Section Four Slides

Botanicals, Microbiome, Biofilms, and Chronic Infections

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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

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

Antifungal therapeutics

Common infectious fungal species

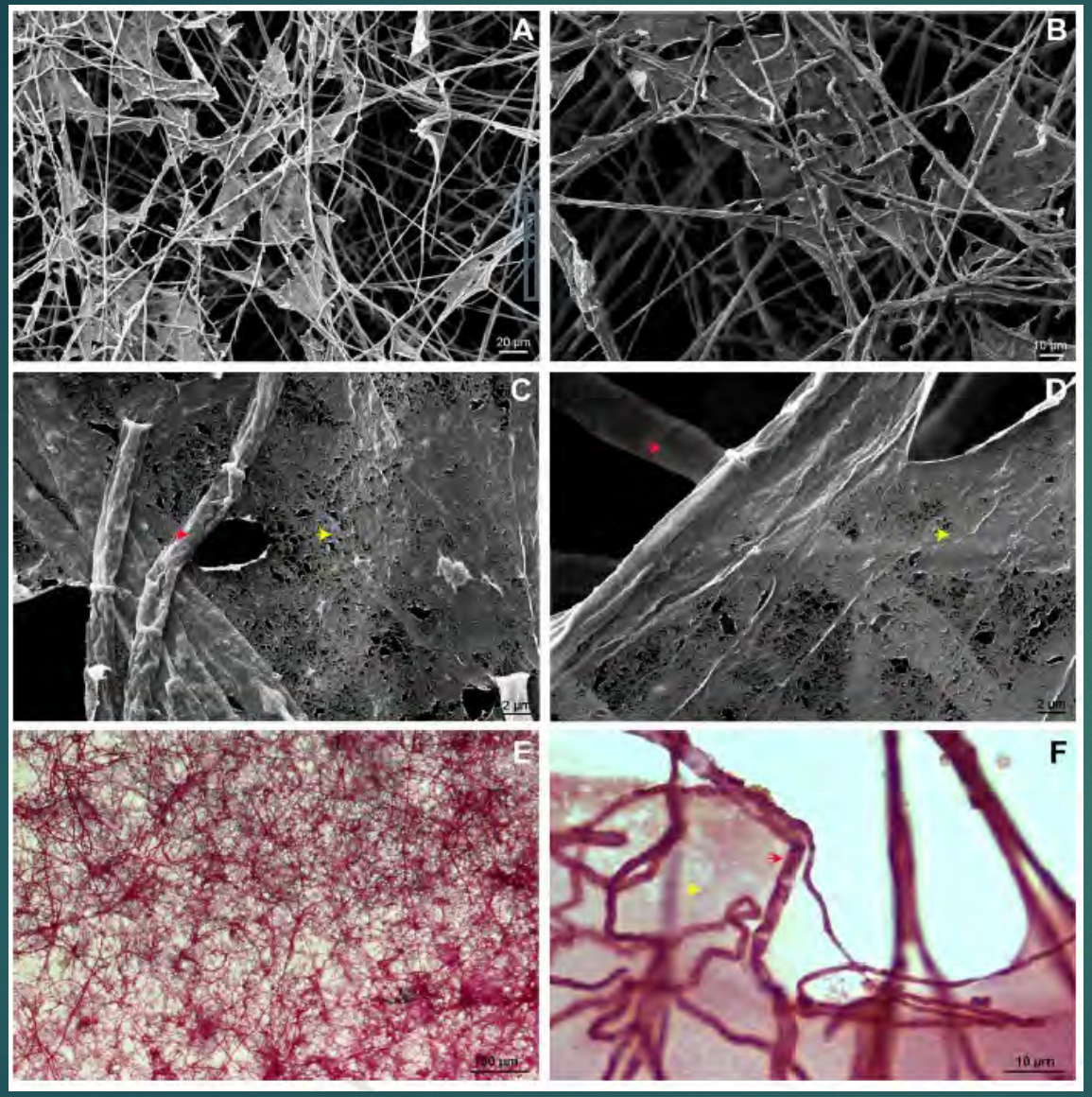
- Candida species. Oral and vaginal infections; hospital-based infections, both topical and systemic
- Trichophyton, Microsporum, Epidermophyton: Ringworm, Tinea, athlete ‘s foot, “jock itch”, and other skin infections.
- Chronic sinusitis: Complex multispecies biofilms with fungal component.
- Biofilm formation has been identified in species of Candida, Cryptococcus, Malassezia, Trichosporon, Saccharomyces, Aspergillus, and Histoplasma.

Costa-Orlandi, C. B., Sardi, J. C. O., Santos, C. T., Fusco-Almeida, A. M., & Mendes-Giannini, M. J. S. (2014). *In vitrocharacterization ofTrichophyton rubrumandT. mentagrophytesbiofilms. Biofouling, 30(6), 719–727.*

Microsporum canis biofilm

- highly structured three dimensional mycelium characteristic of biofilm with expansion in the form of a network of hyphae growing in all directions
- Moreover, polysaccharide extracellular matrix that links one hyphae to another is observed surrounding some areas of this mycelium structure. Formation of biofilm by dermatophytes can be considered as an important factor of fungal virulence

Danielli LJ, Lopes W, Vainstein MH, Fuentefria AM, Apel MA. Biofilm formation by Microsporum canis. Clin Microbiol Infect. 2017 Dec;23(12):941-942. doi: 10.1016/j.cmi.2017.06.006.

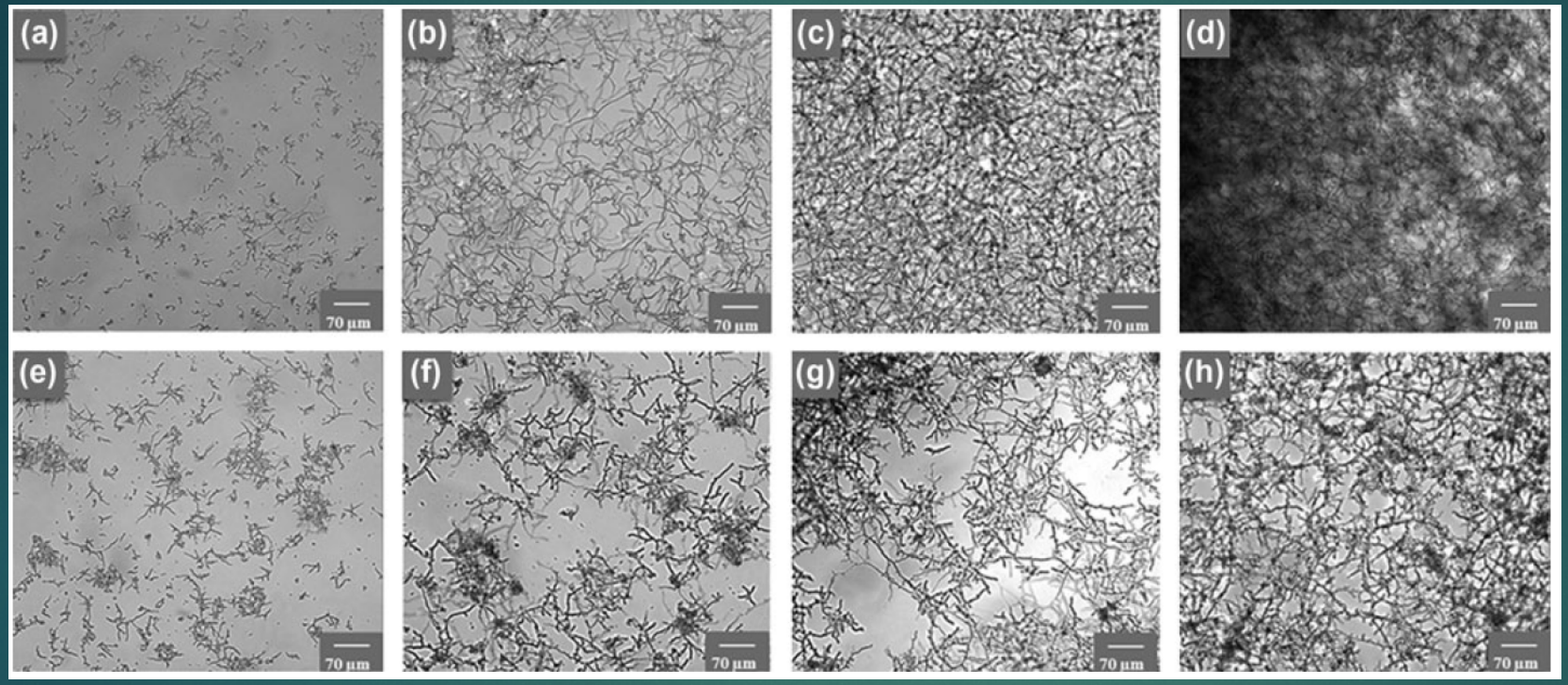


- Highly structured lab-dish biofilm of the dermatophyte Microsporum canis.
- Individual fungi are linked by hyphae, with a polysaccharide in the matrix.

Trichophyton biofilms

- First documentation of biofilms in dermatophytes in 2014.
- Two Trichophyton species were able to form mature biofilms in 72 h.
- Results demonstrated a coordinated network of hyphae in all directions, embedded within extracellular polysaccharide substances in some areas.

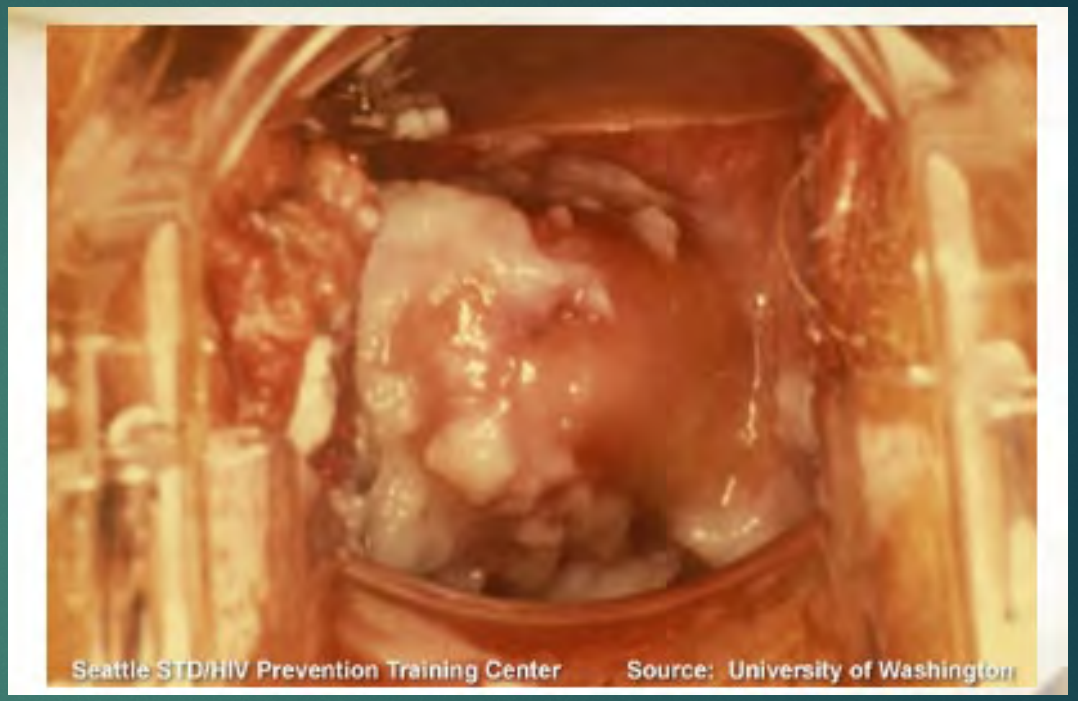
Costa-Orlandi, C. B., Sardi, J. C. O., Santos, C. T., Fusco-Almeida, A. M., & Mendes-Giannini, M. J. S. (2014). *In vitrocharacterization ofTrichophyton rubrumandT. mentagrophytesbiofilms. Biofouling, 30(6), 719–727.*



Trichophyton spp biofilms left to right at 12, 24, 48, and 72 hours.

Costa-Orlandi, C. B., Sardi, J. C. O., Santos, C. T., Fusco-Almeida, A. M., & Mendes-Giannini, M. J. S. (2014). *In vitrocharacterization ofTrichophyton rubrumandT. mentagrophytesbiofilms. Biofouling, 30(6), 719–727.*


SFS



Oral and vaginal candidiasis

Drug Treatments

- Drugs of the Azole class: Diflucan (fluconazole); Nizoral (ketoconazole); Mycelex (Clotrimazole)
- Used topically, orally, or intravenously.
- Local and systemic side effects
- Organisms can rapidly develop resistance. Azole-resistant candida is a major source of hospital-acquired infections in the U.S.
- Candida colonization on surgical implants is typically non-responding to drug treatment and requires surgical removal of the implant.
- Many botanicals or their constituents are equal to or more effective than the azole drugs, especially with longer term use.

Commonly used antifungal botanicals

2019 Survey North American and British Professional Herbalists

- Garlic
- Berberine-containing herbs
- Myrrh
- Calendula
- Thyme, Monarda
- Salvia
- Spilanthes
- Lavendar
- Tea Tree
- Tabebuia - Pau D'Arco.
- Usnea

Professional survey: Topical Candida Treatments

- Mouthwash: Spilanthes, berberine-herb, salvia, small amount cardamom
- Mouthwash: Diluter Calendula tincture with Thymus.
- Children: Chamomile, Monarda, cinnamon and small cardamom
- Swab turmeric and cinnamon
- Vaginal: suppository with coconut oil, turmeric, cardamom, salvia and berberine.
- Oral mouthwash- myrrh, calendula, salvia
- Oral mouthwash: Hydrastis, myrrh

Generalists and specialists

- Berberine
- Alliums
- Myrrh
- Calendula
- Anemopsis
- Thymol/carvacrol
- Spilanthes/Acmella
- Tabebuia (dermatophytes)
- Usnea

Allium species

Garlic and Systemic Candida in mice

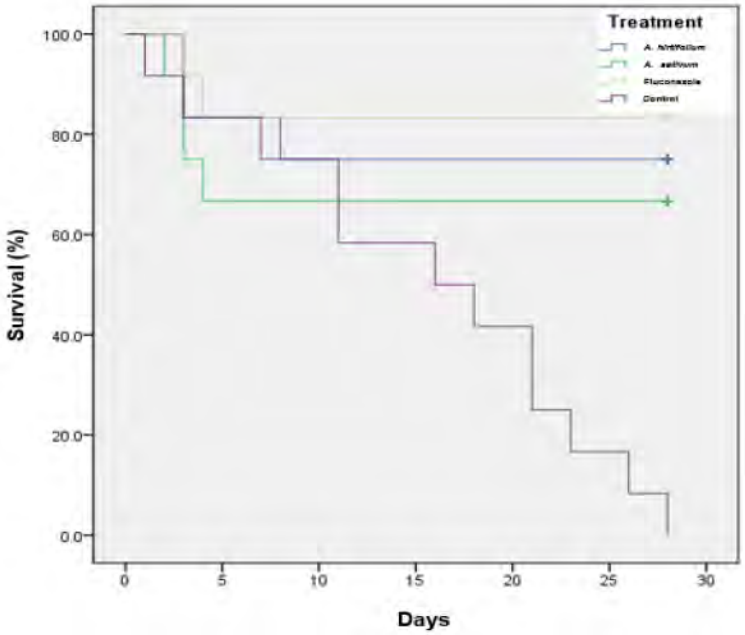


Figure 2. Cumulative mortality of mice infected with *C. tropicalis* treated with *A. hirtifolium, A. sativum* and fluconazole ($p<0.05$).

- Mice with systemic Candida infections treated with aqueous or alcoholic extract of Allium species by IV.
- Aqueous and alcoholic extract about equal, both more effective than the drug.
- The green line represents fluconazole.

Diba A, Alizadeh F. In vitro and in vivo antifungal activity of Allium hirtifolium and Allium sativum. Avicenna J Phytomed. 2018 Sep-Oct;8(5):465-474.

Garlic & fluconazole against Candida culture

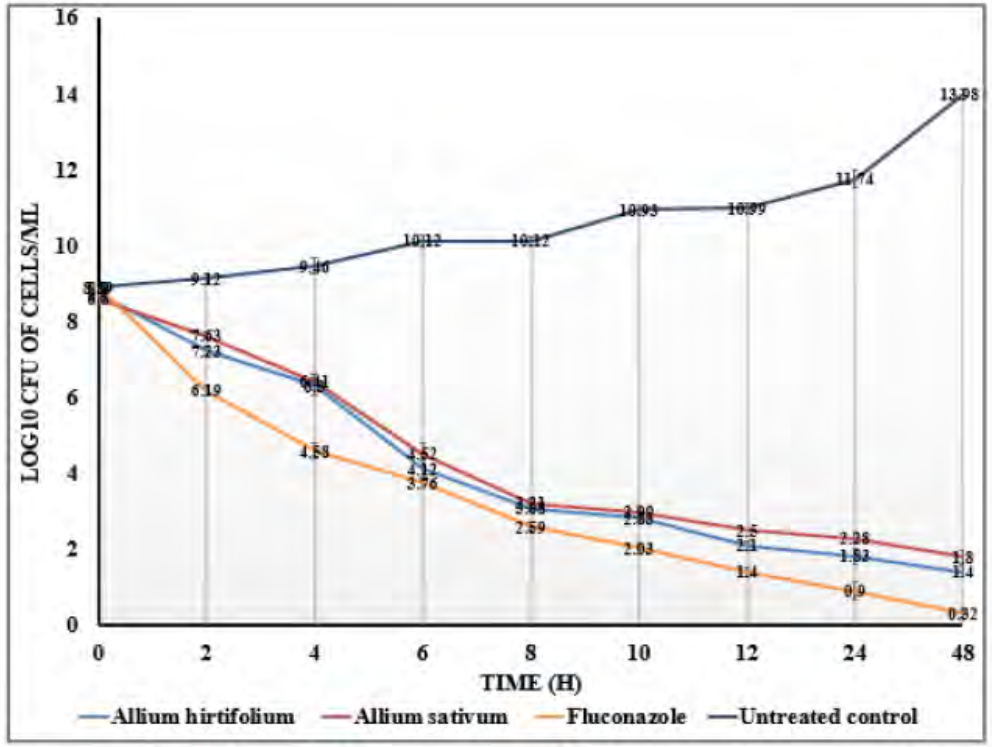


Figure 1. Time-kill curves of *A. hirtifolium* and *A. sativum* against *C. tropicalis* at different time points. Plant extracts were tested at a concentration equal to MIC.

Diba A, Alizadeh F. In vitro and in vivo antifungal activity of Allium hirtifolium and Allium sativum. Avicenna J Phytomed. 2018 Sep-Oct;8(5):465-474.

Garlic effect on Candida biofilms

- Aqueous fresh garlic extract was active against both planktonic and biofilm forms of Candida.
- Fresh garlic crushed in saline water and filtered.
- The MIC for garlic ranged from 62 to 125 micrograms/mL against planktonic Candida.
- Planktonic forms completely eradicated at 1 mg/mL
- Biofilms reduced by about 50% 1 hour after treatment, about 20% after 48 hours.
- The declining efficacy against the biofilm may be due to the rapid degradation of the allicin constituent in garlic. This might be reduced by repeated doses

Garlic antimicrobial pharmacy

- The direct antimicrobial constituent of Garlic is allicin, present in large amount in fresh bulb.
- Upon crushing, the allicin is released via an interaction of the constituents aliin and allinase.
- The half-life of allicin is about 18 hours.
- Famous infection preventing wash by Galen of Roman history was garlic crushed in wine and aged overnight.
- As allicin degrades, it forms into about 30 new sulfur-based constituents with various medicinal properties, NOT including direct antimicrobial, but including immune enhancing (systemic and local) and anti-biofilm effects.

TABLE 1. XTT assay results for adherent and biofilm-associated *C. albicans* treated with FGE

Phase	Mean absorbance at 492 nm ± SD at the following FGE concentration (μg/ml)				
	0	0.5	1	2	4
Adherence phase	3.318 ± 0.107	0.079 ± 0.149	0.000 ± 0.000[a]		
Mature biofilm phase					
1 h treatment	3.049 ± 0.084			1.675 ± 0.231	1.690 ± 0.330[b]
48 h treatment	3.079 ± 0.063			2.714 ± 0.148	2.509 ± 0.243[b]

[a] The *P* value is <0.001 for the mean absorbance values across the concentration range of 0, 0.5, and 1 μg/ml, as determined by ANOVA.
[b] The *P* value is <0.001 for the mean absorbance values across the concentration range of 0, 2, and 4 μg/ml, as determined by ANOVA.

473

- Columns represent dose in mg/mL (not in micrograms/mL as stated in error in the table.
- A higher absorbance figure represents a more dense biofilm

Shuford JA, Steckelberg JM, Patel R. Effects of fresh garlic extract on Candida albicans biofilms. Antimicrob Agents Chemother. 2005 Jan;49(1):473.

Empirical treatment for vaginal Candidiasis

- Case reports of routine treatments over several decades by veteran herbalist/naturopath.
- 2-3 Cloves of garlic (cloves, not bulbs) in a liter of water
- Blend to completely break up the garlic.
- Strain through cheesecloth.
- Use as douche

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	–	–

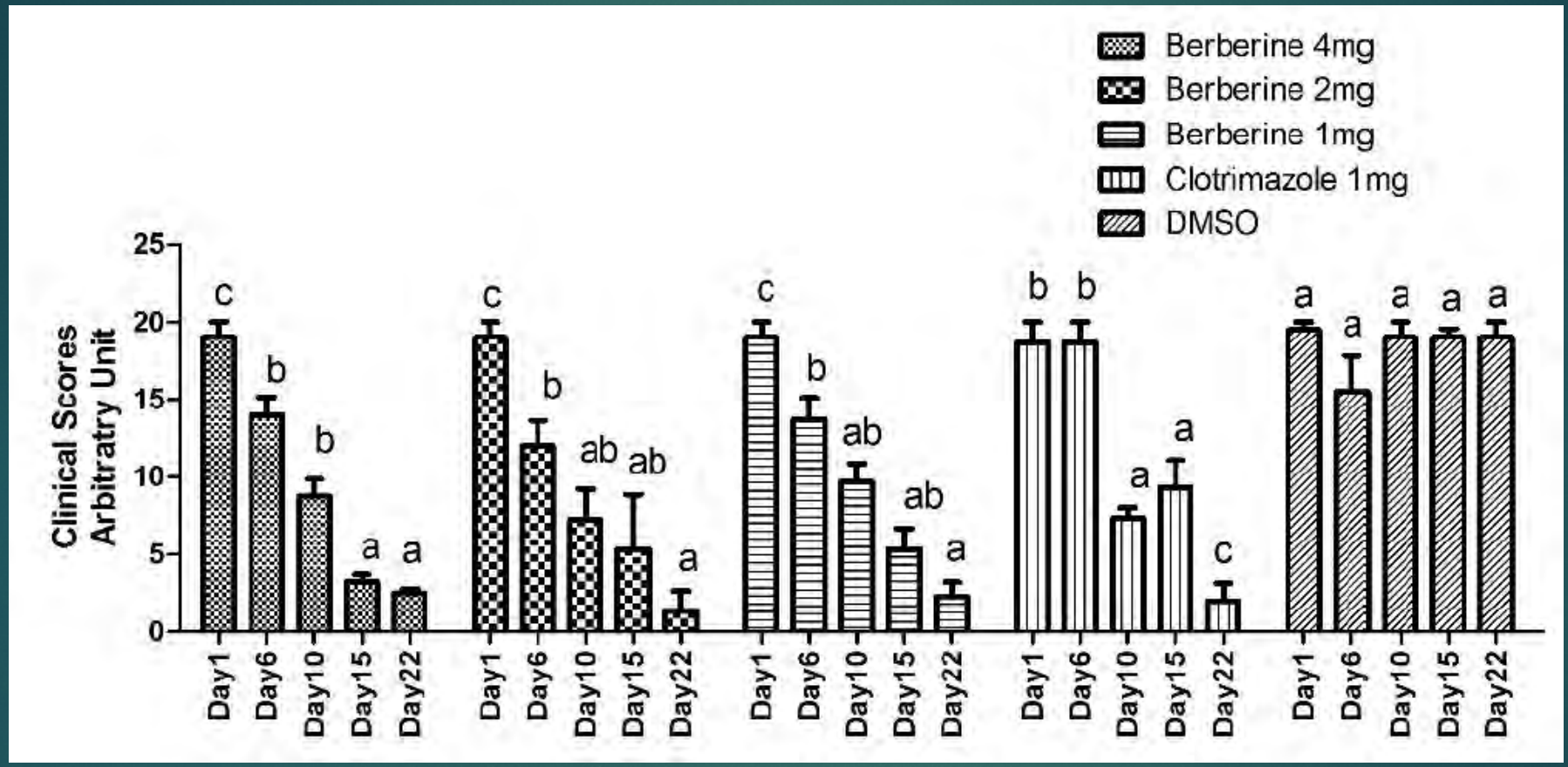
*– = No growth.

*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.

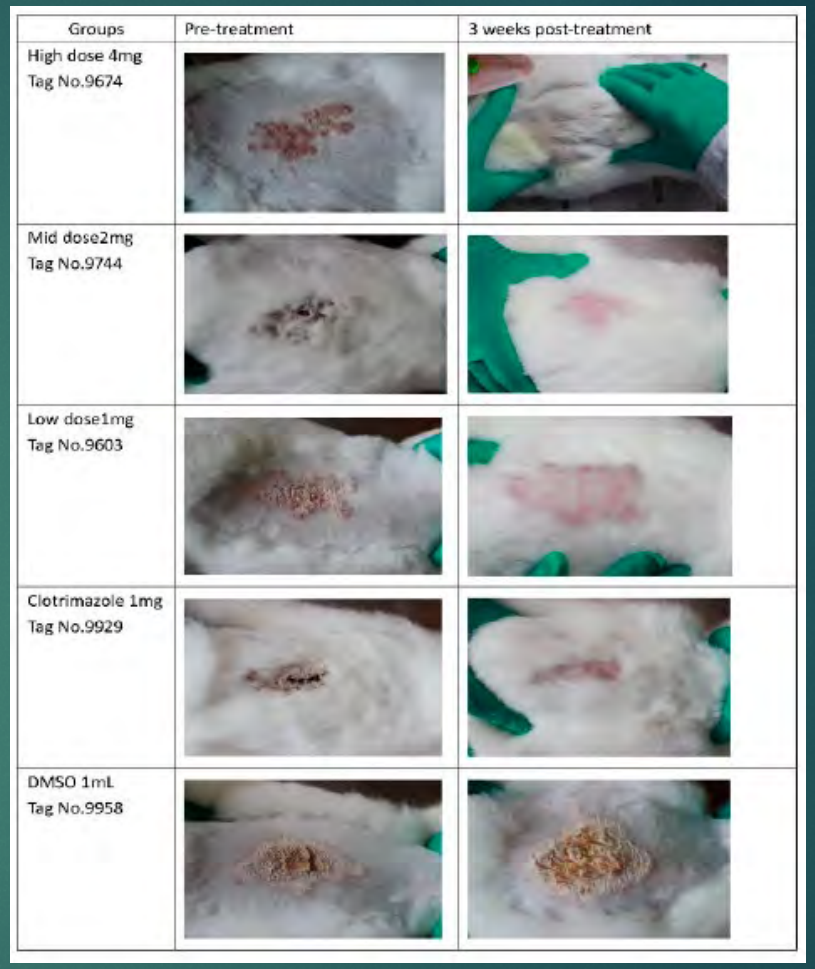
Berberine and fungi

Berberine against Trichophyton mentagrophytes



Topical berberine at any of the doses was as effective but more rapid acting than clotrimazole

- The organism in humans may cause athlete's foot, ringworm, jock itch, nail fungus, beard, skin, scalp infections.
- Major cause of morbidity in veterinary medicine.
- The trial showed similar effect as to the antifungal clotrimazole
- An anti-resistance mechanism for berberine was described.



Xiao CW, Liu Y, Wei Q, Ji QA, Li K, Pan LJ, Bao GL. Inhibitory Effects of Berberine Hydrochloride on Trichophyton mentagrophytes and the Underlying Mechanisms. Molecules. 2019 Feb 19;24(4). pii: E742.

Berberis vulgaris and berberine against dermatophytes

- Methanol and water extracts of Berberis effective against dermatophytes

Table 1: Antidermatophytic effects of various extracts of *B. vulgaris* and its main constituent, berberine, against *T. mentagrophytes*, *T. rubrum*, *M. canis*, and *M. gypseum* by disk diffusion method. Data are expressed as the mean ± SD ($n = 3$).

Dermatophytes	Zone of inhibition (mm)				
	Chloroform extract	Methanolic extract	Aqueous extract	Berberine	Ketoconazole
T. mentagrophytes	24.3 ± 2.15	26.3 ± 1.52	18.3 ± 1.17	51.6 ± 3.08	28.6 ± 1.17
T. rubrum	25.3 ± 2.15	28.6 ± 2.08	21.6 ± 2.15	55 ± 3.08	30.3 ± 2.52
M. canis	32 ± 2.5	37.6 ± 3.08	25.3 ± 2.52	>60	37.6 ± 1.52
M. gypseum	27.6 ± 1.17	30.6 ± 1.52	23.3 ± 1.17	>60	31.6 ± 1.52

Mahmoudvand H, Ayatollahi Mousavi SA, Sepahvand A, Sharififar F, Ezatpour B, Gorohi F, Saedi Dezaki E, Jahanbakhsh S. Antifungal, Antileishmanial, and Cytotoxicity Activities of Various Extracts of Berberis vulgaris (Berberidaceae) and Its Active Principle Berberine. ISRN Pharmacol. 2014 Mar 10;2014:602436.

Coptis and berberine against *Candida* species

Table I. Antifungal activity of crude drugs

Crude drug	A	B	C
Artemisiae capillaris flos	—	—	±
Cinnamomi cortex	±	—	—
Coicis semen	—	—	—
Coptidis rhizoma	++	+++	+++
Forsythiae fructus	—	—	—
Gardeniae fructus	—	—	—
Gentianae Scabrae radix	—	—	—
Geranii herba	—	—	—
Houttuyniae herba	—	—	—
Hydrangeae dulcis folium	—	—	±
Lithospermi radix	—	—	—
Magnoliae cortex	+	—	—
Magnoliae flos	—	—	—
Moutan cortex	—	—	—
Persicae semen	—	—	—
Phellodendri cortex	++	++	+++
Platycodi radix	—	—	—
Rhei rhizoma	—	—	—
Saussureae radix	±	±	+
Scutellariae radix	—	—	—
Smilacis rhizoma	—	—	—
Sophorae radix	—	—	—
Zanthoxyli fructus	—	—	—
Zedoariae rhizoma	—	—	—
Zingiberis rhizoma	—	—	—
Zizyphi fructus	—	—	—

A, *C. albicans*; B, *C. tropicalis*; C, *C. glabrata*.
Antifungal activity of crude drugs are represented on basis of following criteria: an inhibitory zone was formed by adding 0.5%, +++; ditto, 1%, ++; ditto, 5%, +; ditto, 10%, ±; no inhibitory zone was formed by adding 10%, —

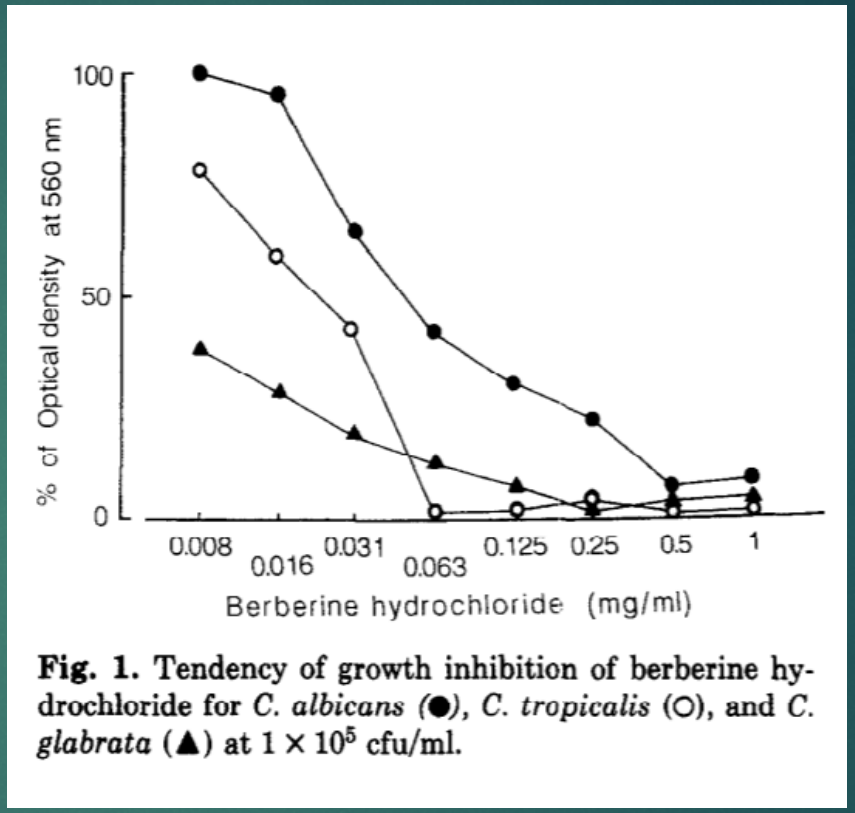


Fig. 1. Tendency of growth inhibition of berberine hydrochloride for *C. albicans* (●), *C. tropicalis* (○), and *C. glabrata* (▲) at 1×10^5 cfu/ml.

Nakamoto, K., Sadamori, S., & Hamada, T. (1990). *Effects of crude drugs and berberine hydrochloride on the activities of fungi. The Journal of Prosthetic Dentistry, 64(6), 691–694.*

Berberine MIC against some fungal species

TABLE 1 Effects of berberine against FLC-resistant strains of *Candida* spp. and *C. neoformans* isolated in Ceara, Brazil

Strain	Origin	GenBank accession no.	MIC (μg/ml)	
			FLC	Berberine
Candida tropicalis 1	Blood	KJ740185	32	8
Candida tropicalis 2	Blood	KJ740181	16	8
Candida albicans 1	Blood	KJ740176	16	8
Candida albicans 2	Blood	KJ740174	32	8
Candida albicans 3	Blood	KJ740179	32	8
Candida parapsilosis 1	Blood	KJ740191	32	8
Candida parapsilosis 2	Blood	KJ740188	16	8
Cryptococcus neoformans 1	Blood	KJ740165	64	16
Cryptococcus neoformans 2	Blood	KJ740167	64	16
Cryptococcus neoformans 3	Blood	KJ740166	64	16
Cryptococcus neoformans 4	Urine	KJ740168	64	16
Candida krusei ATCC 6258			16	4
Candida parapsilosis ATTC 22019			1	16

See following

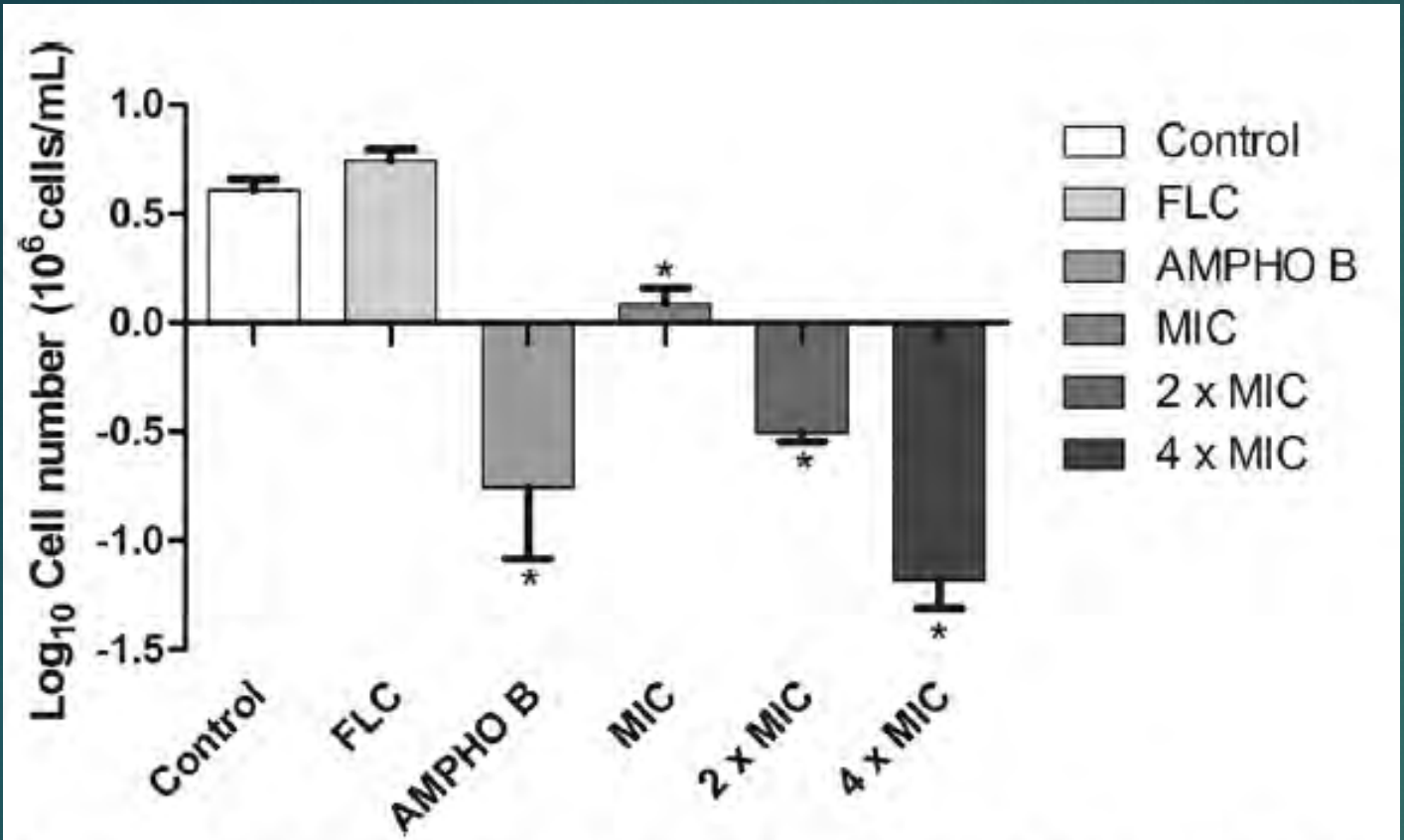


FIG 1 Effect of berberine on the number of viable cells of a *C. albicans* FLC-resistant strain. *, $P < 0.05$ compared to the results for the control, determined by ANOVA followed by the Newman-Keuls test.

Berberine against fluconazole-resistant Candida albicans

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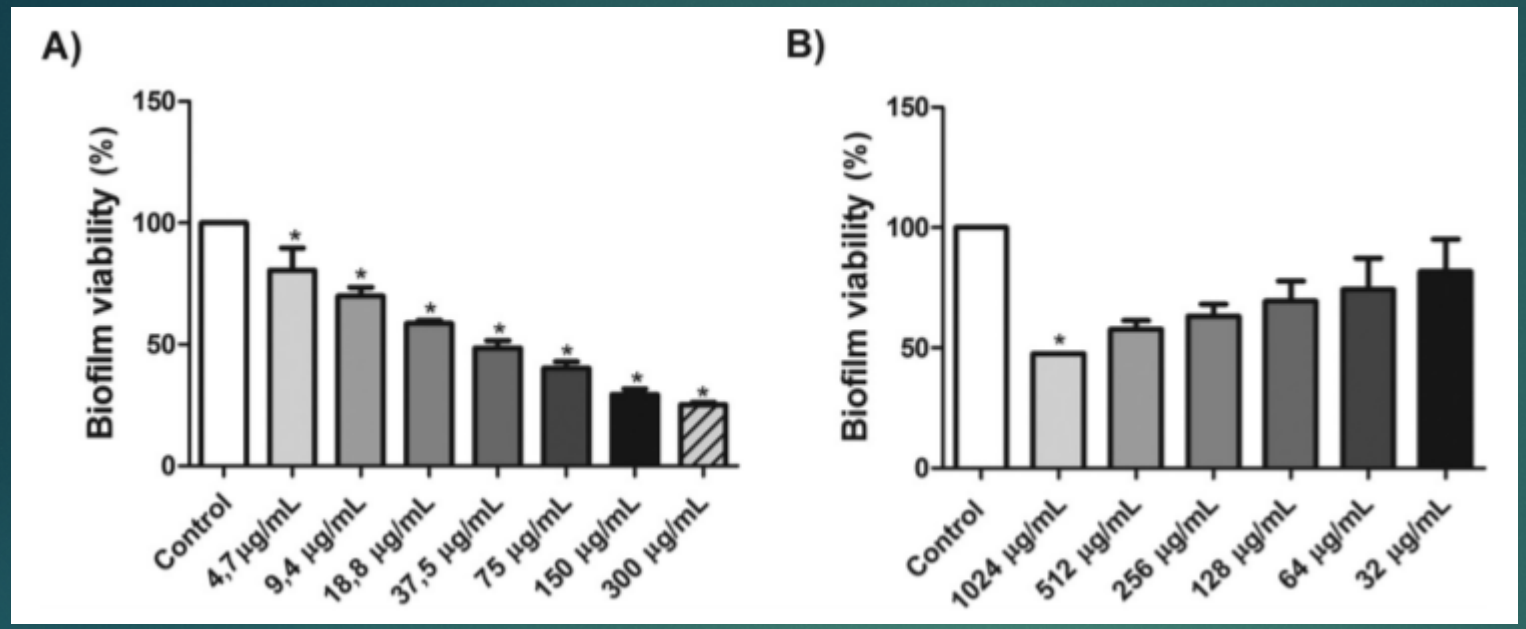


Effect of different concentrations of berberine (A) and fluconazole (B) on the metabolic activity of growing and mature Candida tropicalis biofilms

da Silva AR, de Andrade Neto JB, da Silva CR, Campos Rde S, Costa Silva RA, Freitas DD, do Nascimento FB, de Andrade LN, Sampaio LS, Grangeiro TB, Magalhães HI, Cavalcanti BC, de Moraes MO, Nobre Júnior HV. Berberine Antifungal Activity in Fluconazole-Resistant Pathogenic Yeasts: Action Mechanism Evaluated by Flow Cytometry and Biofilm Growth Inhibition in Candida spp. Antimicrob Agents Chemother. 2016 May 23;60(6):3551-7.

- One study showed synergistic action between berberine and fluconazole in a group of 40 otherwise fluconazole-resistant candida strains.
- The combination was more effective than either one alone.

Quan H, Cao YY, Xu Z, Zhao JX, Gao PH, Qin XF, Jiang YY. Potent in vitro synergism of fluconazole and berberine chloride against clinical isolates of Candida albicans resistant to fluconazole. Antimicrob Agents Chemother. 2006 Mar;50(3):1096-9.

Quercus and tannin

- One study comparing several extracts concluded that the ethanolic extract of Quercus had the strongest effect against Candida species compared to other forms.
- MIC was 40 micrograms/mL
- Minimum fungicidal concentration (MFC) was 450 micrograms/mL

Karimi Poor Fard, M. et al., Antibacterial activities of thymus denaensis, jaft and hydro-alcoholic extract of green hull pistacia atlantica on listeria monocytogenes. J. Med. Yasuj 2012. 17: p. 68-77.

Symptom score changes with treatment

Number of patient in each group = 40

Symptom	Before (clotrimazole)	After (clotrimazole)	Before (Quercus)	After (Quercus)
Discharge	36	8	36	2
Itching	24	4	28	9
Pain/intercourse	19	10	18	11
Edema/redness	23	0	23	0
Positive culture	40	5	40	10
Negative culture	0	35	0	30

One group of women with vaginal candidiasis received clotrimazole vaginal cream (1%) and the other group received vaginal douche of *Q. Brantii* extract. Groups were treated for 7 days. Researchers concluded that the two treatments were functionally equivalent.

Moshfeghy Z, Asadi K, Akbarzadeh M, Zare A, Poordast T, Emamghoreishi M, Najib FS, Sayadi M. Quercus Brantii Lindl. Vaginal Douche Versus Clotrimazole on Vaginal Candidiasis: A Randomized Clinical Trial. J Pharmacopuncture. 2018 Sep;21(3):185-194.

Commiphora/Myrrh

- Myrrh essential oil compared to tincture against Trichophyton, rubrum, T. mentagrophytes, Microsporum canis, M. gypsum, and T. verrucosum.
- The inhibitory effect of myrrh oil and ethanol extract against dermatophytes were 43.1–61.6% and 12.5–27.5%, respectively.
- The MIC and MFC values of myrrh oil were 25–100 and 25–200 micrograms/mL
- For ethanol extract were 25–400 and 25–400 micrograms/mL, respectively.

Mahboubi, M., & Mohammad Taghizadeh Kashani, L. (2015). *The anti-dermatophyte activity of Commiphora molmol. Pharmaceutical Biology, 54(4), 720–725.*

- Myrrh oils may require a much higher concentration against Candida then against dermatophytes
- The MIC and MFC against 52 Candida strains for the essential oil was about 2.5 mg/mL and 5 mg/mL respectively
- Oral strains of Candida were used in the trial

Nikolic M, Smiljkovic M, Markovic T, Cirica A, Glamoclija J, Markovic D, Sokovic M. Sensitivity of clinical isolates of Candida to essential oils from Burseraceae family. EXCLI J. 2016 Apr 19;15:280-9.

Calendula

Table 1. Antifungal activities of the essential oil of flowers of *Calendula officinalis*.

Isolate	Microorganisms	Origin*	Mean zone of inhibition a (mm)	
			Calendula oil15 µl/disc	Nystatin 20 µg/disc
1	*C. albicans*	ATCC 64548	16	12
2	*C. albicans*	orotracheal tube	11	13
3	*C. albicans*	OC – HIV	26	11
4	*C. albicans*	VVC	18	12
5	*C. albicans*	VVC	15	12
6	*C. albicans*	VVC	15	12
7	*C. albicans*	Urine	27	11
8	*C dubliniensis*	ATCC 777	24	11
9	*C. parapsilosis*	ATCC 22019	20	12
10	*C. parapsilosis*	Onychomycosis	14	13
11	*C. parapsilosis*	Paronychia	30	11
12	*C. parapsilosis*	Blood	30	11
13	*C. glabrata*	ATCC 90030	15	12
14	*C. glabrata*	Hands colonization	23	11
15	*C. glabrata*	Hands colonization	28	11
16	*C. tropicalis*	Urine	11	13
17	*C. tropicalis*	Granulomatous lesion	15	12
18	*C. tropicalis*	Urine	21	12
19	*C. tropicalis*	Urine	22	11
20	*C. guilliermondii*	Hands colonization	25	11
21	*C. guilliermondii*	Hands colonization	24	11
22	*C. krusei*	ATCC 6258	15	12
23	*Rhodotorulla sp*	Hands colonization	30	11

* Except to ATCC microorganisms all of others are human clinical isolates OC – HIV: oral candidiasis; VVC: vulvovaginal candidiasis. Mean of inhibition zone by oil of flowers of *Calendula officinalis:* Good activity (11 -18 mm); high activity (20-27 mm); highest activity (28-30 mm).

Calendula e.o. more effective than Nystatin against Candida

Effective against all Candida species tested

Gazim ZC, Rezende CM, Fraga SR, Svidzinski TI, Cortez DA. Antifungal activity of the essential oil from Calendula officinalis L. (asteraceae) growing in Brazil. Braz J Microbiol. 2008 Jan;39(1):61-3.

Calendula equivalent to Fluconazole for Candida

Table 2
Antifungal activity of different *Calendula officinalis* extracts.

Fungi	Antifungal activity of *Calendula officinalis* extracts in term of inhibition zone (IZ) in mm[a]		
	Methanol extract	Ethanol extract	Fluconazole
Candida albicans 0103 (UUC collection)	10 ± 1^{b}	9 ± 1^{b}	7 ± 0^{a}
C. albicans ATCC 90028	9 ± 1^{b}	7 ± 0^{a}	10 ± 1^{b}
Candida krusei ATCC 6258	9 ± 1^{a}	10 ± 1^{a}	14 ± 2^{b}
Candida glabrata ATCC 2001	12 ± 1^{a}	14 ± 2^{a}	12 ± 1^{a}
Candida parapsilosis ATCC 22019	10 ± 1^{a}	10 ± 2^{a}	14 ± 1^{b}
Aspergillus flavus GC 6158	8 ± 1^{a}	7 ± 1^{a}	7 ± 1^{a}
Aspergillus fumigatus 27.5	10 ± 1^{a}	9 ± 1^{a}	12 ± 2^{a}
Aspergillus niger 27.5	9 ± 1^{a}	9 ± 1^{a}	11 ± 1^{a}
Exophiala dermatitidis GC 7895	10 ± 1^{a}	10 ± 1^{a}	11 ± 1^{a}

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

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.*

Calendula superior to Clotrimazole for vaginal candidiasis

Creams of Calendula vs clotrimazole compared

Table 2. Negative mycological test for *Candidiasis* at the follow-ups by the study groups.

Assessment time	*Calendula* (n = 70)	Clotrimazole (n = 74)	p*	OR (95% CI)
10–15 days after the intervention	34 (48.6)	55 (74.3)	.001	0.32 (0.16–0.67)
30–35 days after the intervention	54 (78.7)	25 (33.8)	<.001	3.1 (1.5–6.2)

Note. The data show number (%) unless otherwise specified.

The initial improvement of Clotrimazole, followed by a worsening at 30-35 days implies an initial antimicrobial effect but with a persistent biofilm and possible development of resistance. On the contrary, treatment with Calendula showed continued evidence of biofilm damage and a lack of microbial resistance to the constituents.

Calendula superior to Clotrimazole for symptom frequency

Table 4. Frequency of complaints by the participants at different time points by the study group.

	Baseline			10–15 days after intervention			30–35 days after intervention		
Symptoms	*Calendula* (n = 75)	Clotrimazole (n = 75)	p*	*Calendula* (n = 70)	Clotrimazole (n = 74)	p*	*Calendula* (n = 70)	Clotrimazole (n = 74)	p*
Vaginal discharge	63 (84.0)	62 (82.7)	.827	22 (31.4)	20 (27.0)	.561	17 (24.3)	39 (52.7)	<.001
Vulvar pruritus	60 (80.0)	59 (78.7)	.840	20 (28.6)	31 (41.9)	.095	13 (18.6)	48 (64.9)	<.001
Dysuria	36 (48.0)	36 (48.0)	1.000	15 (21.4)	13 (17.6)	.558	11 (15.7)	13 (17.6)	.766
Abdominal pain	44 (58.7)	43 (57.3)	.869	8 (11.4)	10 (13.5)	.705	8 (11.4)	12 (16.2)	.406
Irritation	37 (49.3)	41 (54.7)	.513	9 (12.9)	14 (18.9)	.321	7 (10.0)	22 (29.7)	.003
Dyspareunia	42 (56.0)	43 (57.3)	.869	10 (14.3)	15 (20.3)	.343	9 (12.9)	16 (21.6)	.165

Note. The data show number (%) unless otherwise specified.

Saffari, E., Mohammad-Alizadeh-Charandabi, S., Adibpour, M., Mirghafourvand, M., & Javadzadeh, Y. (2016). *Comparing the effects of Calendula officinalis and clotrimazole on vaginal Candidiasis: A randomized controlled trial. Women & Health, 57(10), 1145–1160.*

Professional treatment

A Professional herbalist from the UK reports 100% with vaginal candidiasis with the following method.

Equal parts of Tr's Calendula, Allium, Hydrastis, with a couple of drops of Tee tree oil per 100ml of the mixture.

The patient receives 10 ml of this mix and a dropper bottle. They dilute 1 part of this mix to 20 parts of boiled water (Can count out drops

Apply using an empty tampon tube.

Remain lying down for 20 minutes or so. Repeat twice daily gradually increasing the strength of the mix until it starts to smart slightly. Once the thrush has gone phase out the use gradually.

Spilanthes/Acmella

Spilanthes

- Spilanthes acmella = Acmella oleracea
- “Toothache plant” has tingle effect on tongue similar to Echinacea, due to similar constituents
- No evidence of immune enhancement in humans, but positive in animal trials.
- Has anti-inflammatory effects.
- Can be considered an “antifungal specialist,” powerfully fungicidal at low concentrations.

Dubey S, Maity S, Singh M, Saraf SA, Saha S. Phytochemistry, Pharmacology and Toxicology of Spilanthes acmella: A Review. Adv Pharmacol Sci. 2013;2013:423750. doi: 10.1155/2013/423750. Epub 2013 Nov 26. Review.

Spilanthes against *Trichophyton rubrum*

Spilanthes acmella essential oil from flowers tested against the dermal fungus *Trichophyton rubrum*

Acmella EO and its major component d-limonene had mycelia growth inhibitory activity.

The MIC (fungistatic concentration) of the Acmella EO and limonene against *T. rubrum* was observed to be 1 µ/ml and 2 µ/ml respectively.

MFC (fungicidal) values were (4 and 6 µl/ml)

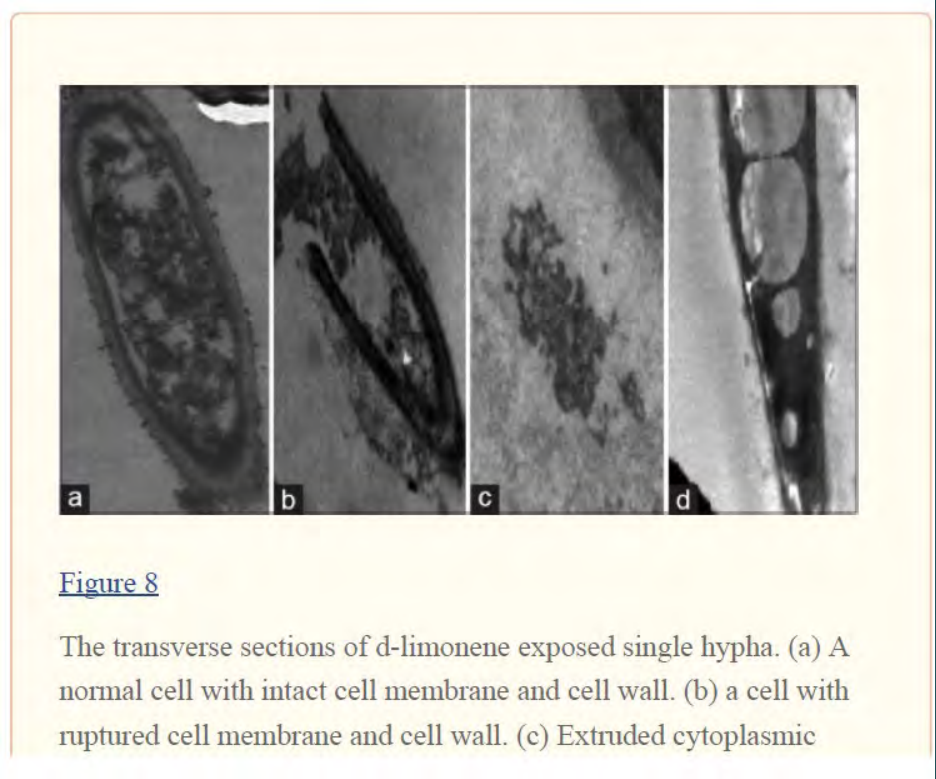
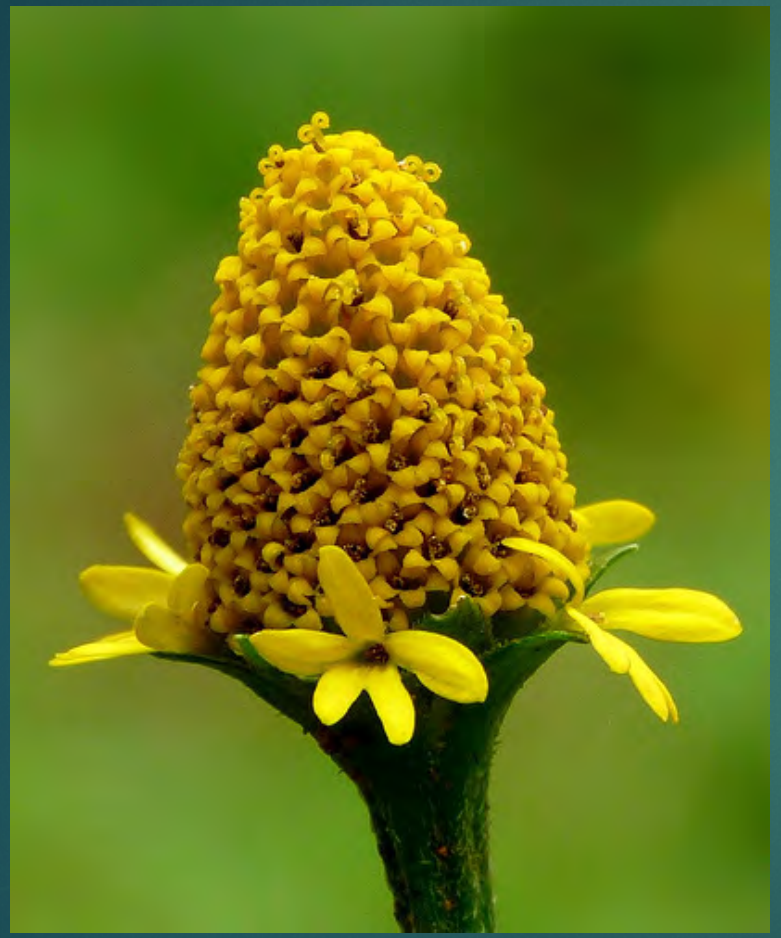


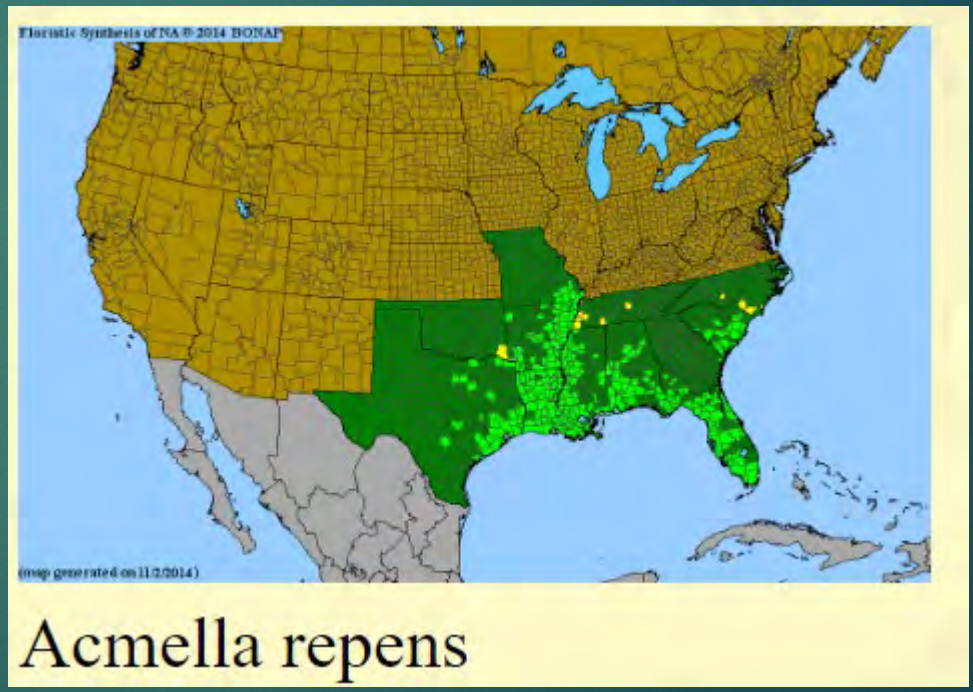
Figure 8

The transverse sections of d-limonene exposed single hypha. (a) A normal cell with intact cell membrane and cell wall. (b) a cell with ruptured cell membrane and cell wall. (c) Extruded cytoplasmic

Padhan D, Pattnaik S, Behera AK. Growth-arresting Activity of Acmella Essential Oil and its Isolated Component D-Limonene (1, 8 P-Mentha Diene) against Trichophyton rubrum (Microbial Type Culture Collection 296). Pharmacogn Mag. 2017 Oct;13(Suppl 3):S555-S560.



Must use flowers for the antimicrobial effect



Acmella repens

In one trial against oral bacteria, Spilanthes tincture killed more than 90% of the bacteria within 10 minutes, a kill rate similar to garlic.

Vlachojannis C, Chrubasik-Hausmann S, Hellwig E, Vach K, Al-Ahmad A. Activity of preparations from Spilanthes oleracea, propolis, Nigella sativa, and black garlic on different microorganisms involved in oral diseases and on total human salivary bacteria: A pilot study. Phytother Res. 2018 Oct;32(10):1992-2001.

Spilanthes case

- Elder with venous insufficiency in lower leg
- Toenail fungus began in top third of nail
- Treated intermittently with *Larrea* to prevent spread.
- The fungus spread all the way down into the nail bed over one year.
- An oral antifungal was prescribed but refused
- Began therapy with a simple of *Spilanthes,* applied intensively and regularly.
- The toenail debris was also regularly removed.
- New fresh nail growth appeared over the next 2 months.

Melaleuca/Tea tree

Tea tree

Traditional use in Australia for a wide variety of skin infections

One hundred and four patients completed a randomized, double-blind trial to evaluate the efficacy of ***10% w/w tea tree oil cream*** compared with 1% tolnaftate and placebo creams in the treatment of tinea pedis.

Significantly more tolnaftate-treated patients (85%) than tea tree oil (30%) and placebo-treated patients (21%) showed conversion to negative culture at the end of therapy ($p < 0.001$

The tea tree oil group (24/37) and the tolnaftate group (19/33) showed significant improvement in clinical condition when compared to the placebo group (14/34; $p = 0.022$ and $p = 0.018$ respectively).

Tea tree oil cream (10% w/w) appears ***to reduce the symptomatology*** of tinea pedis as effectively as tolnaftate 1% but is ***no more effective than placebo*** in achieving a mycological cure.

Tong MM, Altman PM, Barnetson RS. Tea tree oil in the treatment of tinea pedis. Australas J Dermatol. 1992;33(3):145-9.

Melaleuca EO against Candida

- Melaleuca effective against only 11 of 31 strains of Candida, but only at ***high MIC compared*** to thymol and carvacrol (factor of 10+ concentration)
- A 16% solution of the essential oil was used

Bona, E., Cantamessa, S., Pavan, M., Novello, G., Massa, N., Rocchetti, A., … Gamalero, E. (2016). *Sensitivity of Candida albicansto essential oils: are they an alternative to antifungal agents? Journal of Applied Microbiology, 121(6), 1530–1545.*

Melaleuca e.o. at a 16% concentration was ineffective against 27/28 strains of vaginal isolated candia strains

Massa N, Cantamessa S, Novello G, Ranzato E, Martinotti S, Pavan M, Rocchetti A, Berta G, Gamalero E, Bona E. Antifungal activity of essential oils against azole-resistant and azole-susceptible vaginal Candida glabrata strains. Can J Microbiol. 2018 Oct;64(10):647-663.

Tea Tree against equine fungus.

- A randomized open clinical trial was carried out on 60 thoroughbred breeding horses affected by equine ringworm.
- The animals of both groups were topically treated twice a day for 15 days with a 25% mixture of TTO diluted in sweet almond oil.
- All the treated animals showed complete clinical and aetiological healing after 30 days.

Pisseri F, Bertoli A, Nardoni S, Pinto L, Pistelli L, Guidi G, Mancianti F. Antifungal activity of tea tree oil from Melaleuca alternifolia against Trichophyton equinum: an in vivo assay. Phytomedicine. 2009 Nov;16(11):1056-8.

Undiluted oil against dermal fungi

- Researchers tested ***pure undiluted tea tree oil*** against 58 clinical isolates
- Candida albicans, (10), Trichophyton rubrum (8), Trichophyton mentagrophytes (9), Trichophyton tonsurans (10), Aspergillus niger (9), Penicillium species (9), Epidermophyton floccosum (2), and Microsporum gypsum (1).
- The concentrated essential oil showed inhibitory activity against all isolates tested except one strain.
- Researchers did not provide details of the degree of inhibition, in this poorly designed trial.

Concha JM, Moore LS, Holloway WJ. 1998 William J. Stickel Bronze Award. Antifungal activity of Melaleuca alternifolia (tea-tree) oil against various pathogenic organisms. J Am Podiatr Med Assoc. 1998 Oct;88(10):489-92.

- Tea tree oil appears to be ineffective against various fungi at concentrations of the oil less than 25%.
- The only trial reporting definite consistent anti-fungal effects against a variety of species used pure undiluted essential oil.
- Melaleuca oil can cause skin and mucous membrane irritation and sensitization, and this occurs commonly in practice with undiluted Melaleuca oil.

Tabebuia/Pau D’arco

Tabebuia/Pau D'Arco

- MEDLINE search (Pau d'arco OR lapacho OR tabebuia)
- The tea made from the leaves and bark can lower fever.
- Species of the Tabebuia genus have been used empirically as anti-inflammatory, anti-cancer and anti-microbial agents in rural areas of Colombia, Bolivia, Brazil and other Latin American countries.
- The Tabebuia genus is commonly recognized as a therapeutic alternative by rural or remote populations.
- Tabebuia entered into North American herbal practice touted as an antifungal for intestinal and "systemic" candida in the 1980s.

Methanol extract had moderate effect against candida albicans

Table 1. In vitro antifungal activity of dichloromethane and methanol extracts (mg/mL).

Microorganisms	Dichloromethane extracts						Methanol extracts					
	AC	MP	SC	TA	RO	PG	AC	MP	SC	TA	RO	PG
C. albicans	0.015	R	0.03	R	0.007	0.001	R	0.007	0.001	0.003	0.001	0.003
C. dubliniensis	0.03	R	0.06	R	0.015	0.001	R	0.007	0.001	0.007	0.007	0.003
C. parapsilosis	0.015	R	0.007	R	0.03	0.001	R	0.003	0.001	0.003	0.003	0.003
C. tropicalis	0.015	R	0.03	R	0.015	0.001	R	0.003	0.001	0.015	0.003	0.001
C. guilliermondii	0.015	R	0.007	R	0.007	0.001	R	0.001	0.001	0.001	0.003	0.001
C. utilis	R	R	0.001	R	R	0.001	R	0.007	0.001	0.003	R	0.003
C. krusei	0.007	R	0.03	0.06	0.003	0.001	R	0.007	0.001	0.007	0.001	0.001
C. lusitaniae	0.007	R	0.03	R	0.007	0.001	R	0.007	0.001	0.003	0.001	0.001
C. glabrata	R	R	0.001	R	R	0.001	R	0.003	0.001	0.001	R	0.003
C. rugosa	0.007	R	0.03	R	0.001	0.001	R	0.003	0.001	0.003	0.001	0.003

AC, *Arrabidaea chica;* MP, *Mentha piperita;* SC, *Syzygium cumini;* TA, *Tabebuia avellanedae;* RO, *Rosmarinus officinalis;* PG, *Punica granatum*; R, Resistant.

Höfling, J., Anibal, P., Obando-Pereda, G., Peixoto, I., Furletti, V., Foglio, M., & Gonçalves, R. (2010). *Antimicrobial potential of some plant extracts against Candida species. Brazilian Journal of Biology, 70(4), 1065–1068.*

Water and methanol extracts inactive against Candida.

Table 2 (*Continued*)

Plant material	Extract yields (%)			Dose[b]	Growth inhibition zone diameter[c]										
	A	D	M		Af	An	Ca	Ccl	Cn	Fo	Mg	Nc	Pp	Sc	Tm
Tabebuia avellanedae (bark)	21.5	3.8	16.0	A5	–	–	–	–	31	–	19	–	15	–	21
				A10	–	–	–	–	35	–	25	–	18	–	25
				D5	25	–	24	20	39	21	25	15	25	35	24
				D10	31	–	28	24	46	28	32	21	30	35	33
				M5	–	–	–	–	28	–	17	–	17	–	18
				M10	–	–	–	–	33	–	21	–	19	–	22

- ***All forms highly active against Trichophyton and microsporum dermatophytes***

A doses = Water. D doses = dichloroethans M doses = methanol
Ca, Ccl, and Cn = 3 Candida species.
Mg = Microsporum, Pp = penicillium, Tm = Trichophyton

Portillo, A., Vila, R., Freixa, B., Adzet, T., & Cañigueral, S. (2001). *Antifungal activity of Paraguayan plants used in traditional medicine. Journal of Ethnopharmacology, 76(1), 93–98.*

Pau D'Arco

- A total of 57 extracts were screened by the agar-well diffusion technique against *Candida albicans* and *Trichophyton rubrum*.
- The most promising extracts were tested in smaller concentrations and their minimal inhibitory concentrations (MIC) were determined.
- Extracts of *Tabebuia caraiba* were very active against Trichophyton rubrum. MIC values between 170.39 and 23.2/ microg ml
- None of the extracts tested greatly inhibited growth of C. albicans

Melo e Silva F, de Paula JE, Espindola LS. Evaluation of the antifungal potential of Brazilian Cerrado medicinal plants. Mycoses. 2009 Nov;52(6):511-7.

Many Pau D’Arco supplements sold in the United States are purportedly prepared from the sawdust of Brazilian lumber mills from species lacking medicinal properties

Dvorkin-Camiel, L., & Whelan, J. S. (2008). *Tropical American Plants in the Treatment of Infectious Diseases. Journal of Dietary Supplements, 5(4), 349–372.*

Usnea and lichens

Lichens

- A lichen is a composite organism that arises from algae or cyanobacteria living among filaments of multiple fungi species in a mutualistic relationship. The combined lichen has properties different from those of its component organisms. Lichens are by necessity specialists at fighting off bacteria, mold, and fungi.

Greenshield lichen (*Flavoparmelia spp.)*

Wolf lichen (*Letharia vulpina*

Claydonia spp.

Lichen acids in Usnea

- Research into Usnea has tunneled on the ***usnic acid*** constituent, but other acids have similar antimicrobial and antifungal properties.
- Diffractaic acid – ethanol soluble, methanol
- Stictic acid. Soluble in ethanol, methanol.
- Norstictic acid – poorly water soluble, soluble ethanol, methanol
- Usnic acid – More soluble in ethanol than water

Honda, N. K., Pavan, F. R., Coelho, R. G., de Andrade Leite, S. R., Micheletti, A. C., Lopes, T. I. B., … Leite, C. Q. F. (2010). *Antimycobacterial activity of lichen substances. Phytomedicine, 17(5), 328–332.*

Herban Legend Alert

- Usnea does not have to be tinctured in warm or hot alcohol.
- At is true that as the temperature of alcohol rises from room temperature up to the boiling point of water, it will take up a lot more usnic acid.
- By the laws of chemistry, however, once the tincture is back to room temperature, all the usnic acid will fall back out of solution into the marc of the tincture
- In addition, Usnea has a broad spectrum of antimicrobial constituents, and the presence of usnic acid in the product is not essential for biological and antimicrobial activity.

- Lichen acids listed above, from various other lichen species, active in various concentrations against human dermatophytes
- Six *Usnea* species found to inhibit several common dermal fungi
- The ethanolic extract of Usnea was used.

Furmanek, Ł., Czarnota, P., & Seaward, M. R. D. (2019). *Antifungal activity of lichen compounds against dermatophytes: a review. Journal of Applied Microbiology.*

U. filipendula, U. longissima, and U. scabrata are American Usnea species that are morphologically similar to Eurasian U. barbata and all contain about 1-3% usnic acid.

Multiple lichen acids including those in Usnea found to be both antimicrobial, anti mold (aspergillus) and antifungal (Candida)

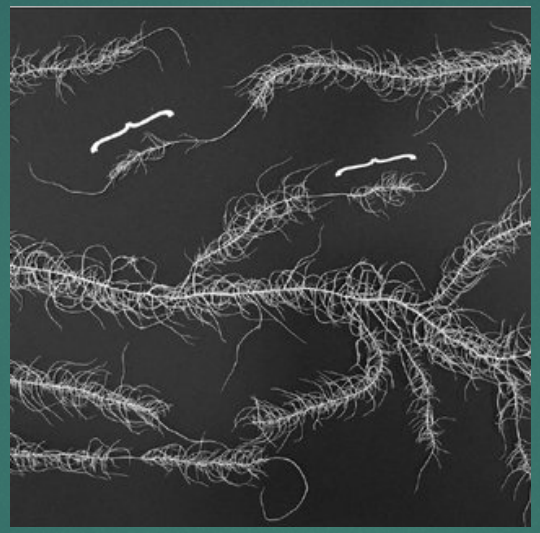
Brakni, R., Ali Ahmed, M., Burger, P., Schwing, A., Michel, G., Pomares, C., … Michel, T. (2018). *UHPLC-HRMS/MS Based Profiling of Algerian Lichens and Their Antimicrobial Activities. Chemistry & Biodiversity, 15(4), e1800031.*

Nishanth KS, Sreerag RS, Deepa I, Mohandas C, Nambisan B. Protocetraric acid: an excellent broad spectrum compound from the lichen Usnea albopunctata against medically important microbes. Nat Prod Res. 2015;29(6):574-7. doi: 10.1080/14786419.2014.953500.

Usnea vs Alectoria

- Usnic acid has been identified in many genera of lichens including Usnea, Cladonia, Lecanora, Ramalina, Evernia, Parmelia and Alectoria.
- **Alectoria** spp. are known to contain up to 6% **usnic acid**, while the thallus of Usnea laevis from the Venezuelan Andes contains only half (nearly 3% **usnic acid**) of that amount (Marcano et al., 1999).
- Alectoria likewise contains a group of other common lichen acids.
- In the native ethnobotany of the Pacific Northwest, Usnea and Alectoria species are used interchangeably

Usnea spp and Alectoria



Usnea (left and middle) Alectoria (right). Usnic acid has been identified in many genera of lichens including Usnea, Cladonia, Lecanora, Ramalina, Evernia, Parmelia and Alectoria.

Dieu A, Mambu L, Champavier Y, Chaleix V, Sol V, Gloaguen V, Millot M. Antibacterial activity of the lichens Usnea Florida and Flavoparmelia caperata (Parmeliaceae). Nat Prod Res. 2019 Jan 24:1-5

Usnea longissima and its plant spirit

- Usnea and related species are most important medicinally for their topical effects against a range of infections, including fungal infections.
- For internal use, Usnea species are expectorant, cool, drying, and somewhat relaxant.
- Although Usnea spp excel as topical disinfectants, we have no evidence that they work internally as “antibiotics” or that they have any effect on the immune system.
- Large or concentrated amounts of usnic acid should not be taken internally. This seems to have only occurred with the abuse of a concentrated weight loss or body building supplement including pure extracted usnic acid.

Thymus and thymol

Thyme and thymol

- Thyme essential oil inhibits candida ***biofilm*** formation with prep at MIC
- Does not affect formed biofilms
- At SIC, Candida sequesters in vacuoles and the fungus effectively detoxifies
- he thymol

Rajkowska K, Nowicka-Krawczyk P, Kunicka-Styczyńska A. Effect of Clove and Thyme Essential Oils on Candida Biofilm Formation and the Oil Distribution in Yeast Cells. Molecules. 2019 May 21;24(10). pii: E1954.

Oregano oil

- Problem with names and species
- Origanum vulgare = Oregano
- Thymus capitatus = Spanish oregano.
- ***Both have predominant Thymol and Carvacrol***
- See “Wild Oregano” Monarda species
- And Satureja (Savory) species
- Both are irritants to skin and mucous membranes and listed as “hazardous” by Tisserand.
- Should be used at dilution, or extracts of the plants used.

(Tisserand R., Balacs T. Essential Oil Safety: A guide for health care professionals. New York: Chruchill Livingstone 1995)

Thymus capitatus oil, Satureja, and Candida

- This trial was on 28 Candida isolates from vaginal candidiasis.
- Azole-resistant strains were determined and used.
- Two most potent plant oils were Oregano and Winter Savory
- ***Study found "medium to high" cytotoxicity to human cells at level that kills Candida***

See next

Thymol/carvacrol containing plants and Candida

696 **Table 3: Results of the minimal inhibitory concentration (MIC) of the tested essential oils (% v/v)**

Candida glabrata strains	*Melaleuca alternifolia* (% v/v)	*Pimpinella anisum* (% v/v)	*Ocimum basilicum* (% v/v)	*Lavandula latifolia* (% v/v)	*Mentha spicata* (% v/v)	*Thymus capitatus* (% v/v)	*Citrus paradisi* (% v/v)	*Satureja montana* (% v/v)
ATCC 15126						0.5		1
29			4			0.5		0.5
30						0.5		1
59						0.5		1
88					4	0.5		1
103						0.25	0.5	1
133					>4	0.5		0.5
136		>4				0.5	0.062	1
137	4	>4	4		4	0.5	0.125	1
159			4			0.5	0.125	0.5
160					4	0.5	0.25	1
165					4	1	0.125	1
174			4		2	0.125	0.125	0.031
192						0.25	0.5	1
196			4		2	0.125		0.25
202			4		2	0.062	0.5	0.5
206						0.25		1
207			>4			0.062	1	0.5
235						0.5	0.5	1
236						0.5	0.5	1
237						0.5	1	1
238				4	2	0.25	0.25	0.5
239						0.5	1	1
240		>4				0.25	1	1
242						0.25		1
243						0.062	0.5	0.5
244				4		0.25	0.5	0.5
245						0.25	0.5	1
246						0.25	0.007	1

Of the plants tested the two plants containing the combination of thymol and carvacrol demonstrated the most potency against candida strains. See ineffectiveness of Melaleuca in column I.

Massa N, Cantamessa S, Novello G, Ranzato E, Martinotti S, Pavan M, Rocchetti A, Berta G, Gamalero E, Bona E. Antifungal activity of essential oils against azole-resistant and azole-susceptible vaginal Candida glabrata strains. Can J Microbiol. 2018 Oct;64(10):647-663.

- Thymol and carvacrol showed the lowest MIC against 3 species of candida. Less than 0.3 micrograms/mL

  Mandras N, Nostro A, Roana J, Scalas D, Banche G, Ghisetti V, Del Re S, Fucale G, Cuffini AM, Tullio V. Liquid and vapour-phase antifungal activities of essential oils against Candida albicans and non-albicans Candida. BMC Complement Altern Med. 2016 Aug 30;16(1):330.

- Thymol and carvacrol showed MICs of 0.125% and 0.0625%. Against Trichophyton infection in horses.

  Mugnaini L, Nardoni S, Pistelli L, Leonardi M, Giuliotti L, Benvenuti MN, Pisseri F, Mancianti F. A herbal antifungal formulation of Thymus serpillum, Origanum vulgare and Rosmarinus officinalis for treating ovine dermatophytosis due to Trichophyton mentagrophytes. Mycoses. 2013 May;56(3):333-7.

- Thymus oils potent effect against Candida strains.

  Pina-Vaz C, Gonçalves Rodrigues A, Pinto E, Costa-de-Oliveira S, Tavares C, Salgueiro L, Cavaleiro C, Gonçalves MJ, Martinez-de-Oliveira J. Antifungal activity of Thymus oils and their major compounds. J Eur Acad Dermatol Venereol. 2004 Jan;18(1):73-8.

- Oil of a Thymus pulegoides species tested
- 7 Candida strains, 5 Aspergillus strains, 2 Trichophyton strains, 2 microsporum strains , and Epidermophyton floccosum
- The oil contained a large percentages of thymol and carvacrol.
- The oil, as well as the two chief constituents, were all effective against all species at a low MIC (,=64 micrograms/mL
- The drug Fluconazole shows inconsistent results, possibly due to resistant strains.

Pinto, E. (2006). *Antifungal activity of the essential oil of Thymus pulegioides on Candida, Aspergillus and dermatophyte species. Journal of Medical Microbiology, 55(10), 1367–1373.*

Salvia spp.

Salvia

- pinene, cineole, camphor, carvacrol, thujone, ursolic acid, oleanolic acid, chlorogenic acid, caffeic acid, labiatic acid, rosmarinic acid, α- and β-amyrin, picrosalvin (carnosol), betulin, flavonoids, tannins, estrogenic substance, saponins, resins

Salvia extract against microorganisms

Table. Number of clinical isolates and reference strains eliminated by each concentration of *Salvia officinalis* extract.

	Concentration (mg/mL)									
Microorganism	**50.00**	**25.00**	**12.50**	**6.25**	**3.13**	**1.56**	**0.78**	**0.39**	**0.19**	**0.09**
Staphylococcus aureus	10[a]	2	0	0	0	0	0	0	0	0
Staphylococcus epidermidis	10	8[a]	1	0	0	0	0	0	0	0
Streptococcus mutans	10[a]	0	0	0	0	0	0	0	0	0
Candida albicans	10	9[a]	1	0	0	0	0	0	0	0
Candida tropicalis	10	10[a]	0	0	0	0	0	0	0	0
Candida glabrata	10	6	3[a]	0	0	0	0	0	0	0

[a]Minimum microbicidal concentration for reference strain (ATCC).

de Oliveira JR, Vilela PGDF, Almeida RBA, de Oliveira FE, Carvalho CAT, Camargo SEA, Jorge AOC, de Oliveira LD. Antimicrobial activity of noncytotoxic concentrations of Salvia officinalis extract against bacterial and fungal species from the oral cavity. Gen Dent. 2019 Jan-Feb;67(1):22-26.

Salvia and vaginal candidiasis

- 111 participants with vaginal candidiasis
- Each received 100 mg vaginal tablet of Clotrimazole and placebo, or 400 mg vaginal tablet of S. officinalis and placebo or a vaginal tablet of S. officinalis and Clotrimazole (SC), once daily for 7 days.
- The Salvia tablet was crude herb, taken once at night on retiring
- Salvia was superior to placebo and equal to Climitrizole on clinical parameters measured.
- Seven days after the end of treatment 29 (80.6%) in the drug group, 35 (97.2%) in Salvia plus drug group, and 33 (94.3%) in Salvia plus placebo group had negative wet test.

Ahangari, F., Farshbaf-Khalili, A., Javadzadeh, Y., Adibpour, M., & Sadeghzadeh Oskouei, B. (2019). *Comparing the effectiveness of Salvia officinalis , clotrimazole and their combination on vulvovaginal candidiasis: A randomized, controlled clinical trial. Journal of Obstetrics and Gynaecology Research.*

Anemopsis/Yerba mansa

Anemopsis

- Traditional treatment for yeast infections and vaginitis in U.S. Southwest and Mexico
- Note historical use in Eclectic medicine for sinusitis ("chronic nasal catarrh"), a condition typically involving a mixed infection by bacteria and fungi.
- Major constituent of essential oil is methyleugenol (>50% plus thymol (>19%)

Acharya RN, Chaubal MG. Essential oil of Anemopsis californica. J Pharm Sci. 1968 Jun;57(6):1020-2.

Medina-Holguín, A. L., Omar Holguín, F., Micheletto, S., Goehle, S., Simon, J. A., & O'Connell, M. A. (2008). *Chemotypic variation of essential oils in the medicinal plant, Anemopsis californica. Phytochemistry, 69(4), 919–927.*

Both thymol and methyleugenol active against both candida and dermatophyte in the same range of effectiveness as conventional medications for these

Table 1. Screening of antifungal activity of alkylphenols against *M. canis* and *Candida* spp. by the agar-well diffusion method.

	Growth Inhibition Zones (mm)							
	Alkylphenols (10,000 µg/mL)						**Controls**	
Strains	thymol	methyl-thymol	eugenol	methyl-eugenol	anethole	estragole	griseofulvin (1000 µg/mL)	amphotericin B (5 µg/mL)
M. canis								
CEMM 01-3-188	35	22	26	20	16	23	55	-
CEMM 01-5-190	40	20	30	20	20	20	47	-
C. albicans								
CEMM 01-3-075	18	18	12	10	11	11	-	14
CEMM 01-3-069	17	13	8	7	9	8	-	10

See next

Also broad spectrum antimicrobial activity of the essential oil

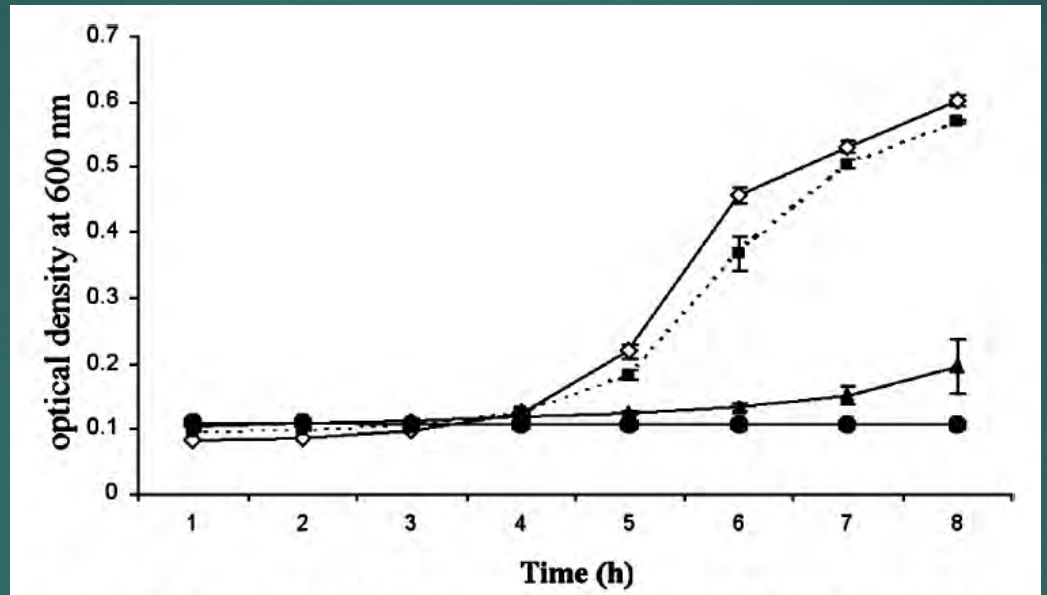


Figure 4. The effects of *A. californica* leaf essential oil and α-pinene on *Staph. aureus* growing in nutrient broth: ◇, media and bacteria; ▲, 0.1% (v/v) leaf essential oil; ■, 0.005% (v/v) α-pinene; ●, 0.01% (v/v) α-pinene.

Medina AL, Lucero ME, Holguin FO, Estell RE, Posakony JJ, Simon J, O'Connell MA. Composition and Antimicrobial Activity of Anemopsis californica leaf oil. J Agric Food Chem. 2005 Nov 2;53(22):8694-8. PubMed PMID: 16248573.

Larrea tridentate, spp./Chaparral

Larrea spp Creosote Bush
Sp: *governadora, hediondilla*

- In the ethnobotany of six native American tribes from the Southwestern U.S, only topical uses are described by five of the six tribes, the other has a single reference among many others to internal use for an unspecified condition or use.
- Reputed to be an internal cancer cure, based on the reported experiences of the Murdoch family in Utah, who began to harvest and market it as a cancer cure, leading eventually to the establishment of the Nature's Way herbal company.
- Internal use has led to cases of liver toxicity, in all cases this was from taking the powder for some extended periods of time.

- The antifungal activity of the ethanolic extracts of ten Argentinean plants used in native medicine was tested against eight fungi and yeasts.
- Extracts of Larrea divaricata, Larrea cuneifolia, and Zuccagnia punctata displayed remarkable activity in the assays against the majority of the test fungi.
- Larrea species showed antifungal activity against all eight fungi tested

See next

Table 2
Radial growth inhibition of alcoholic extracts against filamentous fungi[a]

Fungi[b]	Alcoholic extracts[c]									
	1	2	3	4	5	6	7	8	9	10
A	++	++	−	++	−	+++	−	+	+	−
B	++	++	−	−	−	+++	−	−	−	−
C	++	+	−	+	−	++	+	−	+	−
D	+	−	−	−	−	++	−	−	−	+
E	++	++	−	++	+	++	+	−	+	+
F	+++	++	−	+	+	++	−	−	±	+
G	++	++	−	−	−	++	−	−	−	+
H	++	+	−	+	±	+++	−	−	−	+

[a] The extract concentration (dry matter) in the culture medium was 0.8 mg ml^{-1} in all cases. The inhibition was reported as (−) <10% growth inhibition, (±) between 10 and 20%, (+) between 20 and 40%, (++) between 40 and 80%, and (+++) 80%. ($n = 12$.)

[b] A, *Lenzites elegans*; B, *Schizophyllum commune*; C, *Pycnoporus sanguineus*; D, *Ganoderma applanatum*; E, *Fusarium oxysporum*; F, *Penicillium notatum*; G, *Aspergillus niger*; H, *Trichoderma spp.*

[c] 1, *Larrea divaricata* Cav.; 2, *Larrea cuneifolia* Cav.; 3, *Schkuhria pinnata* (Lam) O. Kuntzi; 4, *Zinnia peruviana* (L), L.; 5, *Equisetum giganteum* L.; 6, *Zuccagnia punctata* Cav.; 7, *Acacia caven* (Mol) Molina; 8, *Phytolacca dioica* L.; 9, *Prosopanche americana* (R.Br) Baillon; 10, *Schinus molle* L.

The Larrea species in columns 1 and 2 are broad-spectrum anti-fungals, active against all fungi tested.

Quiroga, E. N., Sampietro, A. R., & Vattuone, M. A. (2001). *Screening antifungal activities of selected medicinal plants. Journal of Ethnopharmacology, 74(1), 89–96.*

Juglans/Walnut

Fig. 1. Walnut green husks.

Juglans and Candida

- The MIC values of the J. regia L. against Candida strains ranged from 0.006 to 0.195 mg/ml.
- Two C. albicans strains showed a high MIC value (3.125 mg/ml).
- The ***methanol extract*** of Juglans was effective against all 47 Candida strains tested, in the same range of effectiveness as the drug amphotericin B

Noumi E, Snoussi M, Hajlaoui H, Valentin E, Bakhrouf A. Antifungal properties of Salvadora persica and Juglans regia L. extracts against oral Candida strains. Eur J Clin Microbiol Infect Dis. 2010 Jan;29(1):81-8.

Juglans, bacteria and Candida

- Water extract very effective against some bacteria.
- No activity against two Candida speciesNote some contradiction for some candida species

See next

Table 3
Antimicrobial activity of aqueous extracts of walnut green husks from different cultivars

Cultivar	MIC (mg/mL)							
	B. cereus	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*C. albicans*	*C. neoformans*
Franquette	0.1	10	0.1	100	100	100	100	100
	(+ + + +)	(+ + +)	(+ + + +)	(+ +)	(−)	(−)	(−)	(−)
Marbot	1	0.1	0.1	100	100	100	100	100
	(+ + + +)	(+ + +)	(+ + + +)	(+)	(−)	(−)	(−)	(−)
Mayette	0.1	10	0.1	100	100	100	100	100
	(+ + + +)	(+ +)	(+ + +)	(−)	(−)	(−)	(−)	(−)
Mellanaise	0.1	0.1	0.1	100	100	100	100	100
	(+ + + +)	(+ + + +)	(+ + + +)	(−)	(−)	(−)	(−)	(−)
Parisienne	0.1	0.1	0.1	100	100	100	100	100
	(+ +)	(+ + + +)	(+ + + +)	(−)	(−)	(−)	(−)	(−)

No antimicrobial activity (−), inhibition zone < 1 mm. Slight antimicrobial activity (+), inhibition zone 2–3 mm. Moderate antimicrobial activity (+ +), inhibition zone 4–5 mm. High antimicrobial activity (+ + +), inhibition zone 6–9 mm. Strong antimicrobial activity (+ + + +), inhibition zone >9 mm. Standard deviation ± 0.5 mm.

Oliveira, I., Sousa, A., Ferreira, I. C. F. R., Bento, A., Estevinho, L., & Pereira, J. A. (2008). *Total phenols, antioxidant potential and antimicrobial activity of walnut (Juglans regia L.) green husks. Food and Chemical Toxicology, 46(7), 2326–2331.*

Therapeutic generalities

- All herbs here have low MIC. Does not require the use of concentrated essential oils, which may be irritating or cytotoxic to cells. May use dilutions of tinctures and teas effectively
- Soak constituents into the tissues. Use persistently.
- Include topical immune herbs to enhance resistance.
- Use broad spectrum herbs effective against both bacteria and fungi OR add “fungal specialist” herbs to antimicrobial formulas

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