normal oral flora to form chronic biofilms in the middle ear.
- Biofilms readily detected in the exudate after eardrum rupture.
- Traditional treatment with warm infused oil of garlic.
- Antimicrobial/anti-biofilm constituents including oil-soluble *ajoene*.
- Eardrum is permeable to medications and plant constituents.
- Administration in outer ear results in expectoration in the sinuses with garlic flavor to the discharge.



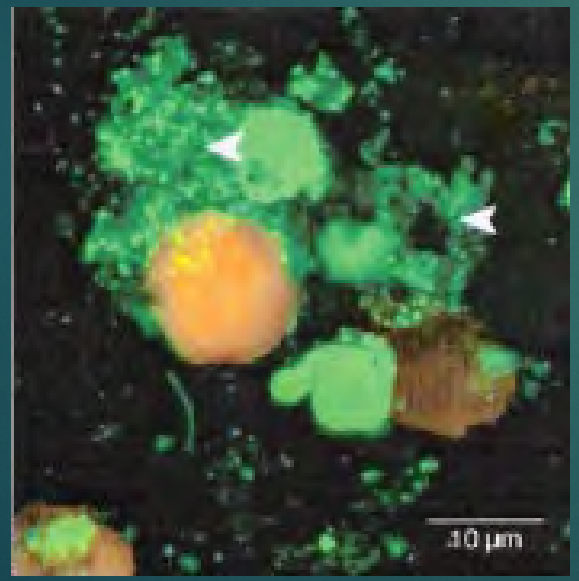
May add the astringent *Verbascum* and antimicrobial *Phytolacca* to the formula.



A, Control at ×5500 magnification. This specimen was obtained at time 0 in an animal that was not inoculated with *Haemophilus influenzae*; B, *H influenzae* microcolonies on middle-ear mucosa 24 hours after inoculation at ×5000; C, mature *H influenzae* biofilm on middle-ear mucosa 5 days after inoculation at ×5000; D, mature biofilm 10 days after inoculation at ×2000. Scale indicated by the 1- or 10-μm bars.

H. influenzae biofilm in middle ear biopsy In animal models on days 0, 1, 5, and 10.

Ehrlich GD, Veeh R, Wang X, Costerton JW, Hayes JD, Hu FZ, Daigle BJ, Ehrlich MD, Post JC. Mucosal biofilm formation on middle-ear mucosa in the chinchilla model of otitis media. JAMA. 2002 Apr 3;287(13):1710-5.

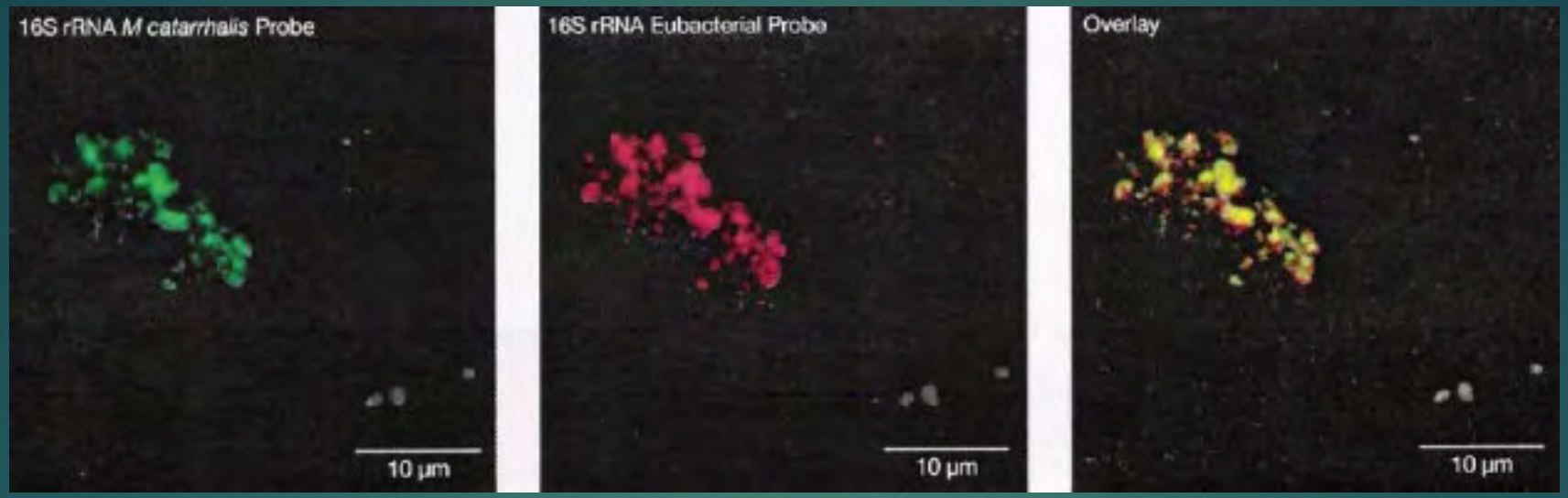


Human middle ear biopsy.
White arrows show biofilms
Green *H. influenza* biofilms from middle ear biopsy.
In both pictures the host cells are yellow, orange, or red

Hall-Stoodley L, Hu FZ, Gieseke A, Nistico L, Nguyen D, Hayes J, Forbes M, Greenberg DP, Dice B, Burrows A, Wackym PA, Stoodley P, Post JC, Ehrlich GD, Kerschner JE. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. JAMA. 2006 Jul 12;296(2):202-11.

Multispecies biofilm in middle ear, *H influenza* (L) *S pneumoniae* (M), overlay (R)

Hall-Stoodley L, Hu FZ, Gieseke A, Nistico L, Nguyen D, Hayes J, Forbes M, Greenberg DP, Dice B, Burrows A, Wackym PA, Stoodley P, Post JC, Ehrlich GD, Kerschner JE. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. JAMA. 2006 Jul 12;296(2):202-11.



Imaging probes for *Moraxella catarrhalis,* eubacteria, and their overlay. A biopsy from the middle ear of a child with otitis media with effusion

Hall-Stoodley L, Hu FZ, Gieseke A, Nistico L, Nguyen D, Hayes J, Forbes M, Greenberg DP, Dice B, Burrows A, Wackym PA, Stoodley P, Post JC, Ehrlich GD, Kerschner JE. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. JAMA. 2006 Jul 12;296(2):202-11.

Otitis externa

- Chronis otitis externa had the presence of biofilms in 23/25 patients.
- Results from the standard treatment with antibiotics and steroids was compared with “chemical peeling” of the biofilm and superficial epithelium of the ear canal.
- Result were similar for the first 3 months.
- At 6 months, 89% of the “peeling group” were symptom free, vs 64% of the antibiotic group
- At 9 months, the corresponding rates were 80% and 60%

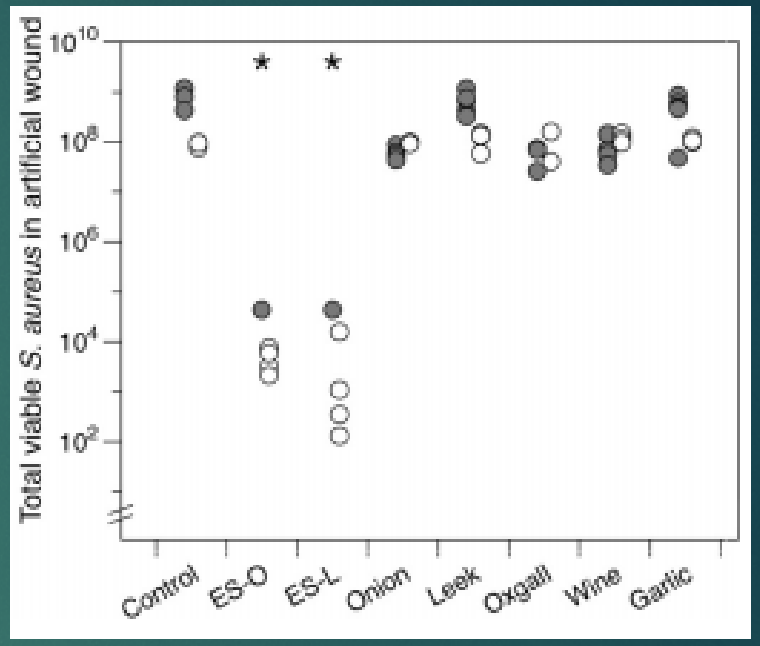
Fusconi M, Petrozza V, Taddei AR, Vinciguerra V, De Virgilio A, Chiarini F, Cirenza M, Gallinelli C, Conte M, de Vincentiis M. Is biofilm the cause of chronic otitis externa? Laryngoscope. 2011 Dec;121(12):2626-33.

Ancient formula from *Balds Leechbook**

- The recipe instructs the reader to crush garlic and a second *Allium* species (whose translation into modern English is ambiguous), combine these with wine and oxgall (bovine bile), and leave the mixture to stand in a brass or bronze vessel for 9 days and nights
- The researchers made two versions of the formula, exactly as described, with *Allium cepa* (onion) in one and *Allium ampeloprasum* (leek) in the other.
- These were tested against *Staphylococcus aureus* in both planktonic and established biofilm form in synthetic wound fluid

* Cockayne O. 1864 –1866. Leechdoms, wortcunning and starcraft: being a collection of documents, for the most part never before printed, illustrating the history of science before the Norman conquest. Rolls series 35th, 3 Vols. Longman, Green, Longman, Roberts, and Green, London, United Kingdom

- Both formulas were 100% bactericidal against planktonic bacteria.
- Both also significantly reduced the biofilm (see chart)
- *None of the elements individually had any effect on the biofilm*.
- The combination of wine, garlic, and leek demonstrated the full effect of the formula
- If onion was used instead of leek, then the bile was necessary also for the full effect of the formula (bile salts = strong antimicrobial)
- Brass had no effect, but because it is sterile, it was probably valuable in medieval times
- Take-home: ***Addition of leek or possibly onion to a topical antimicrobial garlic preparation has strong synergistic anti-biofilm activity.***



Harrison F, Roberts AE, Gabrilska R, Rumbaugh KP, Lee C, Diggle SP. A 1,000-Year-Old Antimicrobial Remedy with Antistaphylococcal Activity. MBio. 2015 Aug 11;6(4):e01129.

Effect of onion oil* on microbes

Table 1. Effect of onion oil on the growth of various bacterial isolates.

Group of bacteria	Bacteria	Inhibition zone (mm)
Gram-negative bacteria	*Escherichia coli*	6
	Klebsiella pneumoniae	12
	Pseudomonas fluorescens	–*
	Serratia rhadnii	–
Gram-positive bacteria	*Bacillus anthracis*	10
	Bacillus cereus	26
	Micrococcus luteus (Sarcina)	16
	Staphylococcus aureus	14

*–: No Inhibition

Table 2. Effect of onion oil at three concentrations (100, 200 and 500 ppm) on the growth of different isolates of dermatophytic fungi.

Dermatophytic fungi tested	Growth zone (cm) at the different concentrations of onion oil			
	Control	100 ppm	200 ppm	500 ppm
Chrysosporium carmichaelii	3.6	3.2	3.0	1.8
C. indicum	4.2	4.0	2.8	1.2
C. keratinophilum	4.0	3.6	2.3	0.6
C. queenslandicum	3.2	2.8	0.9	–
C. tropicum	3.9	3.6	2.1	1.3
Microsporum canis	3.9	3.4	–*	–
M. gypseum	3.6	3.2	–	–
Trichophyton mentagrophytes	4.1	2.9	0.6	–
T. simii	4.2	3.5	–	–

*– = No growth.

*Commercial plant juice in carrier oil

Zohri AN, Abdel-Gawad K, Saber S. Antibacterial, antidermatophytic and antitoxigenic activities of onion (Allium cepa L.) oil. Microbiol Res. 1995 May;150(2):167-72.

Antibiotic drug effects on biofilms

- Antimicrobials whether pharmaceutical drugs or isolated plant constituents, can kill or inhibit some planktonic forms of bacteria.
- Some drugs (macrolides?) or antimicrobial constituents may inhibit quorum-sensing, but generally will not eradicate an established biofilm.
- Antibiotics used on biofilms ***promote resistance genes*** which are then acquired throughout the biofilm.
- Some antibiotics and some plant constituents, when given in sub-inhibitory doses (SIC) ***trigger quorum sensing*** and biofilm formation.
- SIC doses may also promote “Darwinian” evolution of more robust metabolism or other properties of the microorganisms.

Antimicrobial and biofilm effects of herbs used in traditional Chinese medicine

- "Of the twenty-two components of tea decoctions commonly used to treat infections, only Scutellaria, Taraxacum, Tussilago and Glycyrrhiza exhibited antimicrobial activity. The activity, when present, was organism specific, i.e., anti-Staphylococcus aureus, including anti-MRSA activity under aerobic and/or anaerobic conditions. However, with the exception of Scutellaria, ***sub-inhibitory concentrations of the herbs exhibited a pattern of inducing enhanced production of biofilm***."

Lau D, Plotkin BJ. Antimicrobial and biofilm effects of herbs used in traditional Chinese medicine. Nat Prod Commun. 2013 Nov;8(11):1617-20.

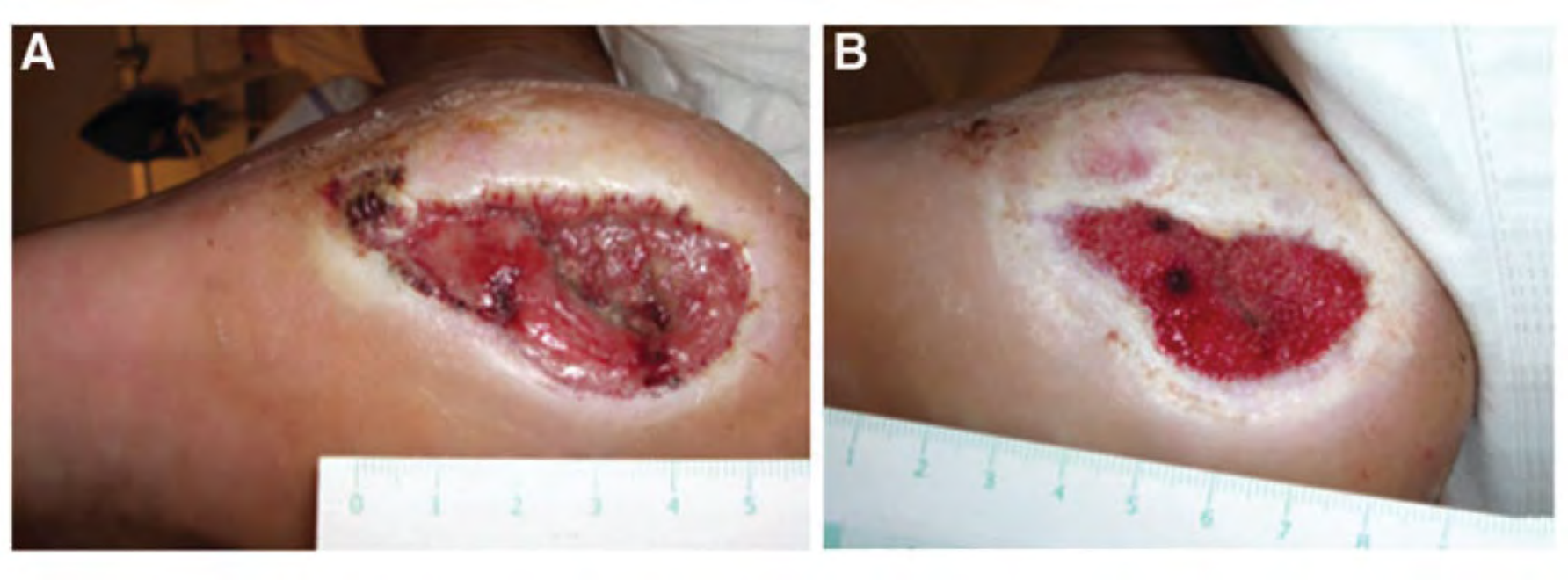
Antimicrobial effect on the microbiome

- Systemic antibiotics can promote the development of resistance in every compartment of the microbiome.
- Systemic antibiotics can force the metabolic evolution of the microbiome to produce more energy (SFCA)
  - Relationship to the fattening of farm animals with SIC of antibiotics
  - Relationship to obesity in U.S. population.
- Systemic antibiotics can reduce the diversity in every compartment of the microbiome.

Media

Vinegar and biofilms

- Acetic acid has an anti-microbial effect against established biofilms both in-vitro and in open wounds.
- It is effective for 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)

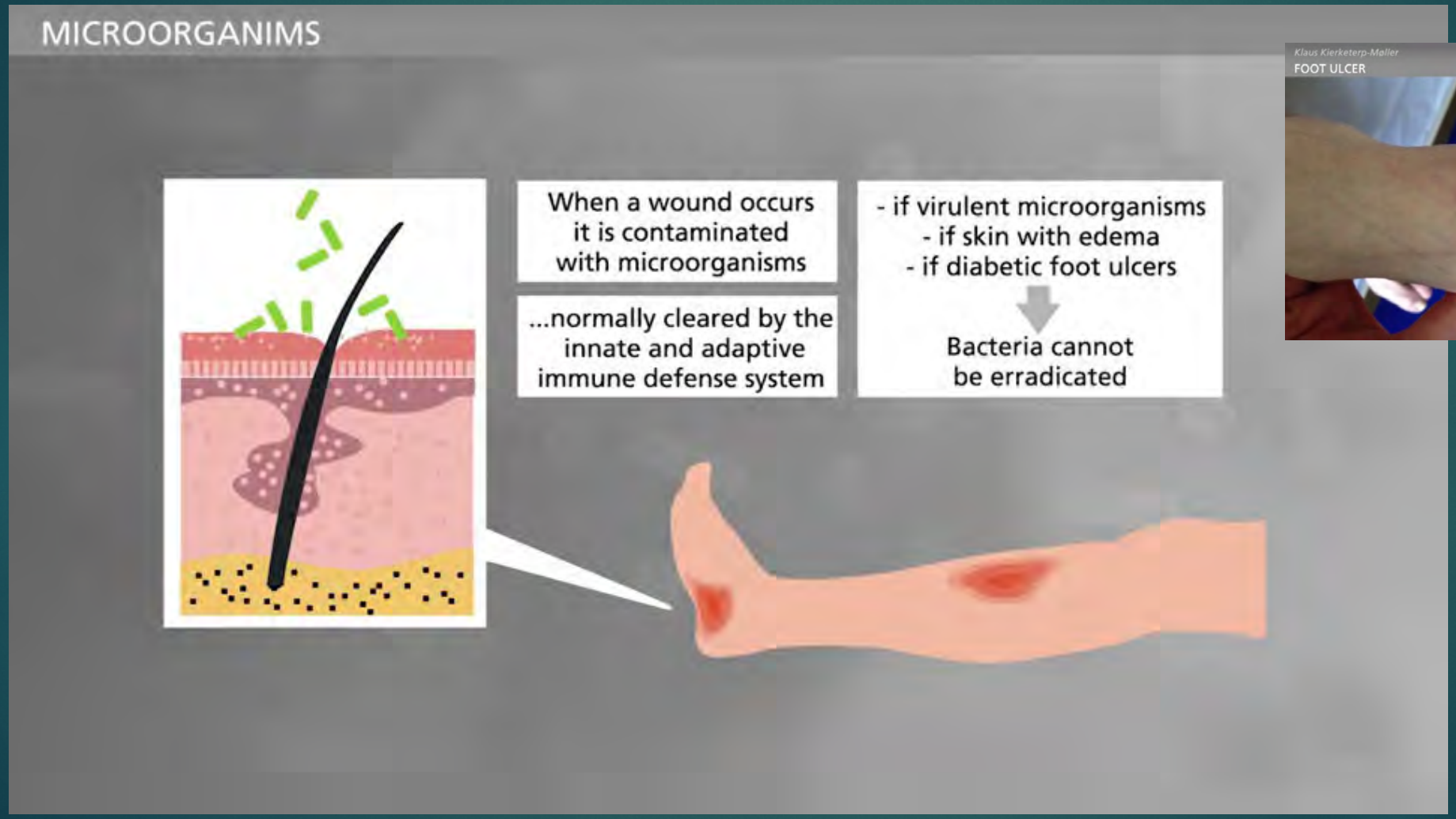


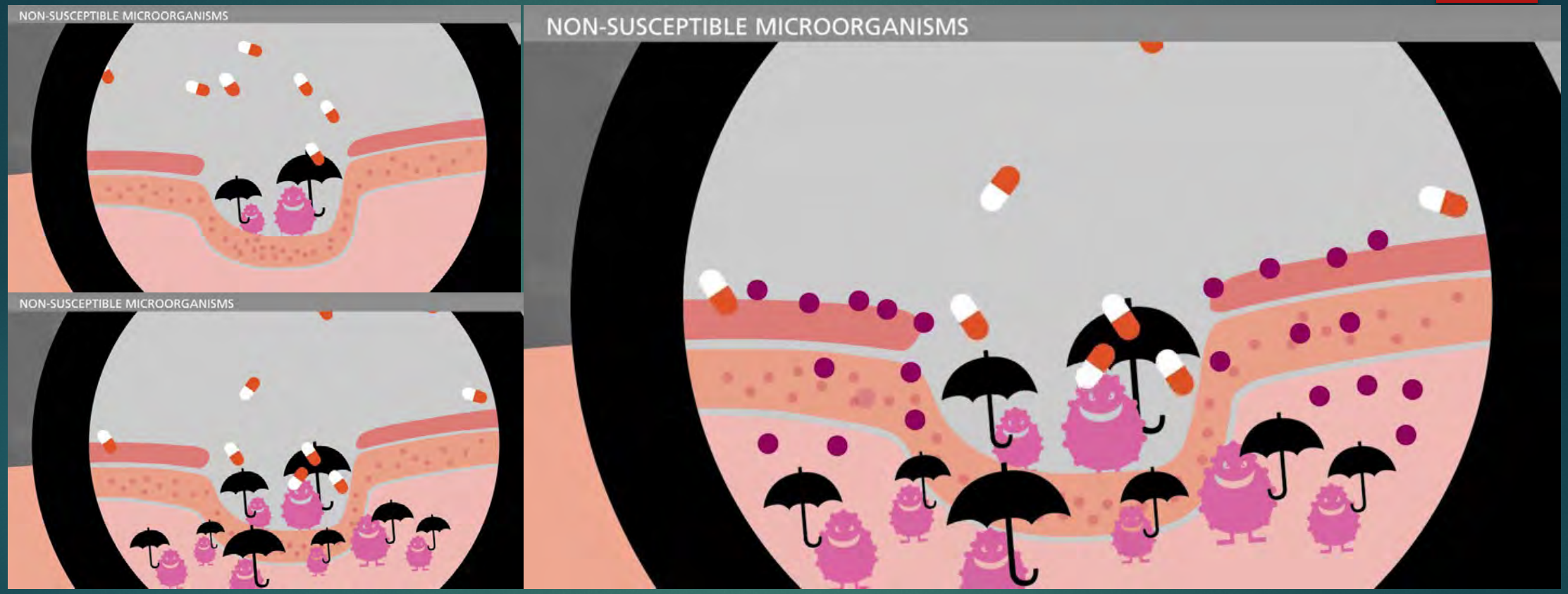
Day 0 vs Day 11 of antibiotic resistant diabetic foot ulcer treated with vinegar. Note complete lack of suppuration.

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





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 ½ was tested against planktonic and biofilm forms of antibiotic resistant *P. aeruginosa and S. aureus*
- Four honeys were tested, including Manuka honey.
- The honey completely eradicated planktonic forms and reduced biofilm forms of both bacteria by 63-91%
- Manuka honey contains essential oil of *Melaleuca alternifolia.*
- *Other essential oils could be used to make a* ***medicated honey****.*

Alandejani T, Marsan J, Ferris W, Slinger 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 cleaning of the teeth.
- Abscesses or infected root canals cannot be addressed with herbs.
- Strong *Echinacea angustifolia* 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	EPI
Quercus alba	4		x	x		x	
Myrrh	4	x	x	x	x	x	x
Myrica	2	x		x		x	
Hydrastis	1			x		x	x
Cinnamomum cassia	1	x	x	x			
Eugenia	1	x		x		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 with vinegar and/or boric acid
  - 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.* Strain the blended preparation through cheesecloth (*allicin* from cut garlic can cause burns)

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, Borumandnia 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;16(7):e19118.

Table 4. Comparison of Laboratory Improvement in Women With Bacterial Vaginosis [a,b]

Group	Garlic	Metronidazole	Total
Lab Improvement	41 (68.3)	33 (55)	74 (61.7)
Lack of Lab Improvement	19 (31.7)	27 (45)	46 (38.3)
Total	60 (100)	60 (100)	120 (100)

[a] Data are presented as No. (%).
[b] Chi square = 2.256 and P > 0.05.

Table 5. Comparison of Treatment Success in Women With Bacterial Vaginosis [a,b]

Group	Garlic	Metronidazole	Total
Successful Treatment	38 (63.3)	29 (48.3)	67 (55.8)
Failure in Treatment	22 (35.7)	31 (51.7)	53 (44.2)
Total	60 (100)	60 (100)	120 (100)

[a] Data are presented as No. (%).
[b] Chi square = 2.737 and P > 0.05.

Table 6. Comparison of Medication Side Effects in Women With Bacterial Vaginosis [a,b]

Group	Garlic	Metronidazole	Total
With Side Effect	9 (15)	20 (33.3)	29 (24.2)
Without Side Effect	51 (85)	40 (66.7)	91 (75.8)
Total	60 (100)	60 (100)	120 (100)

[a] Data are presented as No. (%).
[b] Chi square = 5.502 and P > 0.032.

Calendula vs Flagyl for BV

Variables	*Calendula officinalis* (*n*=40)		Metronidazole (*n*=40)		*P* value before intervention
	Before, *n* (%)	After, *n* (%)	Before, *n* (%)	After, *n* (%)	
Burning					
Yes	13 (32.5)	0	18 (45)	0	0.35
No	27 (67.5)	40 (100)	22 (55)	40 (100)	
Itching					
Yes	9 (22.5)	0	1 (2.5)	0	0.01
No	31 (77.5)	40 (100)	39 (97.5)	40 (100)	
Vaginal discharge					
Yes	11 (27.5)	0	24 (60)	0	0.06
No	29 (72.5)	40 (100)	16 (40)	40 (100)	
Bad odor					
Yes	21 (52.5)	0	16 (40)	0	0.37
No	19 (47.5)	40 (100)	24 (60)	40 (100)	
Dysuria					
Yes	39 (97.5)	0	40 (100)	0	1
No	1 (2.5)	40 (100)	0	40 (100)	
Dyspareunia					
Yes	0	0	3 (7.5)	0	0.24
No	40 (100)		27 (92.5)		
pH*	4.7±0.25 (4.5-5.5)	4.7±0.23 (3.5-4.5)	4.9±0.28 (4.5-5.5)	4.1±0.28 (3.5-4.5)	0.58

*The values are expressed as mean±SD (range). The *P* values are based on the results of Fisher's exact test or independent *t*-test. SD: Standard deviation

A ***methanolic*** extract of Calendula flowers vs a metronidazole cream applied for seven days with evaluation at day 14.

Calendula equivalent to mitronidazole on all measures of progress

Pazhohideh Z, Mohammadi S, Bahrami N, Mojab F, Abedi P, Maraghi E. The effect of Calendula officinalis versus metronidazole on bacterial vaginosis in women: A double-blind randomized controlled trial. J Adv Pharm Technol Res. 2018 Jan-Mar;9(1):15-19.

Vinegar and vaginal 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.

Boric acid and biofilm formation

Bacteria	Biofilm production					
	In the absence of either of the B compounds		In the presence of H_3BO_3		In the presence of $Na_2B_8O_{13}$-$4H_2O$	
	OD	Bf	OD	Bf	OD	Bf
S. aureus ATCC 25923	0.055 ± 0.002	+	0.02 ± 0.003	–	0.013 ± 0.002	–
V. anguillarum No: 218	0.078 ± 0.001	++	0.031 ± 0.003	–	0.019 ± 0.001	–
A. hydrophila ATCC 19570	0.08 ± 0.06	++	0.03 ± 0.002	–	0.02 ± 0.001	–
A. hydrophila No: 219	0.079 ± 0005	++	0.025 ± 0.001	–	0.021 ± 0.001	–
Y. ruckerii No: 217	0.076 ± 0.011	++	0.03 ± 0.001	–	0.025 ± 0.002	–
P. aeruginosa ATCC 27853	0.216 ± 0.014	+++	0.04 ± 0.002	+	0.035 + 0.001	–
P. aeruginosa No: 266	0.09 ± 0.07	++	0.06 ± 0.001	+	0.011 ± 0.001	–
L. garviea M28-K280	0.180 ± 0.012	+++	0.014 ± 0.002	–	0.04 ± 0.001	+
B. melitensis Rev-1	0.05 ± 0.003	+	0.02 ± 0.002	–	0.018 ± 0.001	–
B. abortus No: 31	0.075 ± 0.004	++	0.024 ± 0.002	–	0.022 ± 0.001	–

Bf biofilm formation

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.

Topical wound oil

- *Arctostaphylos*: Powerful anti-microbial, anti-biofilm, efflux pump inhibitors
- *Acalypha:* Powerful anti-biofilm, anti-inflammatory
- *Mahonia:* Powerful antimicrobial via berberine and companion alkaloids; efflux pump inhibitors
- *Hydrastis* leaf: *Antimicrobial via berberine and companion alkaloids; anti-biofilm; efflux pump inhibitors*
- *Commiphora:* Antimicribial, anti-biofilm; efflux pump inhibitors, anti-inflammatory, circulatory stimulant, immune
- *Larrea*: Antimicrobial, anti-biofilm, anti-inflammatory, immune

Infused in warm olive oil.

Unilateral otitis externa of 6 days duration, so severe that the ear canal was swollen shut and the drum not visible. A recurrence of a similar infection 4 months prior. Unresponsive to a garlic oil preparation.

Inflammation reduced after one dose, canal was open by half way through day 3 of treatment 4x per day.

Otitis externa

- Chronis otitis externa had the presence of biofilms in 23/25 patients.
- Results from the standard treatment with antibiotics and steroids was compared with “chemical peeling” of the biofilm and superficial epithelium of the ear canal.
- Result were similar for the first 3 months.
- At 6 months, 89% of the “peeling group” were symptom free, vs 64% of the antibiotic group
- At 9 months, the corresponding rates were 80% and 60%

Fusconi M, Petrozza V, Taddei AR, Vinciguerra V, De Virgilio A, Chiarini F, Cirenza M, Gallinelli C, Conte M, de Vincentiis M. Is biofilm the cause of chronic otitis externa? Laryngoscope. 2011 Dec;121(12):2626-33.

Paronychia

Stock photos showing paronychia and its resolution through discharge.
The topical wound oil on previous slide resolved a paronychia going from the side past the middle of the nail. Consolidation first to the bottom corner of the nail, then clearing by half way through day-3, without any need for drainage.

Topical wound treatment

- *Scutellaria baicalensis*
- *Mahonia* or *Berberis* leaf
- Another berberine containing herb
- *Echinacea*
- *Larrea*

The combined formula has multiple antimicrobial antibiofilm, efflux pump inhibiting, anti-inflammatory Local immune stimulating properties.

Formula from herbalist Sam Coffman of The Human Path school

Topical wound treatment

- Usnea
- Betula
- Calendula
- Artemisia

Each plant has anti-bacterial, anti-fungal, and anti-biofilm properties

Wash for sores, has successfully prevented amputations. Formula from herbalist/wildcrafter Patti Leahy used as standard wash for non-healing wounds and ulcers at Occupy Medical clinic in Eugene, Oregon

Betula antimicrobial and antibiofilm

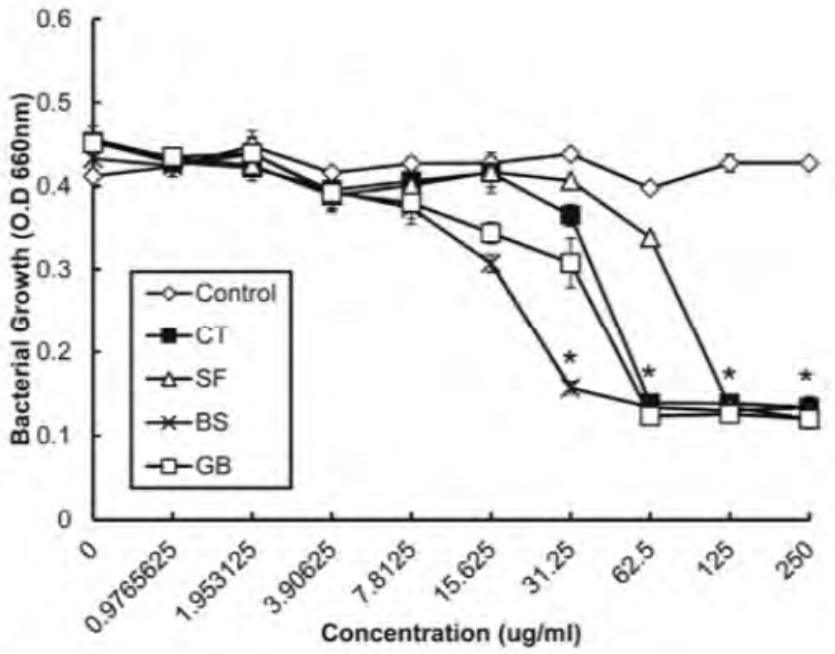


Fig. 1. Susceptibility assay of *S. mutans* for herbal extracts. *S. mutans* was cultured in BHI broth and inoculated into TSB. After cultivating overnight, susceptibility test of *S. mutans* for herbal extracts was performed according to the protocol of CLSI. The experiments were performed three times in duplicate and the representative data express mean and standard deviation. * Significance compared to untreated control bacteria ($P<0.05$). CT, extract of *C. tricuspidata*; SF, extract of *S. flavescens*; GB, extract of *G. biloba*; BS, extract of *B. schmidtii*.

Betula methanol extraction potently inhibits the growth of this Streptococcus species.

See next

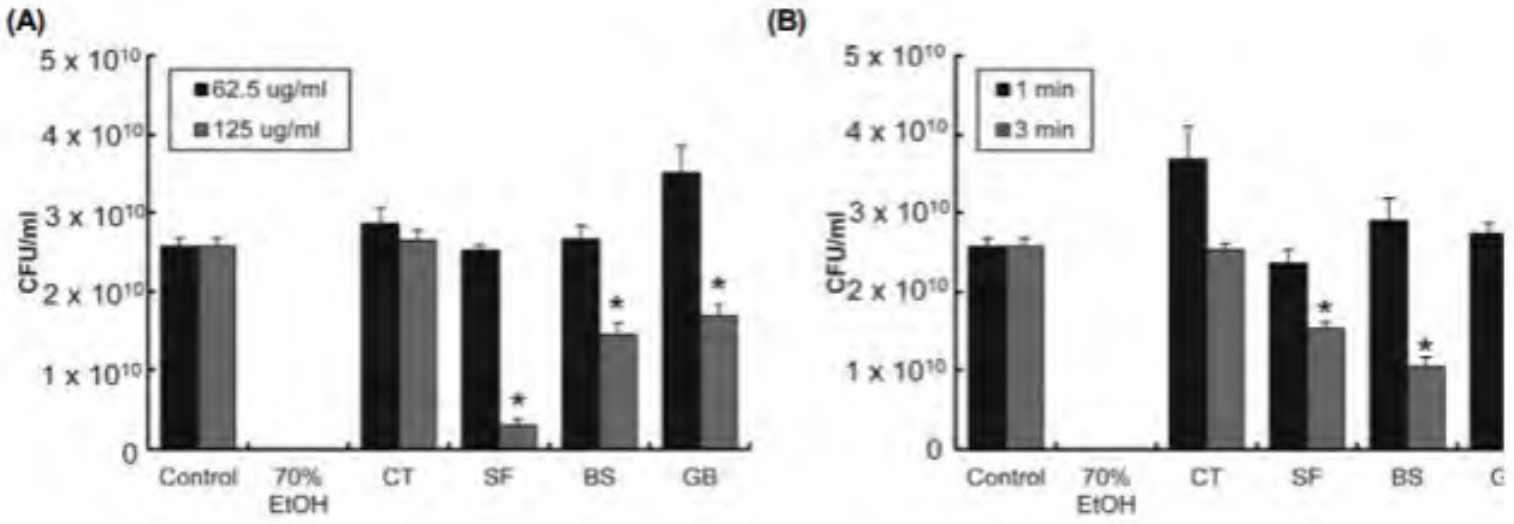


Fig. 2. Anti-biofilm activity of herbal extracts. *S. mutans* was cultured in BHI broth and biofilm was formed for 72 h using fresh BHI broth including sucrose and mannose. *S. mutans* biofilm was treated with the extracts in the indicated concentrations for 1 min (A) or at 62.5 μg/ml for various times (B) and then inoculated on MSB agar plate after disruption of the biofilm. The plate was incubated for 36 h, and colonies were counted. The experiments were performed three times in duplicate and the representative data express mean and standard deviation. * Significance compared to untreated control bacteria ($P<0.05$). CT, extract of *C. tricuspidata*; SF, extract of *S. flavescens*; GB, extract of *G. biloba*; BS, extract of *B. schmidtii*.

Betula extract (BS) applied to preformed streptococcus biofilms for 1-3 minutes show potent ability to break up the biofilm.

Lee, S.-H. (2013). *Antimicrobial effects of herbal extracts on Streptococcus mutans and normal oral streptococci. Journal of Microbiology, 51(4), 484–489.*

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