

MINING NATURE'S CHEMISTRY: ETHNOBOTANY & DRUG DISCOVERY FOR DERMATOLOGIC APPLICATIONS

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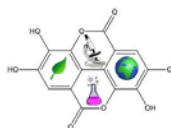
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Twitter/Instagram: [@QuaveEthnobot](#)



Quave Research Group
Medical Ethnobotany & Anti-Infective Drug Discovery

DISCLOSURES

Drug development activities:

- PhytoTEK LLC – CEO/CSO
- Alira Health (LOI with PhytoTEK)

Consulting Activities:

- The Coca Cola Company
- Medline

Contract Research:

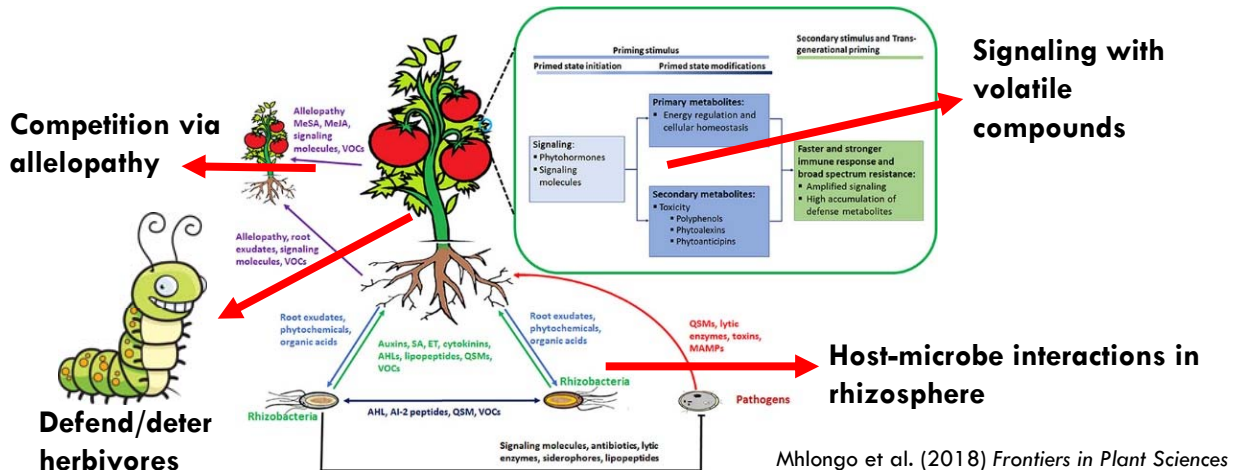
- The Coca Cola Company
- Naturex
- iHealth

OVERVIEW

- ❖ History of plants in medicine
- ❖ Ethnobotany and drug discovery
- ❖ Innovate new solutions for unmet medical needs using medicinal plants

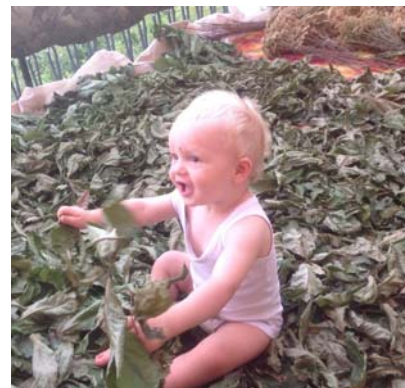


PLANT SECONDARY METABOLISM INFLUENCED BY BIOTIC AND ABIOTIC STRESSORS



HUMANS OBSERVE NATURE: ZOOPHARMACOGNOSY

Study of animals that self-medicate with plants

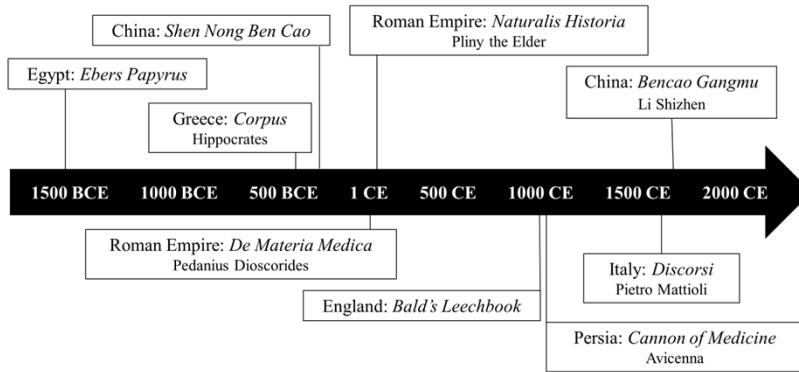


Shurkin (2014) PNAS News Feature

HISTORICALLY, MEDICINE & PHARMACOLOGY WERE BOTANICAL ARTS



Pedanius Dioscorides (1 CE)



Ibn Sina, or Avicenna (980-1037 CE)

PLANTS AS A SOURCE OF MEDICINE



Willow
Aspirin



Foxglove
Digoxin/Digitoxin



Mayapple
Podophyllin/ Etoposide



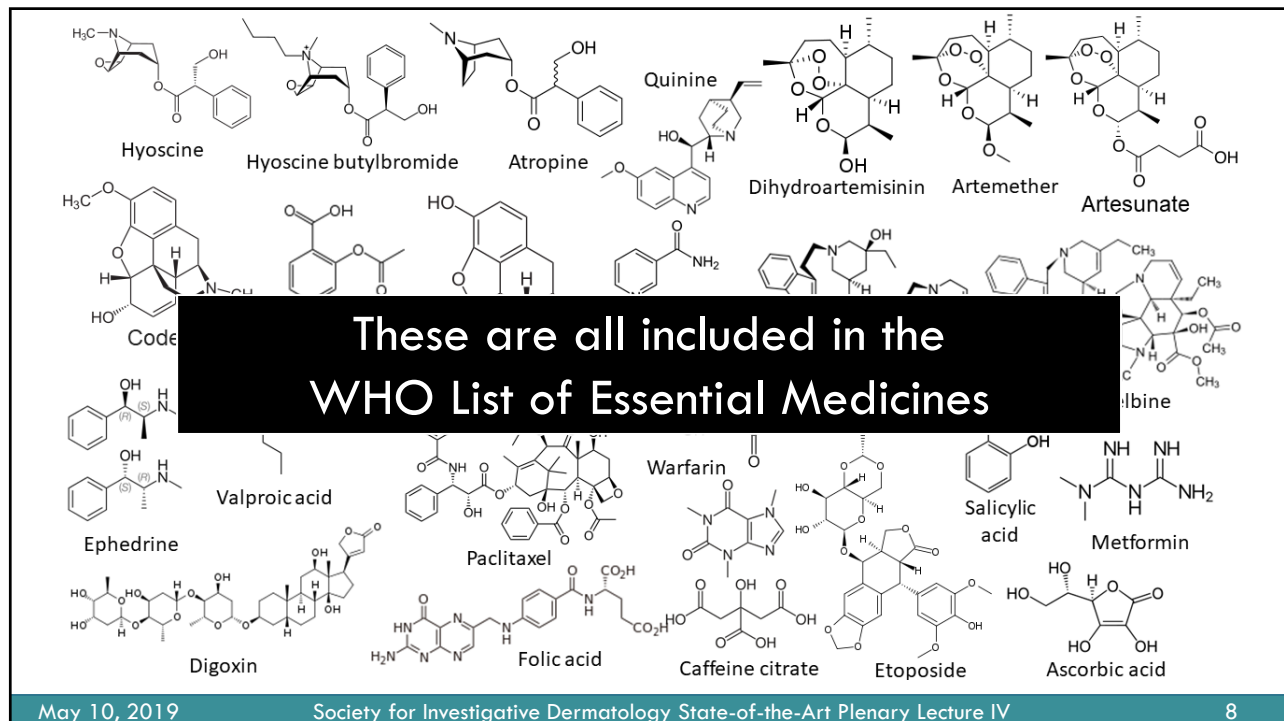
Poppy
Codeine/ Morphine

“At least 28,187 plant species are currently recorded as being of medicinal use.”
-Kew Report: State of the World’s Plants 2017

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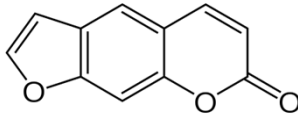
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PSORALENS — PUVA THERAPY



Ruta graveolens



Psoralen

❖ **Chemical class:** Furanocoumarin

❖ **Botanical sources:**

- *Ficus carica* L., Moraceae (fig)
- *Ruta graveolens* L., Rutaceae (rue)
- *Ammi visnaga* (L.) Lam., Apiaceae (bisnaga)
- *Pastinaca sativa* L., Apiaceae (parsnip)
- *Petroselinum crispum* (Mill.) Fuss, Apiaceae (parsley)
- *Foeniculum vulgare* Mill., Apiaceae (fennel seeds)
- *Apium graveolens* L., Apiaceae (celery)

❖ **MOA:** Psoralen intercalates into DNA and on exposure to ultraviolet (UV-A) radiation can form monoadducts and covalent interstrand cross-links (ICL) with thymines, inducing apoptosis.

❖ **Clinical:** Psoralen plus UVA (PUVA) therapy has shown considerable clinical efficacy for psoriasis and alopecia, and less so, for eczema & vitiligo

❖ **Toxicity:** PUVA therapy linked to higher risk of skin cancer

Zhang & Wu. 2018. *Lasers in Medical Science*

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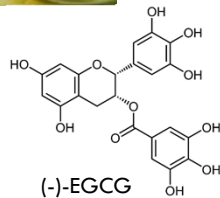
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GREEN TEA — SOURCE OF POLYPHENON E



VEREGEN®
(sinecatechins) Ointment, 15%



(-)-EGCG

Constituents:

Standardized composition of green tea catechins: (-)-EGCG, 65%; (-)-EGC, 4%; (-)-epicatechin, 9%; (-)-epicatechin-3-gallate, 6%; (-)-gallocatechin-3-gallate, 4%; (-)-catechin-3-gallate, 0.2%; gallocatechin, 0.2%; catechins, 1.1% and caffeine, 0.7%

History:

- ❖ 1980: Mitsui Norin (Japan) initiated research on tea catechin
- ❖ 1983: Patent obtained
- ❖ 2 large randomized, double blind studies completed on its efficacy in clearance of *Condylomata acuminata* in 10-16 weeks
 - Side effect: Local irritation
 - Low rate of recurrence
- ❖ 2006: FDA approved as the 1st botanical drug

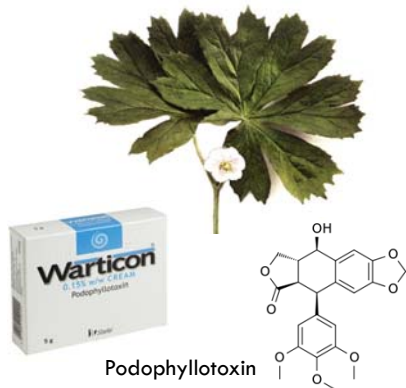
Stockferth & Meyer (2014) *Expert Opinion on Biological Therapy*

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AMERICAN MAYAPPLE — PODOPHYLLOTOXIN



Lipke (2006) *Clinical Medicine and Research*

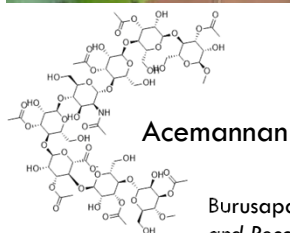
- ❖ **Historic Use:** Native Americans used resin mixture from dried rhizome and roots of as poultice for warts, as snake bite venom antidote
- ❖ **Constituents:** Crude alcohol extract contains podophyllotoxin, 4-demethylpodophyllotoxin, α -peltatin and β -peltatin
- ❖ **Extract** used in treatment of warts:
 - Clinical studies have shown 45% clearance in 3 months; 73% in 9 months for anogenital warts
 - Also used for plantar warts with 84% cure rate
 - Less effective and less cost effective than pure active ingredient, podophyllotoxin
- ❖ **Podophyllotoxin**
 - Clinical trial on 0.5% solution (topical administration) b.i.d. for 1 month yielded 95% reduction in wart area and 84% in wart count compared to 7 and 3% for placebo control
- ❖ **Etoposide**
 - Semi-synthetic derivative of podophyllotoxin, used to treat numerous cancers (testicular, lung, lymphoma, ovarian, neuroblastoma & leukemia)
- ❖ **MOA:** binds to microtubules and causes mitotic arrest in the metaphase of cell division

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ALOE LEAF GEL



Burusapat et al (2018) *Plastic and Reconstructive Surgery*

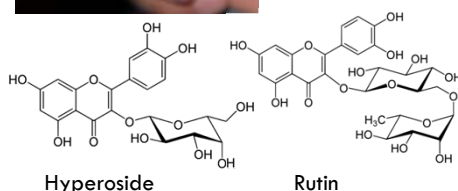
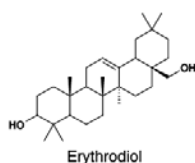
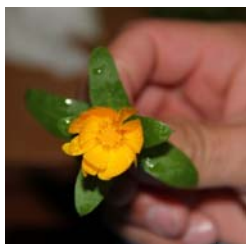
- ❖ **Use:** topical application of gel from fleshy leaves for burn wounds & other inflammatory skin problems
- ❖ **Constituents:** polysaccharides (glucmannans, glycoproteins) and anthraquinone glycosides
 - Polysaccharides are important as soothing and immunostimulating agents
 - Anthraquinone derivatives are antibacterial
- ❖ **Clinical:** *Aloe vera* gel accelerated split thickness skin graft donor-site healing

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MARIGOLD — CALENDULA OFFICINALIS



- ❖ **Use:** Topical applications for wound healing, dry skin, skin inflammations and infections of mucous membrane of mouth
- ❖ **Constituents and properties:**
 - Saponins (based on oleanolic acid structure)
 - Triterpene pentacyclic acids (faradol, arnidol, erythrodiol, and others)
 - Anti-inflammatory
 - Flavonoids (hyperoside and rutin)
 - Wound healing
 - Sesquiterpene and ionine glycosides
 - Essential oil
 - Antimicrobial
- ❖ **Clinical:** Extract effective in achieving epithelialisation in venous leg ulcer healing

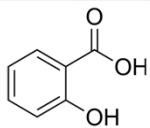
Quave (2018) *Current Dermatology Reports*

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SALICYLIC ACID



Salicylic acid



Salix nigra

- ❖ **Chemical class:** Phenolic acid
- ❖ **Botanical sources:**
 - *Salix* spp., Salicaceae (willow)
 - *Gaultheria* spp., Ericaceae (wintergreen)
 - *Betula* spp., Betulaceae (sweet birch)
- ❖ **History of use:** Pliny the Elder used willow bark for chemical peels (to treat calluses and corns); SA later used to soften and exfoliate stratum corneum
- ❖ **Acne:** Comedolytic agent, useful for topical acne applications (also used to assist penetration of other topical agents)
- ❖ **Warts:** Keratolytic therapy with MOA that slowly destroys virus-infected epidermis and may cause an immune response from the mild irritation caused by the salicylic acid. Available OTC in colloidal base or 40% SA in patch
 - **Advantage:** cheap, limited pain, usually effective
 - **Disadvantage:** weeks-months treatment time, must follow instructions; risk for toxicity in kids
- ❖ **Other names:** 2-hydroxybenzoic acid or orthohydrobenzoic acid
- ❖ **Products:** SA is the only natural product approved for OTC acne creams. Products typically contain between 0.5-5%

Arif (2015) *Clinical, Cosmetic & Investigative Dermatology*

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THE SCIENCE OF ETHNOBOTANY

Ethnobotany (from ethnology, study of culture, and botany, study of plants) is the scientific study of the relationships that exist between people and plants.

Ethnobotany is the science of survival.

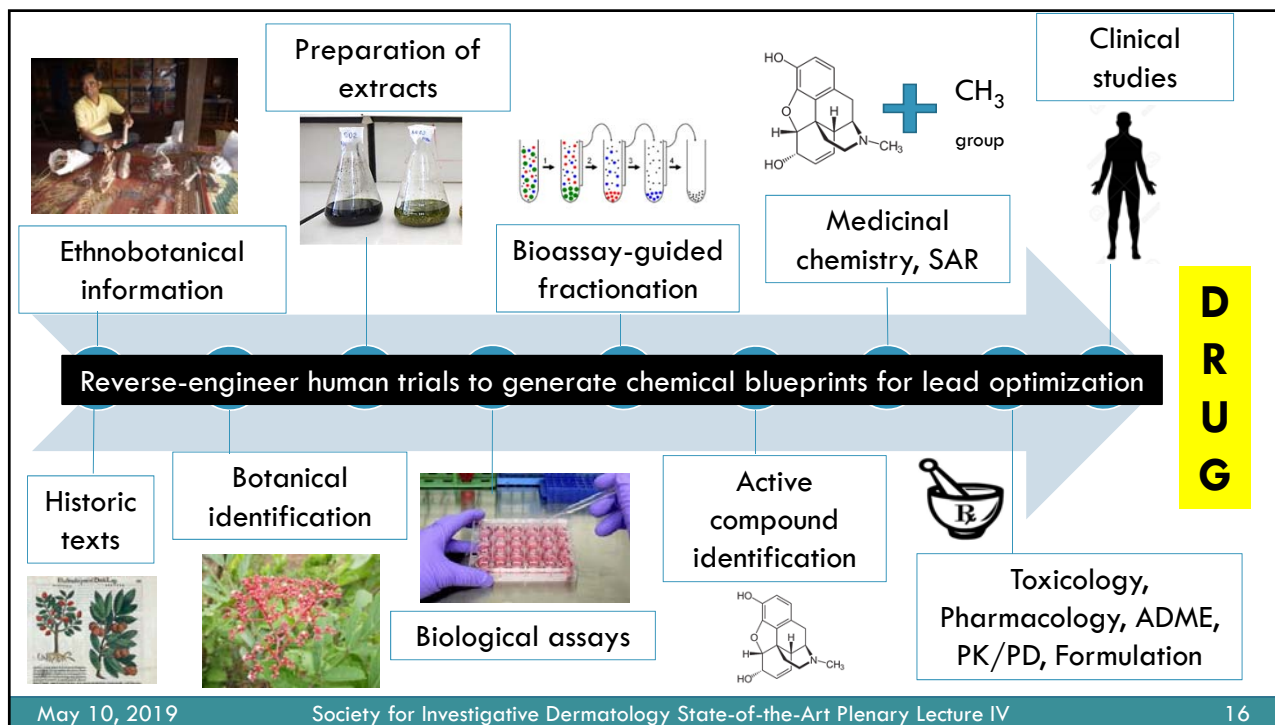


Prance et al. (2007) *Economic Botany*

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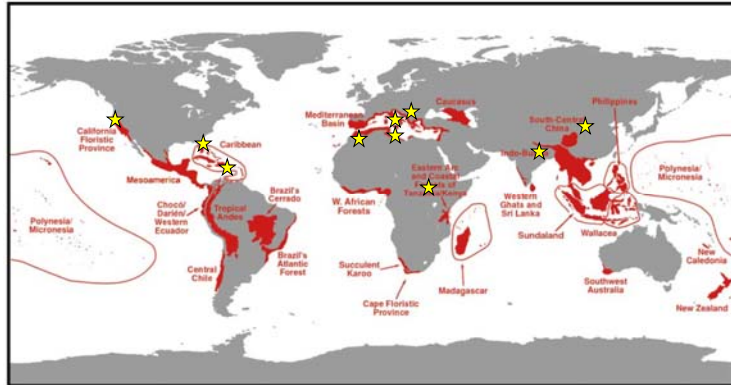
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HOW TO SELECT A FIELD SITE?

- ❖ Global Hotspots of Biodiversity
 - ❖ ~391,000 plants
 - 7% used in traditional medicine
- ❖ As many as 44% of all species of vascular plants confined to 25 hotspots comprising 1.4% of Earth's land surface



The 25 hotspots of biodiversity.
Myers, N., et al. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853-858,

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INTERVIEWS & PLANT COLLECTING

- ❖ Prior informed consent
- ❖ Follow SEB/ISE Code of Ethics
- ❖ Access & Benefit Sharing
- ❖ Permits & international collaborative research agreements



DNA barcoding



Herbarium Vouchers

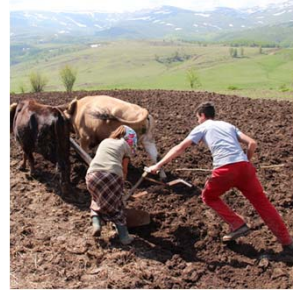
Quave & Pieroni (2015) *Nature Plants*

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ALBANIA, 2012



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KOSOVO, 2015



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FLORIDA, 2016

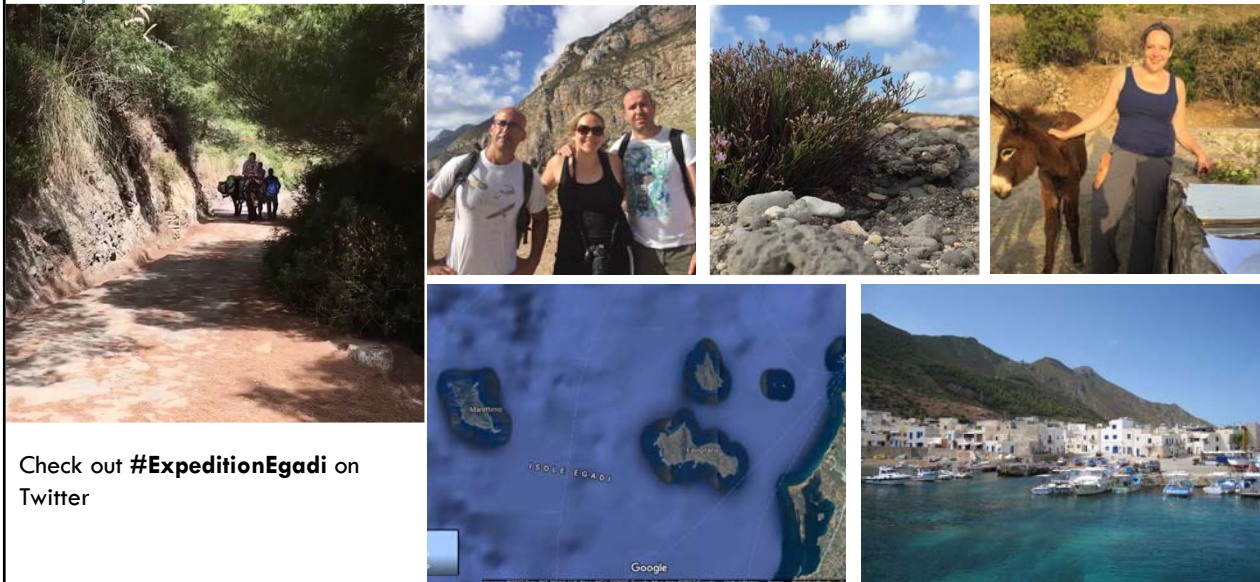


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AEGADIAN ISLANDS, ITALY 2017-2018



Check out [#ExpeditionEgadi](#) on Twitter

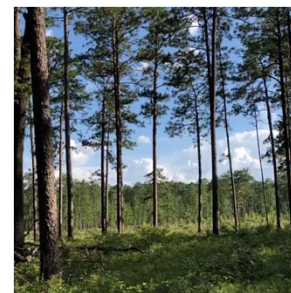
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GEORGIA, 2018

Check out [#ExpeditionChauway](#) on Twitter



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QUAVE NATURAL PRODUCTS LIBRARY (QNPL)

Inspired by traditional medicine. Driven by bioactivity.

>1,900 botanical and fungal extracts

- plus fractions from bioactive leads

Library uniquely targets plants used in human medicine and food

Existing extract library is:

- Biodiverse:
 - 52 orders
 - >600 species
 - Linked to ethnobotanical use data

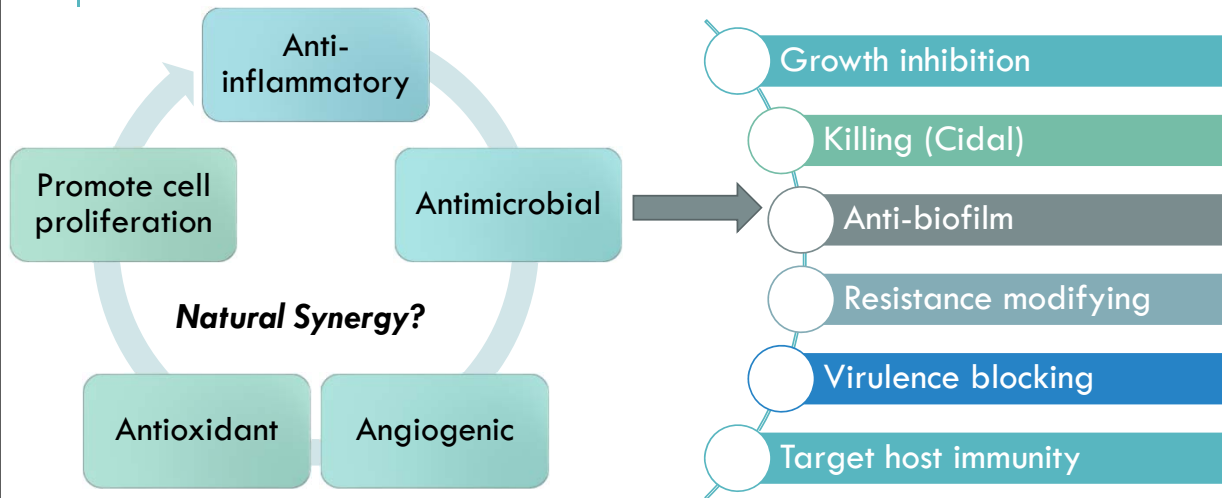


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RELEVANCE OF BOTANICALS TO SKIN TARGETS?



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STAPHYLOCOCCUS AUREUS

Opportunistic pathogen

Leading cause of:

- Bacteremia
- Sepsis
- Brain abscesses
- Medical device infections
- Skin and soft tissue infections (SSTI)

Colonizes nasal passages of 30% healthy adults in US

Commonly implicated in:

- Bone and joint infections
- Surgical site infections
- Pneumonia
- Endocarditis

HA-MRSA vs. CA-MRSA

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STAPHYLOCOCCUS AUREUS TOXINS CAUSE SERIOUS DISEASE



Toxic Shock Syndrome Toxin (TSST-1)

Pyrogenic Toxin
Superantigens



Scalded Skin Syndrome

Exfoliative Toxins



Abscesses, Necrosis, Sepsis

Hemolytic Toxins,
Proteases, Lipases



Atopic Dermatitis

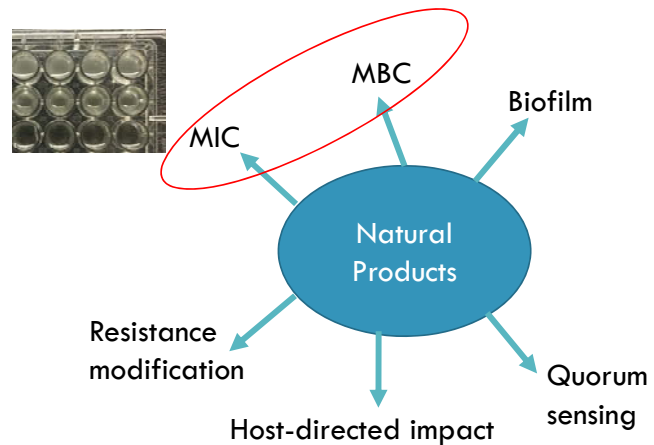
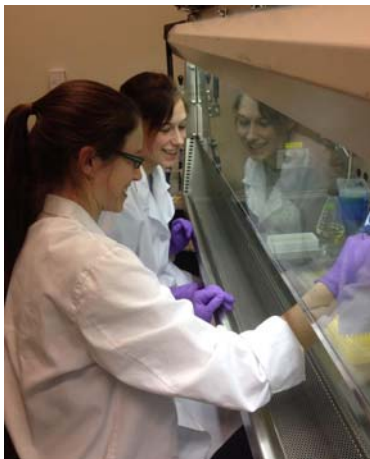
Delta-toxin, Phenol Soluble
Modulins, Hemolytic Toxins

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NEW SOLUTIONS REQUIRE INNOVATIVE & TIMELY SCREENS

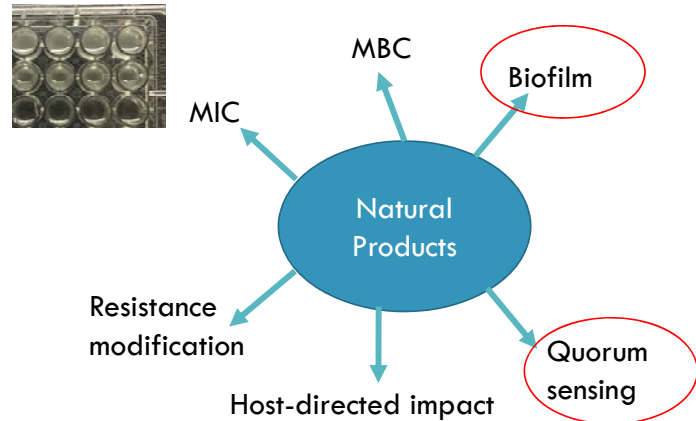
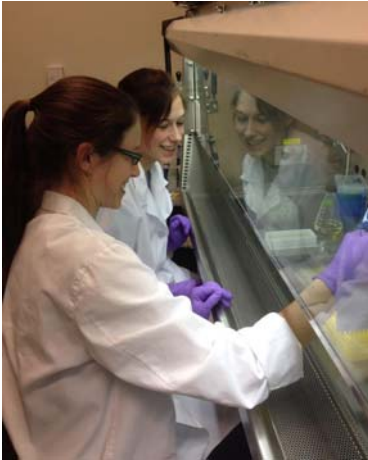


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NEW SOLUTIONS REQUIRE INNOVATIVE & TIMELY SCREENS



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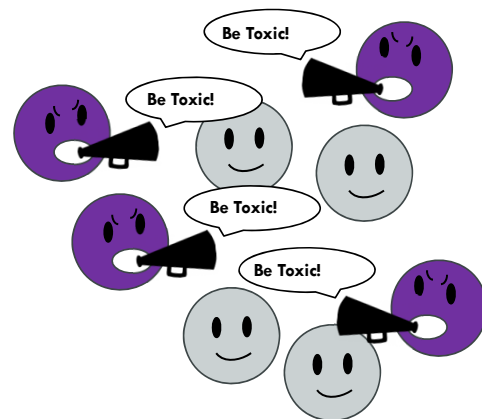
QUORUM QUENCHING APPROACH

Quorum quenching

- “Disarming” bacteria
- Protect the host
- Adjuvant to existing lines of antibiotics

Accessory gene regulator (*agr*) system

- controls virulence

Quave & Horswill. (2014) *Frontiers in Microbiology*

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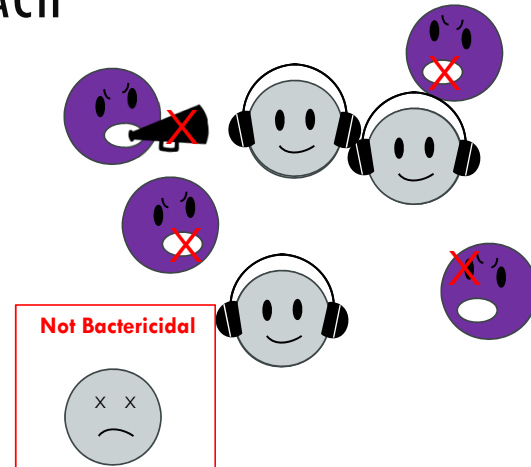
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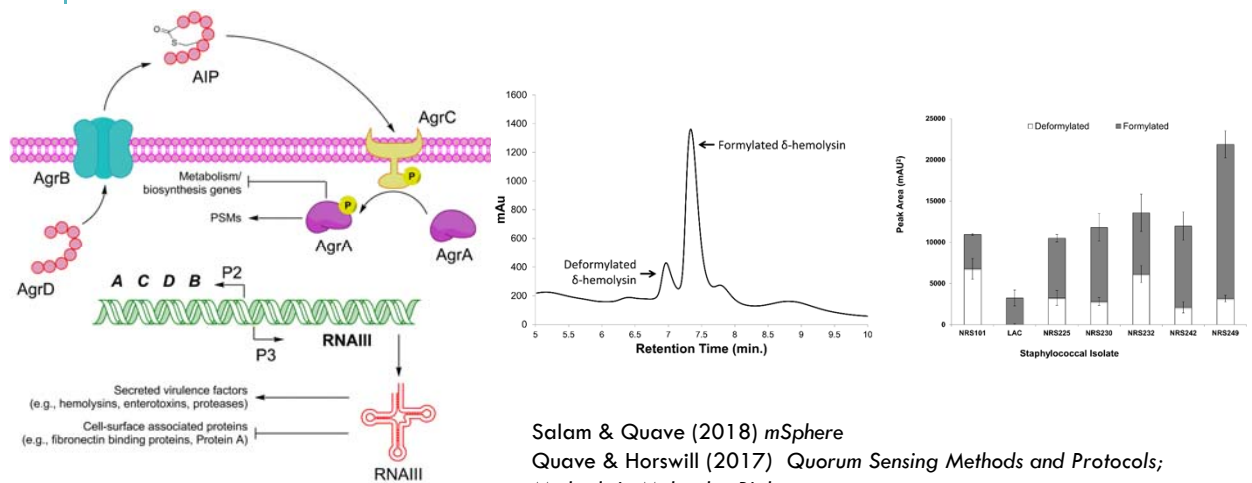
Quave & Horswill. (2014) *Frontiers in Microbiology*

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ACCESSORY GENE REGULATOR SYSTEM (AGR)



Salam & Quave (2018) *mSphere*

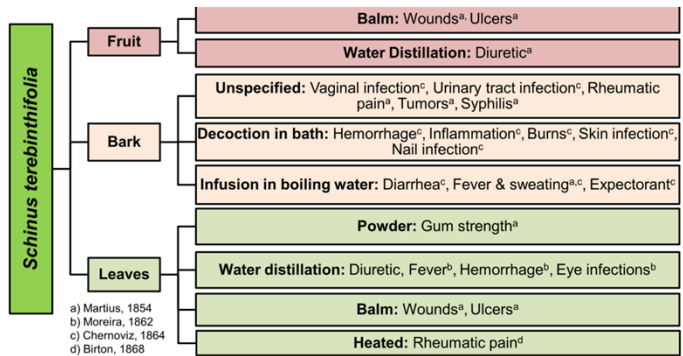
Quave & Horswill (2017) *Quorum Sensing Methods and Protocols; Methods in Molecular Biology*

March 4, 2019

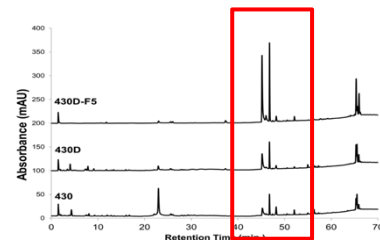
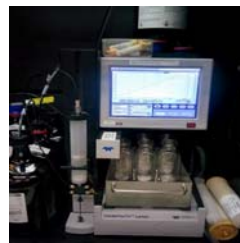
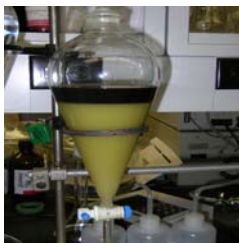
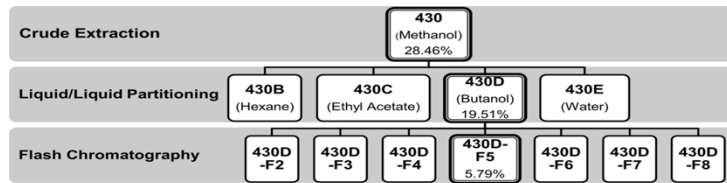
GRC Chemical & Biological Terrorism Defense

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SCHINUS TEREBINTHIFOLIA (BRAZILIAN PEPPERTREE)

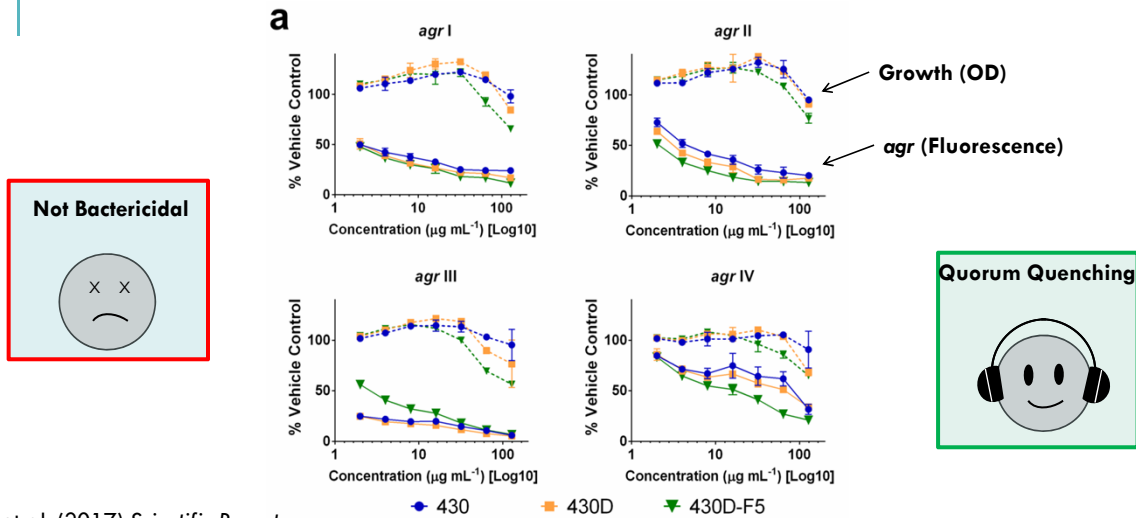


FRACTIONATION SCHEME



agr P3-GFP reporters used to guide fractionation

430D-F5 INHIBITS AGR IN A NON-BIOCIDIDE MANNER



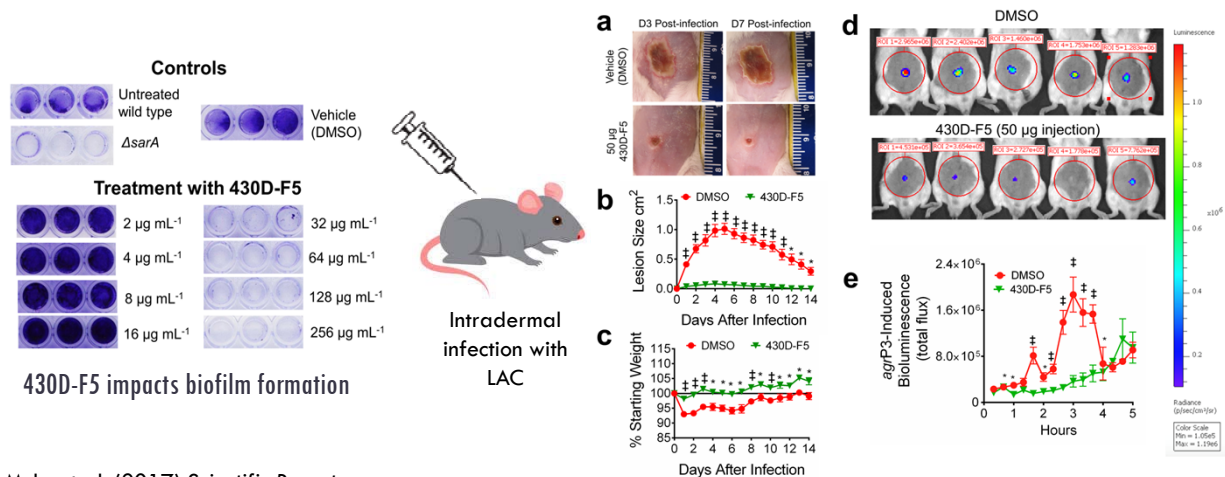
Muhs et al. (2017) *Scientific Reports*

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430D-F5 MEDIATES QUORUM QUENCHING IN VIVO AND ATTENUATES MRSA-INDUCED DERMATOPATHOLOGY IN AN MURINE INTRADERMAL INFECTION MODEL



Muhs et al. (2017) *Scientific Reports*

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430D-F5 HAS LIMITED IMPACT ON GROWTH OF COMMENSAL SKIN MICROFLORA

Species	Strain	MIC	430D-F5	Antibiotic Controls ^a			
				Amp	Clin	Ern	Van
<i>Corynebacterium amycolatum</i>	SK46	IC ₅₀	ND (512)	0.0625	-	0.00781	0.5
		MIC	ND (512)	2	-	2	2
<i>Corynebacterium striatum</i>	FS-1	IC ₅₀	ND (512)	ND (16)	-	1	0.5
		MIC	ND (512)	ND (16)	-	2	0.5
<i>Micrococcus luteus</i>	SK58	IC ₅₀	64	0.125	0.125	0.0625	0.25
		MIC	128	0.125	0.5	0.0625	0.25
<i>Cutibacterium acnes</i>	HL005PA2; HM-493	IC ₅₀	16	-	0.125	0.125	-
		MIC	256	-	0.125	0.5	-
<i>Staphylococcus epidermidis</i>	NIHLM001; HMB96	IC ₅₀	64	0.03125	-	-	1
		MIC	ND (512)	0.0625	-	NT	1
<i>Staphylococcus haemolyticus</i>	NRS116	IC ₅₀	64	ND (32)	-	ND (32)	1
		MIC	ND (512)	ND (32)	-	ND (32)	2
<i>Staphylococcus warneri</i>	SK66	IC ₅₀	64	0.0625	-	-	0.5
		MIC	ND (512)	0.0625	-	-	1
<i>Streptococcus mitis</i>	FO392	IC ₅₀	64	0.03125	-	0.00781	0.5
		MIC	ND (512)	0.0625	-	0.03125	0.5
<i>Streptococcus pyogenes</i>	MGAS1525 2	IC ₅₀	ND (512)	0.0156	0.125	0.0625	-
		MIC	ND (512)	0.0313	0.125	0.0625	-

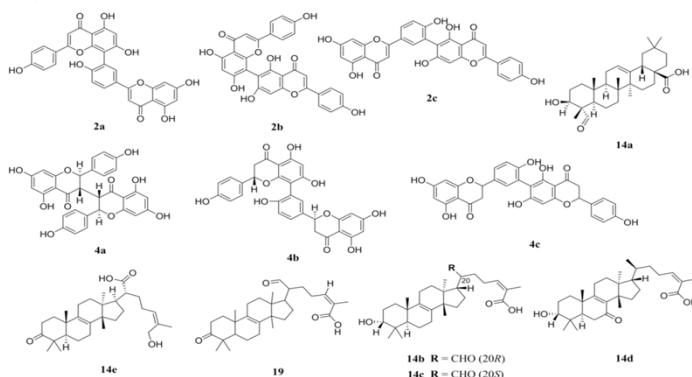
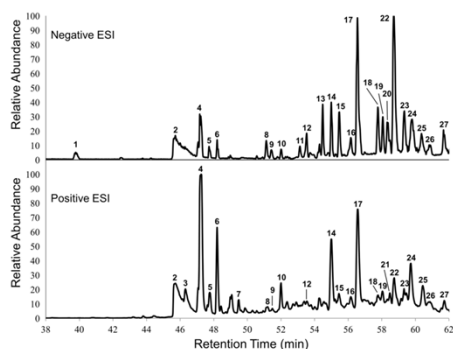
Muhs et al. (2017) *Scientific Reports*

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CHALLENGE: EVEN A SINGLE PLANT TISSUE IS INCREDIBLY CHEMICALLY COMPLEX



Muhs et al. (2017) *Scientific Reports*

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OVERCOMING BOTTLENECKS IN COMPOUND ISOLATION



Salazar et al. (In Preparation)

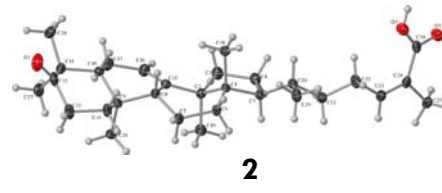
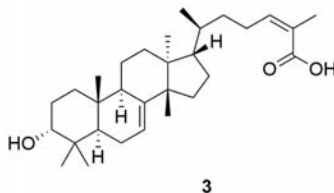
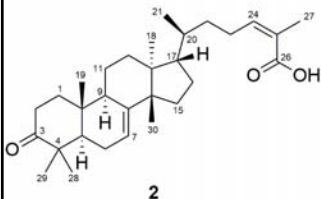
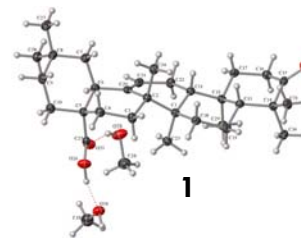
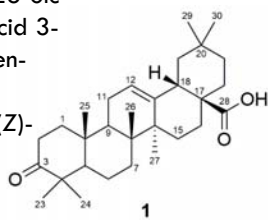
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ISOLATION & IDENTIFICATION OF BIOACTIVE TRITERPENOIDS

3-oxo-olean-12-en-28-oic acid (**1**), oleanonic acid 3-oxo-lanosta-7,24-dien-26-oic acid (**2**) (Z)-masticadienoic acid (Z)-schinol (**3**)



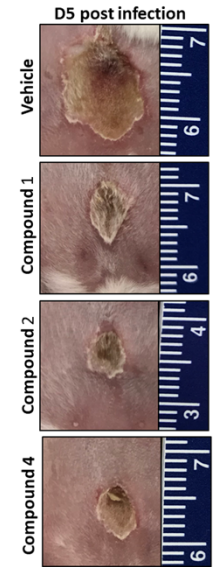
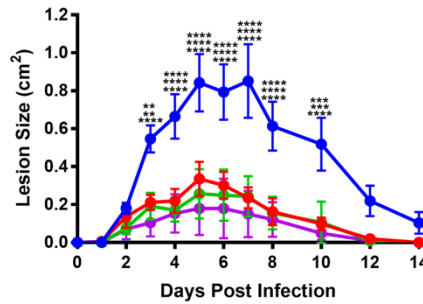
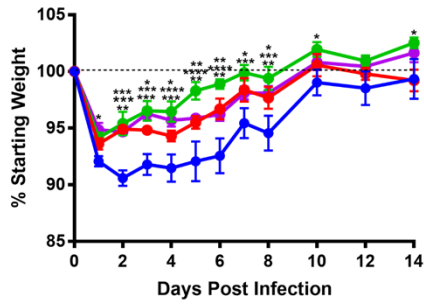
Tang et al. (In Preparation)

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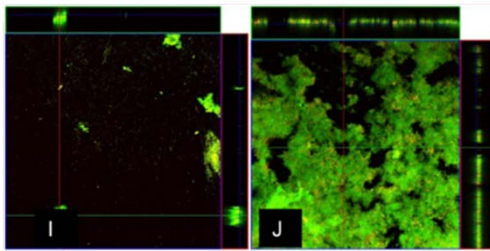
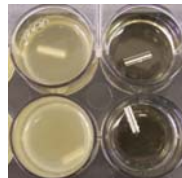
IN VIVO QQ ACTIVITY OF SCHINUS TRITERPENOIDS



Tang et al. (In Preparation) [Collaboration with Horswill lab]

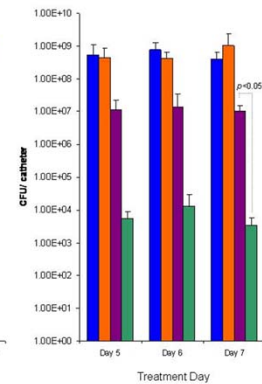
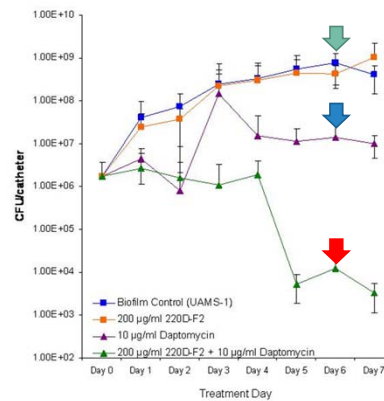
ASSESSING SYNERGY: EXAMPLE OF ELMLEAF BLACKBERRY

Rubus ulmifolius Schott., Rosaceae



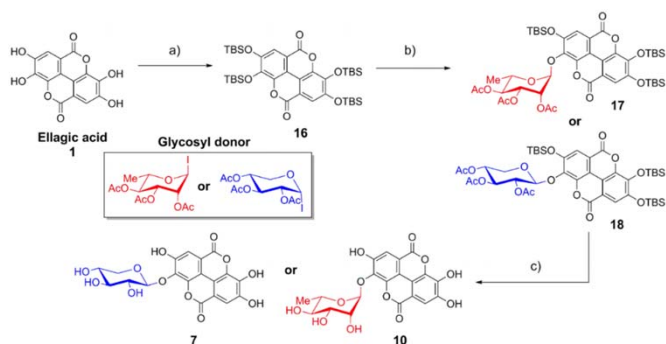
50 µg/mL of 220D-F2

12.5 µg/mL of 220D-F2



Quave et al. (2012) PLoS One

ASSESSING SYNERGY: EXAMPLE OF ELMLEAF BLACKBERRY



- ❖ Sometimes, isolation of compounds causes loss of activity
- ❖ Methods to assess synergy:
 - Recombine parts of the fractions and test
 - Synthesize compounds identified in extract and test combinations
 - Pair mass spectrometry and bioactivity data for PCA analysis of features
- ❖ FDA Botanical Drug Pathway offers options for development of synergistic compositions

Fontaine et al. (2017) *Frontiers in Microbiology*

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DEVELOPMENT OF INNOVATIVE MEDICAL DEVICE SOLUTIONS

A 2,000 year-old clue



Blackberry, Vienna copy of *De Materia Medica*, Dioscorides, Early 6th Century

<https://www.puremend.com/>

PureMend™ LG Alginate, is a 3D printed, highly absorptive, non-occlusive, eco-friendly wound dressing

- Derived from brown seaweed
- Impregnated with proprietary encapsulated lemongrass oil
 - Acts as an all natural antimicrobial
- Proprietary blackberry root extract (220D-F2)
 - Anti-biofilm properties

Indicated for use

Primary dressing in the treatment of moderately to heavily exuding partial- and full-thickness draining wounds

- stage III-IV pressure ulcers
- venous leg ulcers (VLU)
- diabetic foot ulcers (DFU)
- dermal wounds
- surgical incisions
- dehisced wounds
- tunneling wounds
- sinus tracts
- donor sites

*PureMend™ is not yet for sale the US

PureMend™
Eco-Friendly Antimicrobial Wound Dressing

phytotek
Eliminating Infection. Saving Lives.

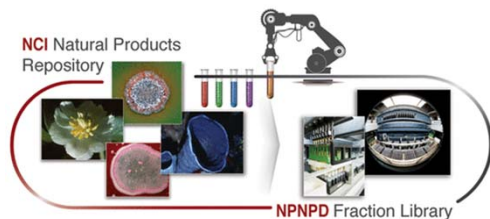
AliraHealth

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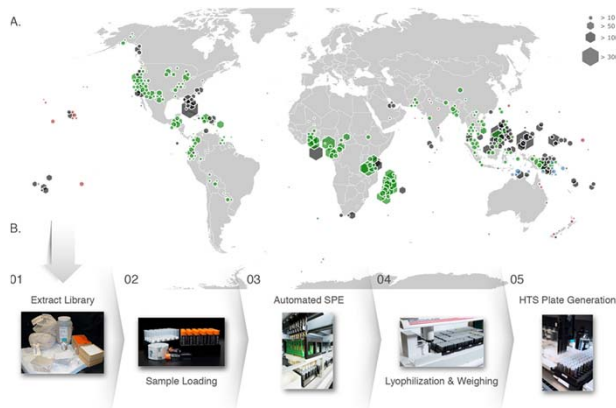
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RESOURCE: NIH/NCI NATURAL PRODUCTS REPOSITORY



- >230,000 unique extracts
- Sourced from plants, marine organisms and microbes
- HTS-amenable library of >1,000,000 fractions
- 1st set of 150,000 plated fractions **now available** (428 384-well plates) under MTA



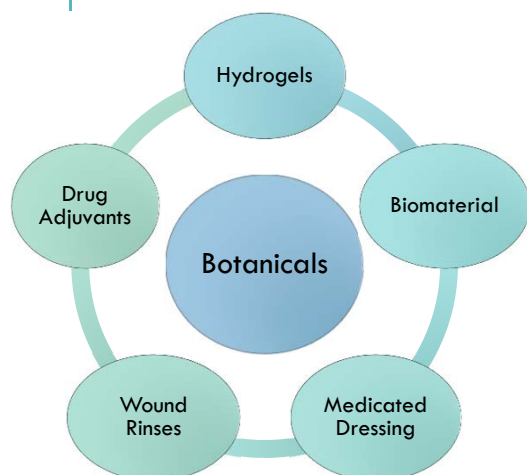
Thornburg et al. (2018) ACS Chemical Biology

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CONCLUSIONS



- ✓ Botanicals offer potential for “natural” mechanisms for skin care, repair and therapy
- ✓ Enriched plant extracts may present opportunities for synergy, acting on multiple targets
- ✓ There is a huge amount of chemical space to explore among plants
- ✓ Tools of ethnobotany can be used as a lens to identify promising leads
- ✓ Eligible for regulatory approval as Botanical Drugs by FDA pathway
- ✓ More research is needed on understudied plants commonly used in traditional medicine for dermatological conditions

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