Australian essential oils and plant medicines for treatment of resistant infections

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Summary

- Overview – antibiotic resistant infections
- Essential oils for superbugs
- Tea tree oil
- Other Melaleuca spp.
- Eucalyptus
- Lemon myrtle
- Lemon-scented tea tree
- Wound healing
- Oral hygiene
- Skin penetration enhancement
- Microbial biofilms
- Quorum sensing
- Medicinal honey
- Essential oil blends
- Antimicrobial plant extracts
- Indigenous Plants for Health Association (IPHA)

Antibiotic resistant infections

- According to von Radowitz (2015), 400,000 people have died from "superbugs" a figure expected to double over the next decade
- In the USA alone, according to official estimates at least 2 million people acquire antibiotic resistant infections annually, of which 23,000 people die (CDC, 2013).
- As we know the problem lies partly in the indiscriminate use of antibiotics in humans, as well as in farm animals.

Methillicin Resistant Staph. aureus

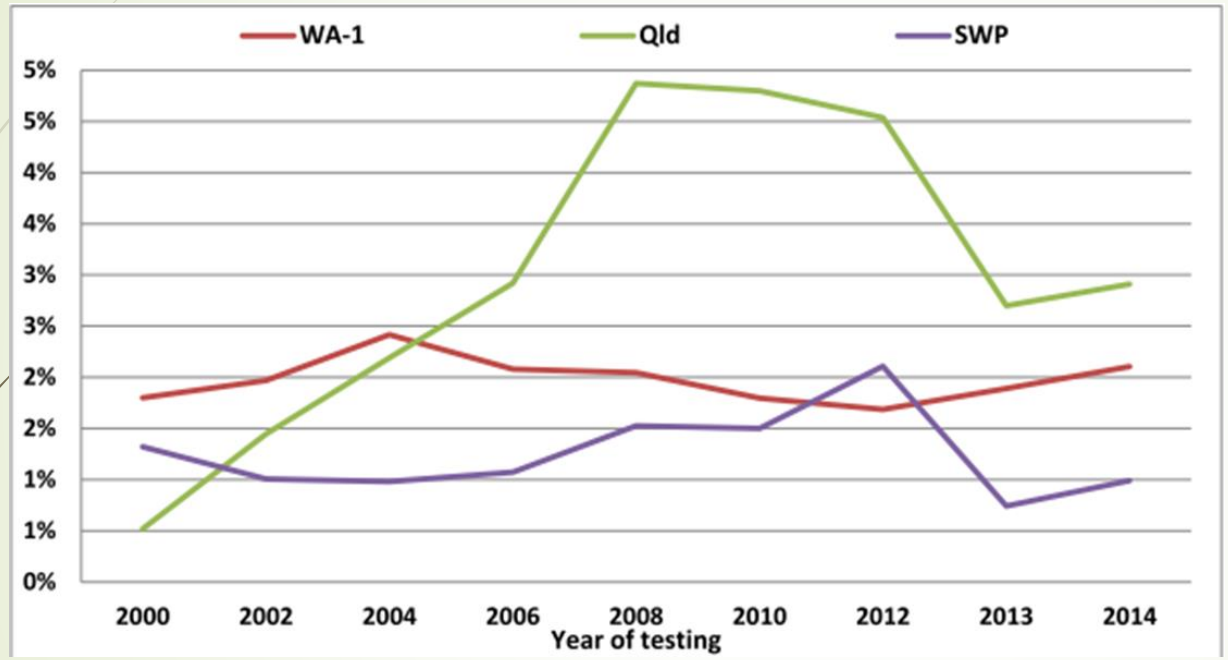
- These are clones of golden staph. which can cause invasive disease, due to the presence of virulence factors. It is a gram positive (gm+ve) bacteria

- **Type 1 – Hospital associated MRSA**
  - affects people exposed to health care environments
  - often older patients with comorbidities: pneumonia, bacteremia, invasive infections

- **Type 2 – Community associated MRSA**
  - CA-MRSA affect previously healthy younger patients and can cause skin and soft tissue infections, necrotizing pneumonia and severe sepsis.
  - CA-MRSA clones are known to be more virulent than HA-MRSA
  - Queensland clone, a Panton-Valentine leukocidin (PVL) positive MRSA, is most prevalent in Australia
  - The USA300 Clone is more prevalent in the US. Extra-virulent strain resistant to more antibiotics (Tisserand, 2015)

- **Type 3 – livestock associated MRSA**
  - 86% of pig farmers in Germany colonised with MRSA
  - Golden staph is one of the few bacteria that cross-infect between humans and animals (Junie, Jeican, Matroș, & Pandrea, 2018)

AGAR Surveys: Community-associated MRSA, trends of the three dominant clones in community-onset infections, 2000–2014; percentage of all *S. aureus*



Turnidge et. al. 2016 Australian Commission on Safety and Quality in Health Care.

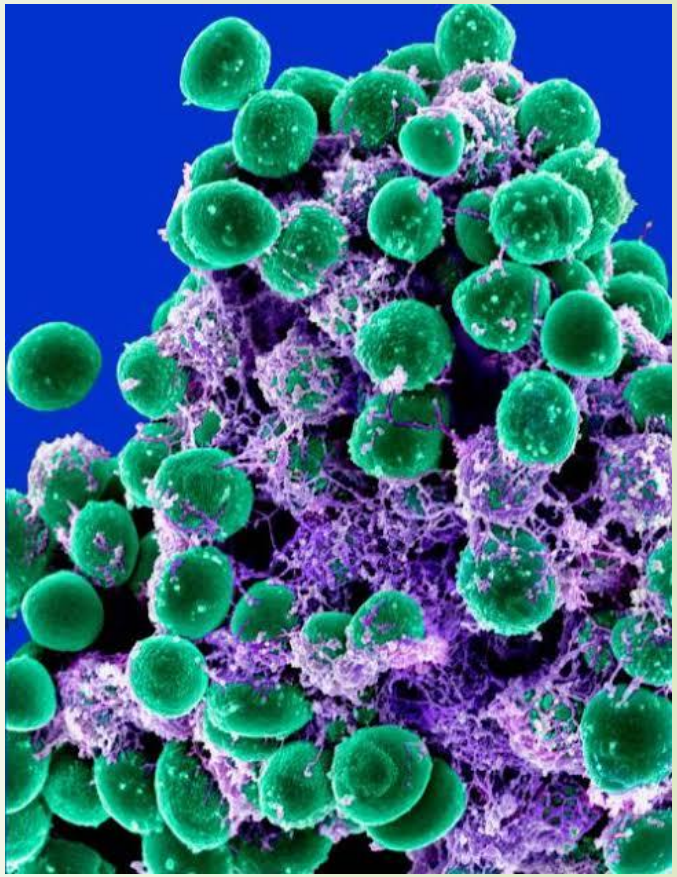
Multidrug Resistance in *S. aureus*

Antibiotic	MSSA (1930)	MRSA (1994)	Resistance mechanism
Penicillin	S	R	+ (1945)
Streptomycin	S	R	+ (1948)
Tetracycline	S	R	+ (1950)
Methicillin	S	R	+ (1961) *mecA*
Oxacillin	S	R	+
Cephalothin	S	R	+
Cefotaxime	S	R	+
Imipenem	S	R	+
Chloramphenicol	S	R	+
Ciprofloxacin	S	R	A
Clindamycin	S	R	+
Erythromycin	S	R	+
Gentamycin	S	R	+
Rifampin	S	R	A
Vancomycin	S	S	*A (1997)* VISA
Vancomycin	S	S	*+ (2002) vanA*
Teichoplanin	S	S	+
Trimeth/Sulfa	S	R	A

Tomasz, 2006

Staphylococcus epidermidis

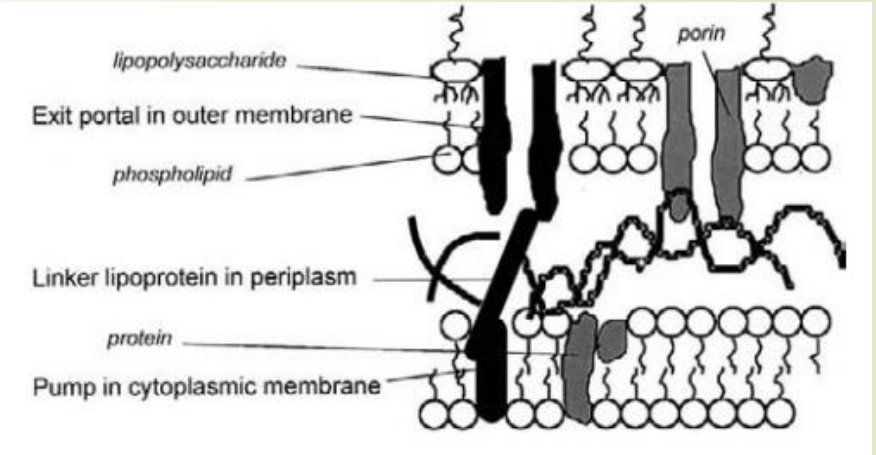
- Regarded as an important opportunistic pathogen.
- It is now the most frequent cause of nosocomial infections, at a rate about as high as its more virulent cousin *S. aureus*
- *S. epidermidis* represents the most common source of infections on medical devices, eg intravenous catheters
- *S. epidermidis* shows significant, genome-wide adaptation to the biofilm mode of growth (Otto, 2009).



Pseudomonas aeruginosa

- Gram negative (gm–ve) bacteria
- Ability to acquire resistance, via mutations, to all relevant treatments
- Multiple mechanisms for resisting antibiotic therapy
  - Cell wall impermeability
  - Three component efflux pump
  - Quorum sensing
    - regulates and coordinates population-wide group behaviors in infection processes
    - biofilm formation, secretion of virulence factors
    - Gene signaling: elastase (Las), rhamnolipid (Rhl), and Pseudomonas quinolone signal (PQS) systems

(Livermore, 2002; Tümmler, 2019)



Three component efflux pump (Livermore, 2002)

Mechanisms for multiple drug resistance

Resistance mechanism	Example
Enzymes that destroy or inactivate the antibiotic	Acetyltransferase, adenyltransferase, β-lactamases (β-lactams), phosphoryltransferases (aminoglycosides, chloramphenicol), thioltransferase (fosfomycin)
Modified target	Altered peptidoglycan cross-link (glycopeptides), altered penicillin-binding proteins (β-lactams), methylation of adenine residues prevents the antibiotic binding to the 50S ribosomal subunit (macrolides-lincosamides-streptogramins B), mutation generating a reduction on binding to active site(s) (oxazolidinones and quinolones), production of proteins that attach to the ribosome and modify the conformation of the active site (tetracyclines)
Efflux	Efflux pumps (aminoglycosides, chloramphenicol, fluoroquinolones, β-lactams, macrolides, and tetracyclines), novel membrane transporters (chloramphenicol, quinolones, and tetracyclines)
Decreased membrane permeability	Altered membrane permeability due to porin loss or other barriers (aminoglycosides, fusidic acid, quinolones)
Overproduction of the target	Excess peptidoglycan (glycopeptides), overproduction of dihydrofolate reductase (trimethoprim)

Reproduced from Faleiro & Miguel in Rai, M. & Kon, K. (eds) 2013. *Fighting multidrug resistance with herbal extracts, essential oils and their components*. Academic Press/Elsevier London P.71

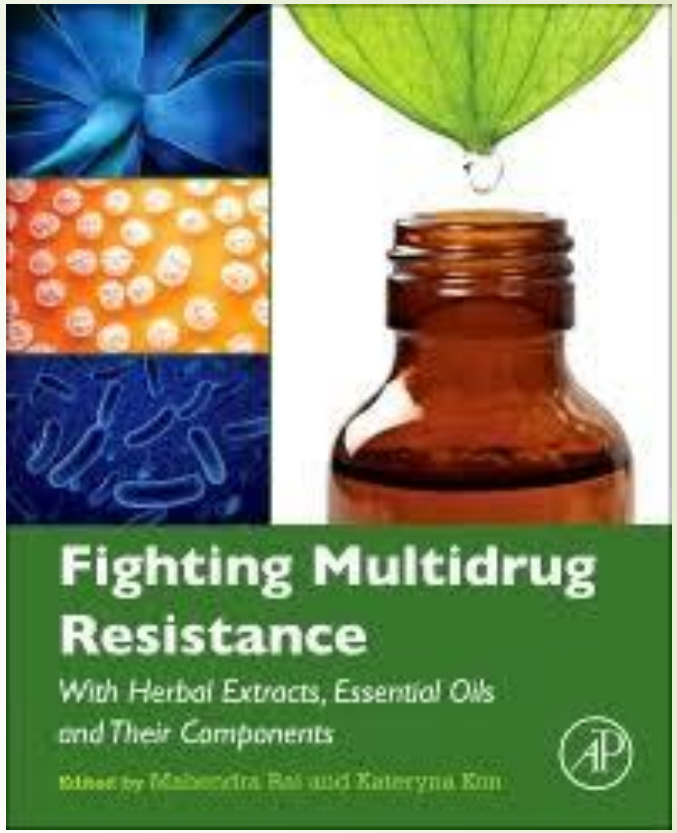
Superbugs. What can we do?

- With little incentive for developing new antibiotics, and with fewer of the currently available drugs effective, there is renewed interest in the research community for testing plant extracts and essential oils to help fill the vacuum.
- Aromatherapists and herbalists are well positioned to make an impact on the conundrum using their tried and tested treatments.
- However practitioners and patients alike should acknowledge that replacing antibiotics with herbs or essential oils is no solution in itself.
- As with all dis-ease states, wholistic approaches are required.
- According to the Australian herbalist and author Peter de Ruyter (2013), dealing with multi-drug resistance requires a paradigm shift in which lifeforce concepts are valued as part of the healing process

Alternative antimicrobial therapies

Therapy	Description
Peptide antibiotics	Ribosomally synthesized peptides produced by all organisms "nature's antibiotics"
Bacterial interference	Interactions between pro-/prebiotics and bacteria
Vaccines	Targeting bacterial proteins that bind to human extracellular matrix components (Staphylococcus)
Bacteriophage "phage"therapy	Capable of disrupting bacterial biofilms, low toxicity
Antimicrobial metals	Nanosilver particles used in wound care
Medicinal grade honey dressings	Contain Methylgloxal (MGO), however all honey has antibacterial properties
Herb and plant extracts	Multiple antimicrobial components: essential oils, resins, saponins, alkaloids etc.
Isolated phytochemicals	Higher potency, inherent safety issues
Essential oils	Rich in antimicrobial terpenoids

(Adapted from Edward-Jones, 2013)



Essential oils for superbugs

Essential oils

- Essential oils are complex natural mixtures, their main constituents, terpenes and phenylpropanoids, being responsible for their biological properties.

- Essential oils, along with resins (solid substances that include essential oils mixed with various plant acids and terpenes), have long been known to act as antimicrobial agents, a major reason for their use in embalming by Egyptians in antiquity (Faleiro & Miguel, 2013).

- Besides antibacterial and antifungal activities, essential oils have antiviral, insecticidal, anti-tumour, analgesic, anti-inflammatory and antioxidant properties

- Being complex biochemical mixtures, there may be lack of consistency in composition due in part to geographical location, climatic conditions, time of harvesting, extraction method and the presence of chemotypes within many species

Tea tree oil - *Melaleuca alternifolia*

- TTO is a traditional medicine used by the Bundjalung Aborigines of northern New South Wales. Crushed leaves of "tea trees" were inhaled to treat coughs and colds or were sprinkled on wounds, after which a poultice was applied. In addition, tea tree leaves were soaked to make an infusion to treat sore throats or skin ailments

- With a broad spectrum of antimicrobial activity against yeast and bacteria, coupled with additional properties of stability and non-irritancy, tea tree oil is regarded as the ideal medication for use in topical formulations for irritation of mucous membranes of the vagina and anal canal.

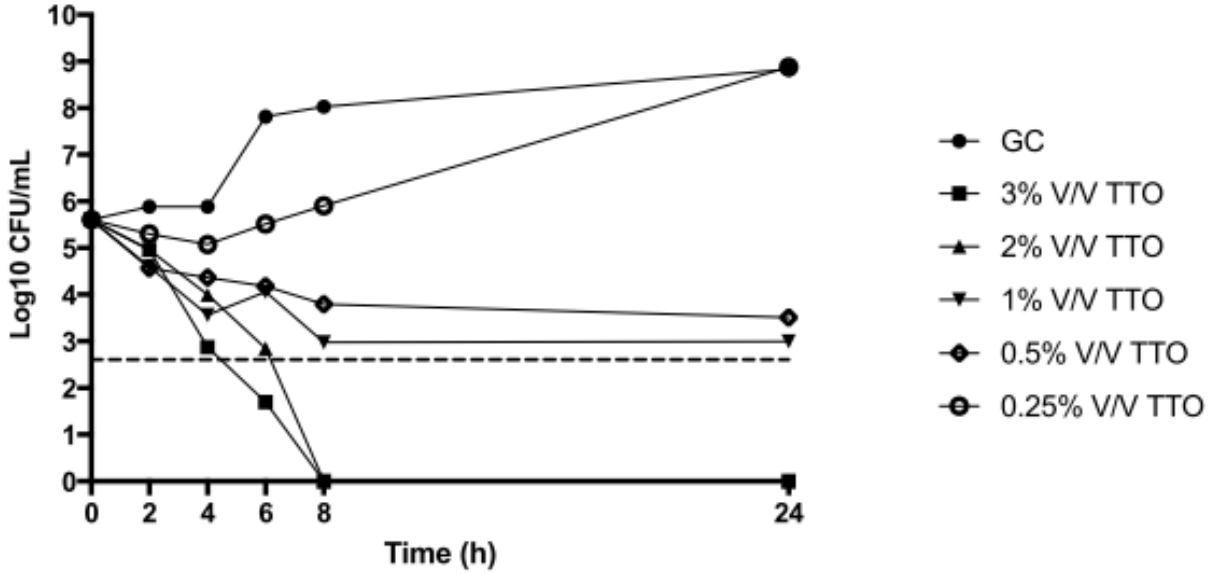
Tea tree oil and MRSA

- Carson and co-workers demonstrated significant activity against strains of MRSA that were resistant to the standard topical application mupirocin (Carson et al 1995).
- Clinical evidence for this activity was demonstrated in a 2004 UK study in which tea tree 10% cream and 5% body wash proved efficacious for treatment of skin and nasal MRSA carriage, although the effect on the nasal mucosa was less potent than mupirocin.
- Topical preparations containing TTO can be considered in regimens for eradication of methicillin-resistant *S. aureus* in hospitals (Dryden, Dailly & Crouch 2004).
- Using a standard European suspension testing procedure, 5% TTO achieved a >104 -fold reduction in *P. aeruginosa* cell numbers after a 5-min contact time. TTO (5%) in 0.001% Tween 80 was significantly more active against *E. coli* and *P. aeruginosa* than against *S. aureus* and *A. baumannii.* After a 1-min contact time, 5% TTO in 0.001% Tween 80 achieved a >104 -fold reduction in *E. coli* and *A. baumannii* cell numbers, respectively.
- The authors conclude that TTO handwashes might prove useful in hospital settings because of their efficacy in different testing conditions, and because of the increasing acceptability of TTO formulations as being less damaging to the skin than conventional handwashes (Messager, Hammer, Carsona & Riley, 2004).
- The enzyme-linked assay (ELISA) also demonstrated suppression of TNF-a production in cells activated by staph enterotoxin (Shi et al. 2016).

Tea tree oil and MRSA

Synergism with antibiotics

In agreement with the MIC/MBC data, killing studies of TTO alone against MRSA showed a concentration-dependent effect, with an absence of bacterial growth at concentration of 2%; on the other hand, only a bacteriostatic effect was noted at the concentrations of 1% and 0.5% and no activity was observed at 0.25% (Figure 2).



TTO in combination with each reference antimicrobial showed a high level of synergism at sub-inhibitory concentrations, especially with cefazolin/oxacillin/amikacin against both MSSA and MRSA

Olivia et al., 2018

Synergism – TTO and antibiotics

		Antibiotics	Bacteria tested	Effect
Melaleuca alternifolia	Myrtaceae	Tobramycin	*E. coli, S. aureus*	Synergy against both strains tested
		Ciprofloxacin	*K. pneumoniae, S. aureus*	Against *K. pneumoniae*: synergy at three ratios, antagonism at another; against *S. aureus*: antagonism at all ratios;
		Gentamicin	*A. baumannii, B. cereus, B. subtilis, E. faecalis, E. coli, K. pneumonia, P. aeruginosa, Salmonella* ser. Typhimurium, *S. marcescens, S. aureus, Y. enterocolitica*	Mostly indifferent effect

Kon & Rai, 2013

TTO and acne

- Several studies have demonstrated that TTO reduces lesion numbers in *Acne vulgaris*, equivalent to standard treatments including 5% benzoyl peroxide and 2% topical erythromycin, with low rate of adverse effects (Hammer, 2017)

- In a recent phase II pilot study of mild to moderate acne using tea tree gel and face wash, TTO provided significant improvement and well tolerated (Malhi et al. 2016).

Summary of clinical studies evaluating tea tree oil (TTO) products for the treatment of acne.

Treatment group	Trial design	Product application	Efficacy (mean reduction in total lesion count[a]) (%)	Tolerability (frequency of adverse events)	Outcomes
(1) TTO 5% gel (n = 58) (2) BP 5% (n = 61)	Double-blind[b]	Twice daily (left on) for 8 weeks	(1) 29.3 (2) 45.9	(1) 44% (2) 79%	Both treatments significantly reduced inflamed lesions, although BP better than TTO. Treatments equivalent for reducing non-inflamed lesions and erythema
(1) TTO 5% gel (n = 30) (2) Erythromycin 2% gel (n = 30)	Investigator-blind	Twice daily (left on) for 6 weeks	(1) 55 (2) 40	Rates not stated; rates for groups not significantly different	TTO significantly better than 2% erythromycin at reducing lesion numbers
(1) TTO 5% gel (n = 30) (2) Placebo (n = 30)	Double-blind	Twice daily (washed off) for 6 weeks	(1) 43.6 (2) 12.0	(1) 10% (2) 6.7%	TTO significantly better than placebo at reducing lesion numbers. Significant decrease in total lesion count and acne severity index after TTO treatment but not placebo
(1) TTO 5% gel (n = 46) (2) TTO 5% gel + Perfact tablet (n = 46) (3) Perfact tablet alone (n = 48)	Open-label	Gel applied once daily; tablets taken twice daily for 4 weeks	(1) 62.1 (2) 73.7 (3) 73.0	No serious adverse events reported	All treatments significantly reduced lesion number compared with baseline. No statistics performed comparing all groups
(1) TTO 5% extract (n = 34) (2) LFCO 5% extract (n = 34)	Double-blind	Twice daily for 8 weeks	(1) 38.2[c] (2) 65.3	(1) 31.3% (2) 12.6%	Inflammatory lesions significantly reduced by both treatments; LFCO better than TTO. LFCO also reduced non-inflammatory lesions
(1) Baseline + mixture of TTO 3% and lavender oil 2% (n = 27) (2) Baseline only (n = 27)	Not stated	Oils applied twice daily (washed off) for 4 weeks. Baseline not stated	(1) 9.2 (2) 4.8	(1) 3.7% (2) 0%	Numbers of inflammatory lesions significantly reduced compared with baseline
TTO 0.1% + *Ramulus mori* extract 0.01% (n = 20)	Case-controlled	4 Weeks	28.7	Not stated in English abstract	Numbers of inflammatory lesions significantly reduced compared with baseline

BP, benzoyl peroxide; LFCO, *Lactobacillus* fermented *Chamaecyparis obtusa*.

Several studies have shown that application of TTO products reduces the number of lesions in those with mild-to-moderate acne.

Comparative trials showed that TTO products were better than placebo and were equivalent to comparators including 5% benzoyl peroxide and 2% topical erythromycin.

Hammer, 2015

Tea tree oil - Mechanisms of action

- Antibacterial activity of TTO has been attributed to increased membrane permeability induced by the main active constituent terpinen-4-ol, leading to leaking of potassium ions. Loss of intracellular ions disrupts cellular homoeostasis, inhibits respiration and metabolic processes within cells (Cox et al., 2000; Jones, 2015)

- Production of extracellular vesicles, inhibition of cellular respiration (Faliero & Miguel, 2013)

- Pathology from golden staph is due to the release of virulence factors (a-hemolysin) and endotoxins, TTO was shown to inhibit a-hemolysin and enterotoxins A & B in vitro

- TTO also inhibits growth of *C. albicans* by increasing the permeability of cells walls (Carson et al 2006)

- *Pseudomonus aeruginosa*, a gm-ve pathogenic bacteria, has increased resistance to TTO, by virtue of reduced outer membrane permeability and active efflux systems (Longbottom et al., 2004)

Other *Melaleuca* species

- The oils of several other species are also strongly bactericidal:
  - *Melaleuca quinquenervia* (encompassing the nerolidol, linalool and 1,8-cineole chemovars) (Siddique et al. 2018)
  - *Melaleuca ericifolia* (Wilkinson and Cavanagh, 2005).
  - *Melaleuca teretifolia* - citral and 1,8-cineole rich oils in two novel chemovars of in Western Australia (Southwell et al. 2003)

Paperbarks

M. ericifolia

M. teretifolia

M. quinquenervia

Eucalyptus

- Although lacking the broad-spectrum antimicrobial actions of TTO, *Eucalyptus* essential oils have been shown to inhibit both gm+ve (*S. aureus*) and gm–ve (*E. coli*) bacterial strains (Salehi et al., 2019)
- In a pulmonary tuberculosis case study following inhalation of oil distilled from *E. globulus,* the patient was asymptomatic and the sputum culture TB negative (Sherry & Warnke, 2004).
- While cineole may not be one of the primary antimicrobial components, it may permeabilize bacterial membranes and facilitate the entry of other, more active components (Otto, 2009).
- A high cineole chemotype of *E. radiata* was shown to inhibit gm+ve multi-drug resistant pathogens (Mulyaningsih et al. 2011).
- *E. stageriana* has a rich lemon odour due to a high content of citral. In one study this oil was found to inhibit all microorganisms for which it was evaluated, at a potency four times that of a standard antibiotic (Barbosa, Filomeno & Teixeira, 2016).

Eucalyptus globulus

The antimicrobial activity of the essential oils of *Eucalyptus* spp. against multidrug-resistant bacteria.

	ECL		ERL		EGL		EGF[§]	
Microorganisms	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive								
MRSA								
MRSA NCTC 10442*	2	4	4	>4	4	>4	0.25	0.06
USA300	2	4	>4	NT	>4	NT	0.5	1
1678/98	4	>4	>4	NT	>4	NT	0.125	0.5
635/93	2	4	>4	NT	4	>4	0.25	0.25
2387/00	2	4	4	>4	4	>4	0.12	0.12
1000/93	1	2	4	>4	2	4	0.12	0.25
BL7127/98	4	>4	>4	NT	4	>4	0.5	0.5
MR131/98	4	>4	>4	NT	>4	NT	0.5	0.5
MR134/93	>4	NT	>4	NT	4	>4	0.5	2
MR1150/93	>4	NT	>4	NT	>4	NT	1	2
VRE								
VRE *E. faecalis* ATCC 51299*	>4	NT	>4	NT	>4	NT	1	2
VR902291	>4	NT	>4	NT	>4	NT	0.5	0.5
VR902316	>4	NT	>4	NT	>4	NT	0.5	0.5
VR902247	>4	NT	>4	NT	>4	NT	1	2
VR902267	>4	NT	>4	NT	>4	NT	1	2
Gram-negative								
*Escherichia coli**	>4	NT	>4	NT	>4	NT	8	NA
*Pseudomonas aeruginosa**	>4	NT	>4	NT	>4	NT	>8	NT
*Klebsiella pneumonia**	>4	NT	>4	NT	>4	NT	>8	NT
*Acinetobacter baumannii**	2	2	1	1	2	2	1	1

EGF, *Eucalyptus globulus* fruits; EGL, *E. globulus* leaves; ECL, *E. citriodora* leaves; ERL, *E. radiata* leaves; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration.

(Mulyaningsih, Sporer, Reichling & Wink, 2019)

Eucalyptus EOs active against MDR bacteria, their constituents, bacterial targets and MIC

Eucalyptus globulus (Tasmanian bluegum)	Fruit oil: aromadendrene 31.17, 1,8-cineole 14.55, globulol 10.69	MRSA; VRE; *Escherichia coli*; *Pseudomonas aeruginosa*; *Klebsiella pneumoniae*; *Acinetobacter baumannii*	0.25–1 mg/mL; 0.25–1 mg/mL; 8 mg/mL; > 8 mg/mL; > 8 mg/mL; 1 mg/mL
	Leaf oil: 1,8-cineole 86.51, α-pinene 4.74, γ-terpinene 2.57	MRSA; VRE; *E. coli*; *P. aeruginosa*; *K. pneumoniae*; *A. baumannii*	2–> 4 mg/mL; > 4 mg/mL; > 4 mg/mL; > 4 mg/mL; > 4 mg/mL; 2 mg/mL
Eucalyptus radiata (Narrow-leaf peppermint gum)	1,8-cineole 82.66, α-pinene 3.68, α-terpineol 7.03	MRSA; VRE; *E. coli*; *P. aeruginosa*; *K. pneumoniae*; *A. baumannii*	4–> 4 mg/mL; > 4 mg/mL; > 4 mg/mL; > 4 mg/mL; > 4 mg/mL; 1 mg/mL
Eucalyptus citriodora (Lemon-scented gum)	Citronellal 90.07, citronellol 4.32, β-caryophyllene 1.46	MRSA; VRE; *E. coli*; *P. aeruginosa*; *K. pneumoniae*; *A. baumannii*	2–> 4 mg/mL; > 4 mg/mL; > 4 mg/mL; > 4 mg/mL; > 4 mg/mL; 2 mg/mL

From Faleiro & Miguel in Rai, M. & Kon, K. (eds) 2013. *Fighting multidrug resistance with herbal extracts, essential oils and their components*. Academic Press/Elsevier London P.71

Corymbia citriodora – lemon-scented gum

- The antimicrobial activity of *C. citriodora* essential oil against twelve bacteria and two yeasts was determined via both the disc diffusion method and the disc volatilization method. Higher antimicrobial activity was observed in the liquid phase.

- The minimum inhibitory concentration (MIC) was found to vary from 0.6 to 20 µl/ml for bacteria while for yeasts it was equal to 1.25 µl/ml. The most sensitive strains were the gm+ve *Staphylococcus epidermidis* and the two yeasts, while the most resistant was *Pseudonomas aeruginosa* (Tolba, Moghrani , Aboun & Maachi 2018)

- Silver nanoparticles synthesized with the ethanol leaf extract of *E. citriodora* have been reported as having a significant antibacterial activity against clinically multidrug-resistant (MDR) *Acinetobacter baumannii* isolated from pneumonia patients (Salehi et al., 2019)
  - *A. baumannii* is a gm–ve bacteria (coccobacillus) causing hospital acquired infections. It has been linked to wound and urinary tract infections, pneumonia and septicemia.

Eucalyptus extracts

- Extracts of *E. globulus, C. maculata* and *E. viminalis* demonstrated significant inhibition of seven micro-organisms that cause food poisoning, acne and athlete's foot including MRSA, but with no inhibition of gm-ve bacteria.
  - The antimicrobial constituents were found to include the chalcone and two flavonoids based on eucalyptin (Takahashi, 2004).
- Standardized extract (high in 1-8 cineole) Myrtol® has been shown effective for treating respiratory infections, including sinusitis, bronchitis and COPD
- The production of silver nanoparticles (AgNPs) by using leaf extract of *E. camaldulensis* has revealed promising cytotocic effects against gm-ve (*P. aeruginosa* and *E. coli*) and gm+ve (*S. aureus and Bacillus subtilis*) bacteria (Salehi et al., 2019).
- *E. tereticornis* showed profound antimicrobial activity, including against multi-drug resistant *Escherichia coli* and *Staphylococcus aureus (*Maji, Dandapat, Ojha et al., 2010)
- Freeze-dried aqueous leaves of tallow wood (*E. microcorys*) demonstrated significant antimiocrobial action against gm+ve and gm-ve pathogenic bacteria comparable to the antibiotic ciprofloxacin, as well as potent inmhibition of three species of fungal pathogens (Bhuyan et al., 2017)

Eucalyptus camaldulensis

Eucalyptus microcorys

Backhousia citriodora – lemon myrtle

- The composition of the leaf oil is 90-95% citral, with some variable presence of citronellal, myrcene, methyl heptenone, linalol, and α- and β-cyclocitrals.
- Citral is a potent antimicrobial and sedative, a useful agent in viral, bacterial and fungal infections.
- In one study the *B. citriodora* essential oil and a leaf paste were found to inhibit growth of bacteria and fungi, including human pathogens *Clostridium*, *Pseudomonas* and a hospital isolate of methicillin resistant *S. aureus* (MRSA). Interestingly, the essential oil was more potent than citral alone (Wilkinson et al, 2003).
- Methanolic extracts of *B. citriodora* demonstrated potent antibacterial and antifungal effects using a disc diffusion and growth time course assay. These findings established the susceptibilities of a broad range of microbes to *B. citriodora* - Both gm+ve and gm -ve were equally susceptible, though weaker than the reference antibiotics tested (Cock, 2013)
- In addition to being an effective antibacterial agent, lemon myrtle EO is an excellent antifungal agent. This indicates the potential of using lemon myrtle EO as a surface disinfectant and as a natural preservative in the food industry (Sultanbawa, 2016).

http://www.anbg.gov.au/gnp/gnp14/backhousia-citriodora.html

Antimicrobial effects compares favourably with tea-tree oil

(Hayes & Markovich 2002)

MIC values (as %, v/v) for lemon myrtle oil and tea tree oil (Hayes & Markovitch, 2002)

Organisms	Lemon myrtle oil	Tea tree oil	Citral
S. aureus	0.05	0.2	0.03
E. coli	0.03	0.2	0.03
P. aeruginosa	2.0	>2.0	2.0
C. albicans	0.03	0.2	0.03
A. niger	0.1	0.4	0.1
MRSA	0.2	0.3	0.2
K. pneumoniae	0.2	0.3	0.2

Leptospermum petersonii – lemon-scented tea tree

- The common chemotypes of this EO are rich in antimicrobial citrus aldehydes
- Most research focused on antifungal activity:
  - *L. petersonii* eo. (CT1) produced 100% inhibition of 3 species of Aspergillus, while several species of Eucalyptus and Melaleuca provided little or no inhibition. The most active constituents were neral and geranial (Kim & Park, 2012).
  - In vivo study: Animals with aspergillosis disease induced by exposure to *Aspergillus fumigatus* L. were treated with oil. Animals that completed the treatment had no trace of fungal infection, and no adverse effects were observed.
  - Conclusions: The significant reduction in fungal burden in the lungs of infected animals by the volatiles of *L. petersonii* oil was larger than that reported for conventional antifungal drugs of choice. (Hood, Burton, Wilkinson & Cavanagh 2010).
  - Antifungal activity against dermatophytes: *Microsporum, Trichophyton, Epidermophyton* (Williams, 2011)
- "*L. petersonii* displays noteworthy antibacterial activity, and it remains a mystery why this species has been neglected in the scientific literature". (Van Vuuren, Docrat, Kamatou & Viljoen, 2014)
- May be used to enhance the antimicrobial action of Calendula and Hypericum infused oils

Essential oil profiles for 5 chemotypes

Constituent	CT 1	CT2	CT3	CT4	CT5
Neral	31.3	13.5		0.5	
Geranial	45.4	22.8		0.3	
Citronellal	6.8	46.2			
∂-Terpineol	31.3	13.5		0.5	
Nerol	0.7	0.2			38.3
Geraniol	2.7	2.4	4.8		21.2
Terpinolene				17.6	7.3
a-Pinene	12.3	0.1	0.1	9.6	0.6
Terpinene				26.5	11.5

Antimicrobial synergism *Leptospermum, Kunzea* EOs

Antimicrobial activity (mean MIC expressed in mg/ml) of *L. petersonii*, *L. scoparium* and *K. ericoides* essential oils.

Test Organism	*L. petersonii*	*L. scoparium*	*K. ericoides*	Control[a]
Staphylococcus aureus ATCC 12600	4.00	4.00	8.00	$6.250\ e^{-4}$
Staphylococcus epidermidis ATCC 2223	**2.00**	4.00	8.00	$1.563\ e^{-4}$
Mycobacterium smegmatis ATCC 14468	**1.50**	**2.00**	**2.00**	$3.906\ e^{-5}$
Enterococcus faecalis ATCC 29212	8.00	4.00	12.00	$6.250\ e^{-4}$
Streptococcus pyogenes ATCC 8668	**0.50**	**1.00**	**2.00**	$1.563\ e^{-3}$
Streptococcus agalactiae ATCC 55618	**2.00**	**0.50**	**2.00**	$1.563\ e^{-3}$
Streptococcus pneumoniae ATCC 49247	**2.00**	8.00	8.00	$7.813\ e^{-4}$
Brevibacterium brevis ATCC 8246	**1.00**	**1.00**	**1.00**	$1.563\ e^{-4}$
Brevibacterium agri ATCC 51663	**0.06**	**0.06**	**1.00**	$1.563\ e^{-4}$
Brevibacterium laterosporum ATCC 64	**0.25**	**0.25**	**1.00**	$1.563\ e^{-4}$
Propionibacterium acnes ATCC 11827	**1.00**	**1.00**	4.00	$6.25\ e^{-4}$
Klebsiella pneumoniae ATCC 13883	8.00	8.00	8.00	$7.813\ e^{-5}$
Pseudomonas aeruginosa ATCC 9027	4.00	4.00	4.00	$1.563\ e^{-4}$
Moraxella catarrhalis ATCC 23246	4.00	**2.00**	8.00	$3.125\ e^{-4}$
Cryptococcus neoformans ATCC 90112	**1.00**	**1.00**	**1.00**	$3.125\ e^{-3}$
Candida albicans ATCC 10231	**2.00**	8.00	4.00	$3.125\ e^{-3}$

Noteworthy activity is given in bold.

[a] Ciprofloxacin was used as the control for bacteria and amphotericin B for the yeasts.

Van Vuuren, Docrat, Kamatou & Viljoen, 2014)

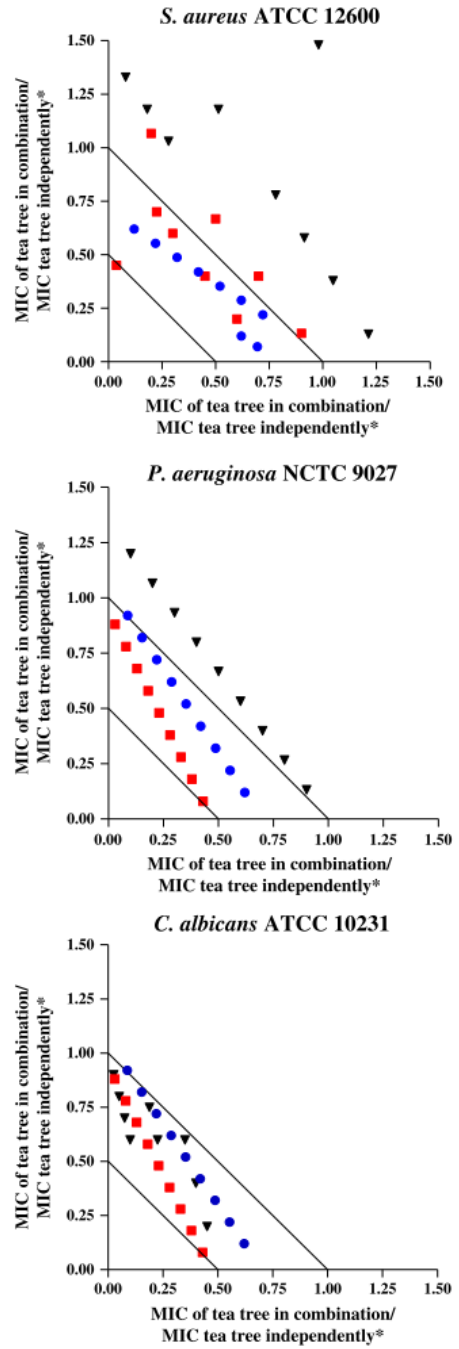


Fig. 1. Isobolograms representing interactions of tea tree oils where * = ▼ is the combir tion of *L. petersonii* with *L. scoparium*; ● is the combination of *L. petersonii* with *K. ericoic* and ■ is the combination of *L. scoparium* with *K. ericoides*.

Antiviral essential oils

HIV

- Essential oils from eucalyptus, tea tree, thyme and their major monoterpene compounds: a-terpinene, g-terpinene, a-pinene, p-cymene, terpinen-4-ol, a-terpineol, thymol, citral and 1,8-cineole, were examined for their antiviral activity against herpes simplex virus type 1 (HSV-1) in vitro. These essential oils were able to reduce viral infectivity by >96%, the monoterpenes inhibited HSV by about 80%.
  - Both the essential oils and monoterpenes exhibited high anti-HSV-1 activity by direct inactivation of free virus particles. All tested drugs interacted in a dose-dependent manner with herpesvirus particles thereby inactivating viral infection.
  - Among the analysed compounds, monoterpene hydrocarbons were slightly superior to monoterpene alcohols in their antiviral activity, a-pinene and a-terpineol revealed the highest selectivity index. However, mixtures of different monoterpenes present in natural tea tree essential oil revealed a **ten-fold higher selectivity index and a lower toxicity** than its isolated single monoterpenes (Astani, Reichling, & Schnitzler, 2010).
- In Japan Hirobe and co-workers demonstrated antiviral effects for *B. citriodora* leaf capsules against HIV and Cytomegalovirus (Archer, 2004).

Influenza

- The development of an effective and less toxic anti-influenza virus drug is urgently needed due to continual appearance of drug-resistant mutants.
- The essential oil from *E. globulus* have shown a significant virucidal activity against influenza virus following exposures to oil vapors over only 10 min. In addition, this activity was observed without measurable adverse effect on the epithelial cell monolayers (Salehi et al., 2019)

Herpes

- Externally *C. citriodora* is used for *Herpes* lesions and applied to the skin as an insect repellant. It may be used as a simple infusion or a 1:5 tincture from crushed dried leaves, with a dose of 1-4mls.

Wound Healing and skin infections

- In a small investigative study TTO assists with the accelerated healing of abscessed wounds and cellulitis. TTO appears to be a safe complementary modality in the treatment of these common infections (Chin & Cordell, 2013).

- TTO also shows promise as a topical anti-inflammatory which, combined with its strong topical bactericidal and fungicidal activity, recommended as an aid to accelerated wound healing (Hart et al., 2000; Brand et al., 2001; Koh et al., 2002).

- Reports suggest that repeated use of formulations containing TTO does not lead to dermatological problems, nor affect the original protective bacterial flora of the skin (Carson and Riley, 1995)

- The antibacterial activity of some skin-wash formulas containing TTO as well as pure TTO was evaluated against *Staphylococcus aureus, Acinetobacter baumannii, Escherichia coli and Pseudomonas aeruginosa.* "Our findings suggest that TTO-containing handwash formulations may help reduce the skin carriage of potentially pathogenic organisms by healthcare staff and thus reduce transmission of nosocomial infections in hospital settings" (Messager et al., 2005).

- Results of one study revealed that TTO has potential to be incorporated into chitosan to make films for wound-healing applications (Dos Santos et al., 2019)

Oral hygiene

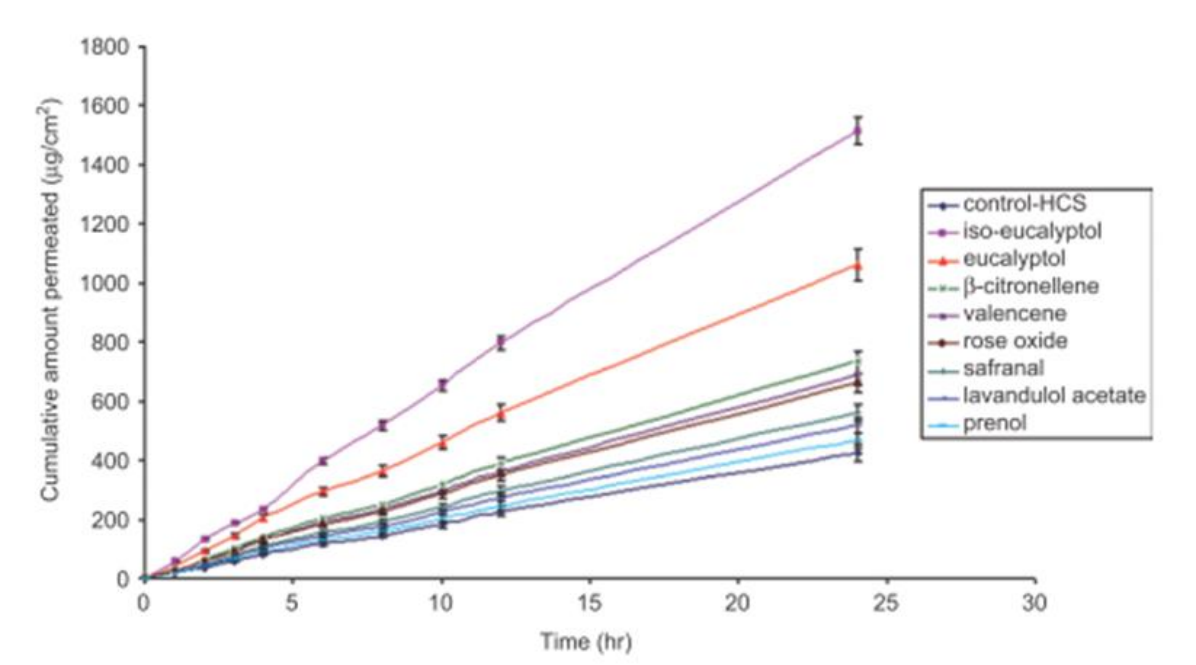
- Bacterial communities inhabit the oral cavity in a symbiotic manner and form dental biofilm. However, excessive formation of biofilm in combination with immune response leads to intra-oral diseases, such as dental caries, gingivitis, and periodontitis (Kriebel et al., 2018)

- In a study by Warnke et al, all tested essential oils and oil mixtures showed good to moderate antibacterial and antimycotic effects against the tested strains. Inhibition zones of 6 mm to 49 mm were obtained.
- Among the pure oils the greatest inhibition zones were obtained for lemongrass oil (up to 49 mm) and thyme oil (up to over 30 mm) with reference to all the tested strains.
- Of the oil mixtures, KMPT (Eucalyptus oil based) showed the greatest inhibition zones with reference to Staph. and Candida strains. In the tests with streptococci, obvious inhibition zones for KMPT and Salviathymol were an expression of their good antimicrobial activity.
- Even the tested resistant strains, such as MRSA and *Candida krusei*, reacted sensitively to the essential oils and oil mixtures, as confirmed by the obvious inhibition zones. (Warnke et al., 2009)

Skin penetration enhancers

- Essential oils and their terpene constituents may be acceptable natural alternatives to synthetic skin penetration enhancers.
- Linalool oxide and pinene interfered with the stratus corneum in human melanoma cells, though via different mechanisms (Vaddi, Ho, Chabn & Chan, 2003)
- Eucalyptus oil is an effective skin-penetration enhancer, it may also yield additive antibacterial actions in combination with other skin antiseptics.
- 1,8-cineole temporarily disrupts intercellular lipids, allowing entry into the skin of otherwise poorly penetrable substances (Hendry et al., 2009)
- Due to the popularity of these essential oils, their safety is well documented, and found to be of relatively low toxicity compared with most synthetic penetration enhancers.

Skin penetration enhancers



Permeation profile of valsartan across HCS in the absence and presence of various terpenes (1% w/v) in vehicle.

Iso-eucalyptol has been found to be an effective penetration enhancer for diffusion of valsartan, a lipophilic drug through rat skin.

Ahad, Aqil, Kohli, Sultana, Mujeeb & Ali, 2011.

Nerolidol – antimicrobial, skin-penetration enhancer

Antibacterial activity

Staphylococcus aureus FDA 209P, 14 strains of methicillin-susceptible *S. aureus* (MSSA) and 20 strains of methicillin-resistant *S. aureus* (MRSA)	Broth-dilution with shaking method (BDS)	Exhibited dose-related inhibition against 34 clinical isolates of *S. aureus*. Inhibitory dose 50% (ID_{50}) ranged from 5.0 to 22.0 μg/mL and from 2.6 to 10.6 μg/mL against MSSA and MRSA respectively.	Suggested the aliphatic chain of nerolidol mediates the antibacterial activity by damaging the bacterial cell membrane
Staphylococcus aureus FDA209P	Broth dilution with shaking (BDS) method and quantitation of the leakage of K^+ ions using K^+-selective electrode	Treatment of nerolidol caused a dose-dependent increase in amount of K^+ ions leakage from bacterial cells.	Mediates the antibacterial activity via cell membrane-distrupting mechanism and hence resulting in the leakage of K^+ ions from bacterial cells
Staphylococcus aureus FDA209P	Broth dilution with shaking (BDS) method and quantitation of the leakage of K^+ ions using K^+-selective electrode	(i) Caused a dose-dependent increase in K+ ions leakage from bacterial cells (ii) Exhibited minimum inhibitory concentration at 40 μg/mL	
Staphylococcus aureus ATCC 6538	Broth microdilution method (MIC)	(i) Exhibited anti-microbial activity with MIC ranged from 125–500 μg/mL	-
Staphylococcus aureus and *Streptococcus mutans*	Broth dilution method	Exhibited antibacterial activity against *S. aureus* and *S. mutans* with MIC measured at 200 and 25 μg/mL respectively	-
Salmonella enterica, *Staphylococcus aureus* and *Aspergillus niger*	Disc-diffusion and broth dilution methods	(i) Exhibited antibacterial activity against *S. enterica*, *S. aureus* and *A. niger* with MIC, MBC and MFC values measured ranging from 3.9–15.6 μg/mL, 31.3–62.5 μg/mL and 62.5 μg/mL respectively	-
Escherichia coli and *Staphylococcus aureus*	Agar-disc diffusion assay	Nerolidol (*cis*-nerolidol and the racemic mixture of *cis*- and *trans*-isomers) potentiated the action of antibiotics: (i) amoxicillin/clavulanic acid against *S. aureus* and (ii) amoxicilline/clavulanic acid, ceftadizine and imipenem against *E. coli*	-
Escherichia coli ATCC 25922 and *Staphylococcus aureus*	Disc-diffusion assay	(i) Nerolidol concentrations ranged from 0.5 to 2 mM enhanced the susceptibility of *S. aureus* to ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline, and vancomycin (ii) Nerolidol (1 mM) enhanced the susceptibility of *E. coli* to polymyxin B	-

Chan et al., 2018

Meleleuca quinquenervia

Skin penetration activity

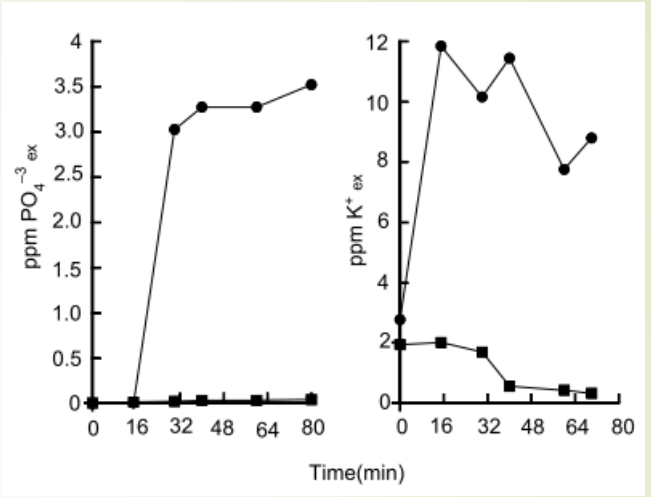
In vitro diffusion studies and stratum corneum-water partitioning studies	Increased diffusion rate by over 20-fold for transdermal delivery of drugs such as 5-fluorouracil	Nerolidol exhibits a chemical structure that allows it to align within the lipid lamellae of the stratum corneum in order to disrupt the organization of stratum corneum
Solubility studies, *ex vivo* permeation studies and histopathological studies	The enhancement effect is increased with the increasing lipophilicity; the rank of order (nerolidol > farnesol > limonene > linalool > geraniol > carvone > fenchone > menthol) in facilitating transdermal delivery of alfuzosin hydrochloride	
In vitro permeation studies	Exhibited the highest permeation enhancing ability with a 3.2-fold increase in permeation of selegiline hydrochloride across the rat skin, followed by the effect of carvone (2.8-fold increase) and anethole (2.6-fold increase)	-
In vitro skin permeability studies	Most effective terpene enhancer for percutaneous permeation of four different drug models (nicardipine hydrochloride, hydrocortisone, carbamazepine, and tamoxifen) when compared to fenchone, thymol and limonene	-

Anti-biofilm activity

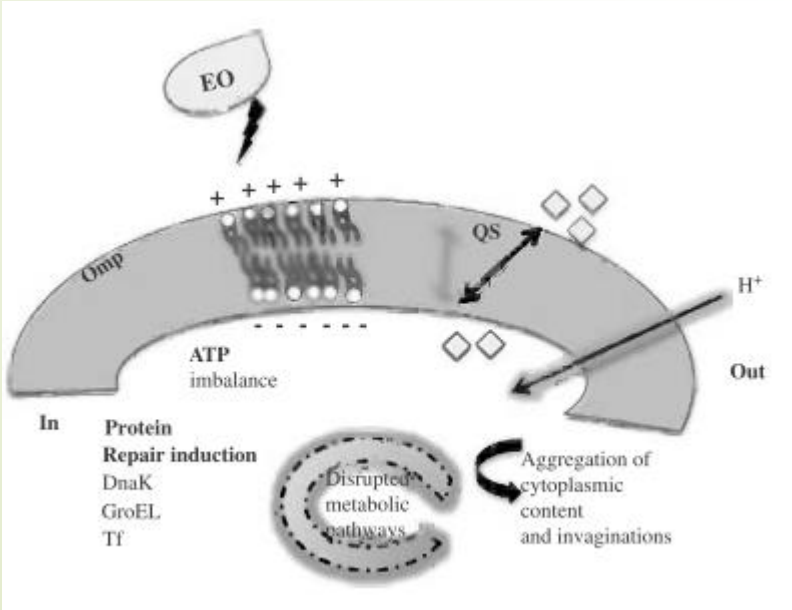
Staphylococcus aureus	Crystal violet biofilm assay	*Cis*-nerolidol at 0.01% (*v*/*v*) inhibited *S. aureus* biofilm formation by > 80 %; *trans*-nerolidol at similar concentration exerted 45% inhibition	-
Candida albicans	MTT assay	Concentrations of 0.06%–1.0% inhibited biofilm formation by 30% and 50% after 24 and 48 h incubation respectively	-
Candida albicans	MTT assay	1.0% of *cis*,*trans*-nerolidol exerted 76.1% reduction in the viability of pre-formed biofilm while only 67.0% reduction observed from 1.0% *cis*-nerolidol	-

Essential oils – antimicrobial mechanisms

Addition of oregano essential oil and the major constituents thymol and carvacrol to cultures of *P.aeruginosa* and *S. aureus* increased permeability, leading to significant leakage of potassium and phosphate ions, protons and amino acids.
Reduction of pH within cytoplasmic cells of the bacteria was observed, as well as depletion of intracellular ATP pools (Lambert et al., 2001)



Extracellular concentration of k and phosphate ions in *P. aeruginosa*, without and with oregano essential oil (Lambert et al., 2001)

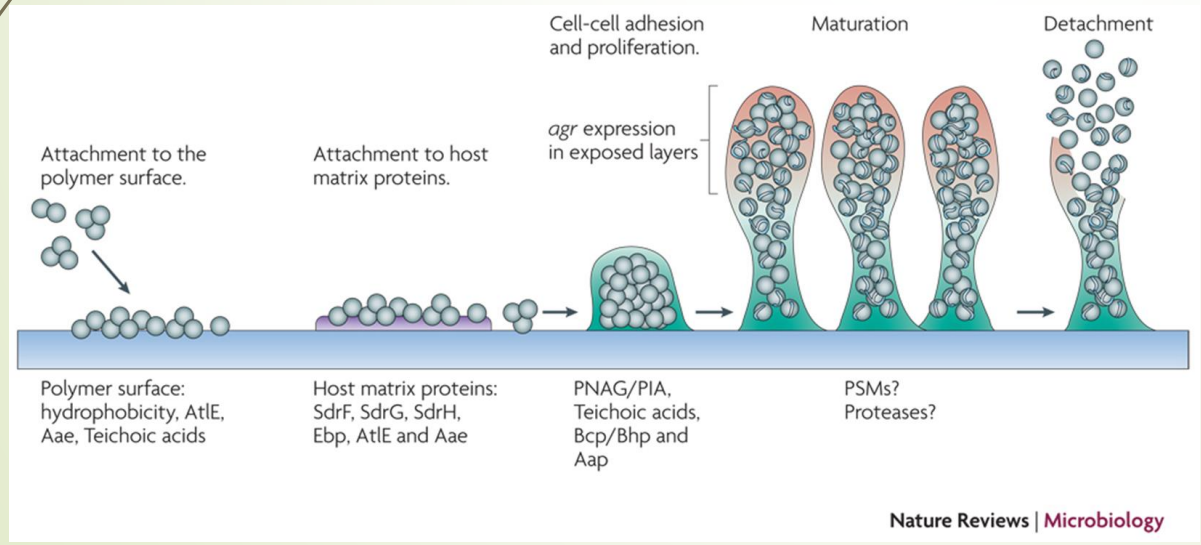


Bacterial cell structures and cellular processes disrupted by the activity of essential oils or their components. Essential oil-treated cells are more permeable to protons, experience an ATP imbalance, Metabolic pathways can be affected.

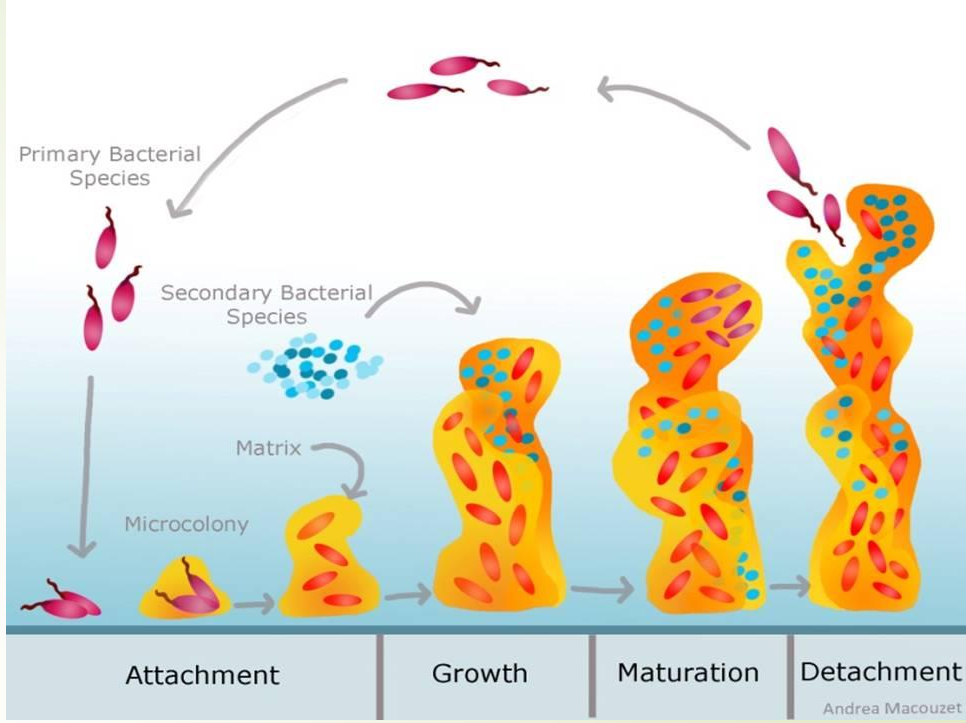
Image from Faleiro & Miguel in Rai, M. & Kon, K. (eds) 2013. *Fighting multidrug resistance with herbal extracts, essential oils and their components*. Academic Press/Elsevier London P.71

Microbial biofilms

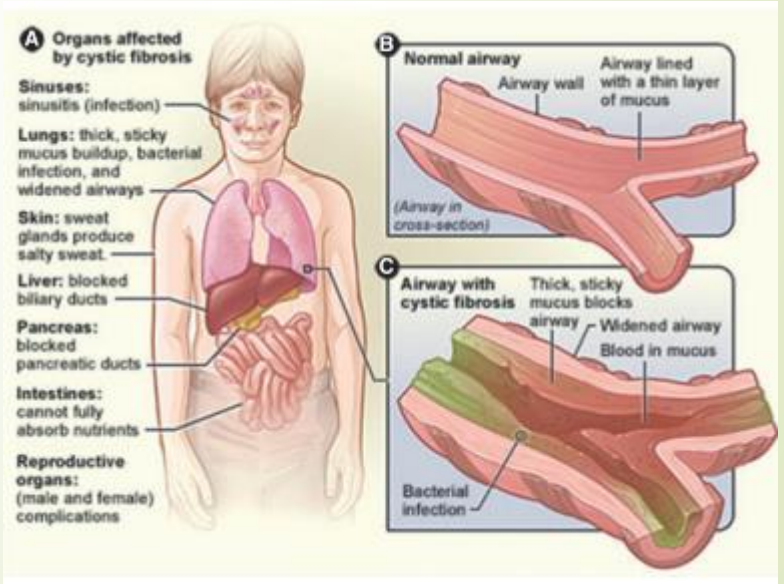
- Biofilms are complex microbial communities where the microorganisms are linked on a surface, wrapped by a polymeric matrix of exopolysaccharides (EPS). The biofilm structure, as well as the communication mechanisms between microorganisms (quorum sensing), makes treatment more difficult.
- Bacteria can adhere to cells and tissues as well as to solid surfaces (e.g. floor or equipment found at a farm, slaughterhouse, or processing plant)



Biofilm development in *S. epidermidis* (Otto, 2009)



http://www.cresa.cat/blogs/sociedad/en/espanol-biofilms-bacterianos-por-que-deberia-importarnos/



Biofilm in Cystic fibrosis (Bjarnsholt, 2013)

Essential oils and Biofilm strategies

- *M. alternifolia* nanoparticles were tested against *Candida* species biofilms. This study revealed that TTO nanoparticles increase the antimicrobial activity of TTO, and reduce the biofilm formation of many *Candida* species, enabling and making it a viable alternative against biofilm infections (Souza et. 2017)

- Nanoemulsions containing *E. globulus* oil have been reported to possess antimicrobial and antibiofilm activities against the gm-ve bacterium commonly found in immunocompromised patients *(P. aeruginosa* and *C. albicans)*

- Hendry et al. demonstrated synergistic interactions between high 1,8-cineole eucalyptus oil and the disinfectant chlorhexidine digluconate against gm+ve and gm–ve drug resistant microorganisms, including those grown in biofilm cultures (Hendry et al., 2009).

- Eucalyptus EO, TTO and thymol have demonstrated their potential synergistic activities in combination with chlorhexidine digluconate against biofilms of different *S. epidermis* strains (Solorzano-Santos & Miranda-Novales, 2012)

- Application of 4% w/v TTO in vivo resulted in no change to the total microbial load of diabetic foot ulcers complicated by biofilm. The authors concluded wound solutions should not be used as a sole therapy and clinicians should consider multifaceted strategies that include sharp debridement as the gold standard (Johani et al. 2017)

Nanoparticles

Nanoparticles are natural or synthetic particles that are measured in nanometres

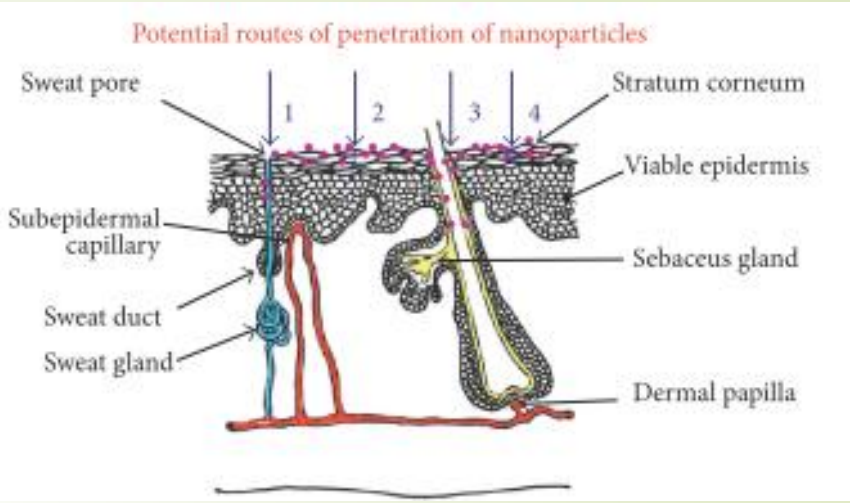
- 1 nm = 1cm/1 million

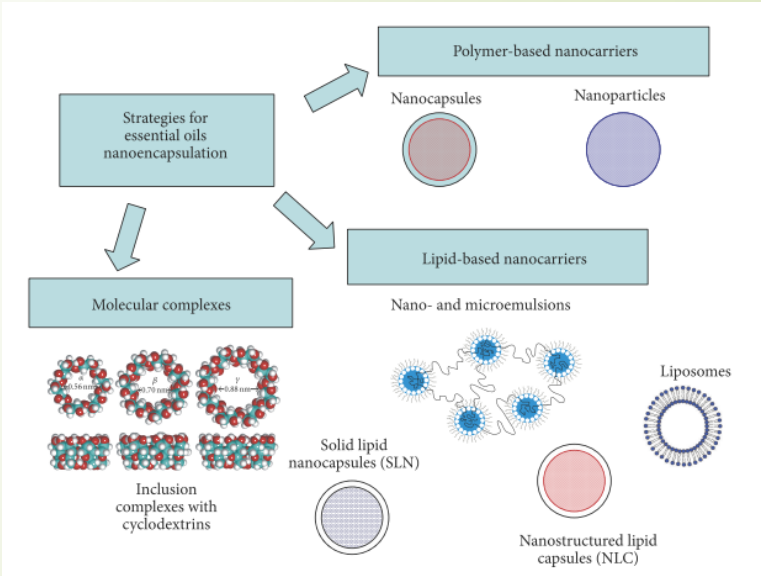
Uses include in food colouring, toothpaste, sunscreens, tattoos, emulsifying agents eg ice cream, mayonnaise.

In pharmaceutical products they are purported to facilitate absorption, increase efficacy and reduce toxicity. They increase solubility of essential oils

Safety? Little is known about the long term effects on human health.

Environmental concerns eg sunscreens contaminating water (Macia & Chrzanosski, 2018).





Nanoparticles - colloidal delivery systems

- Alginate/cashew gum nanoparticles
- Nano-structured lipid carriers - liposomes
- cyclodextrins
- Zein nanoparticles (liquid dispersion method)
- Chitosan
- Silver

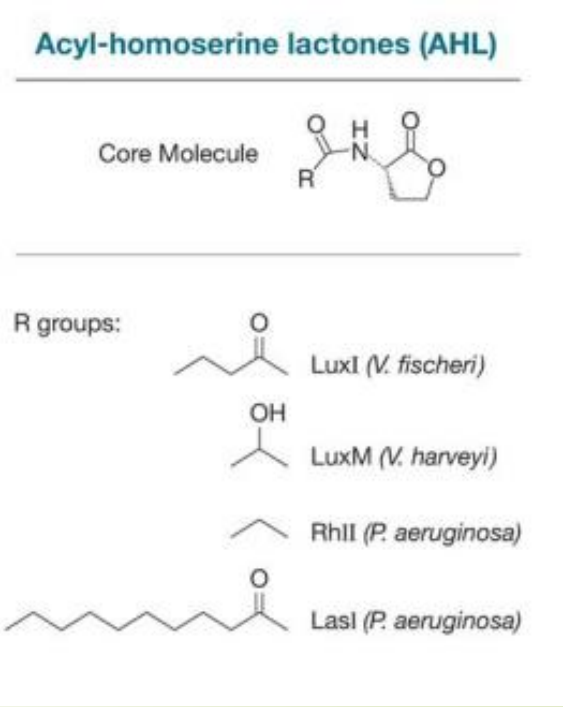
Images from Bilia et al 2014

QUORUM SENSING

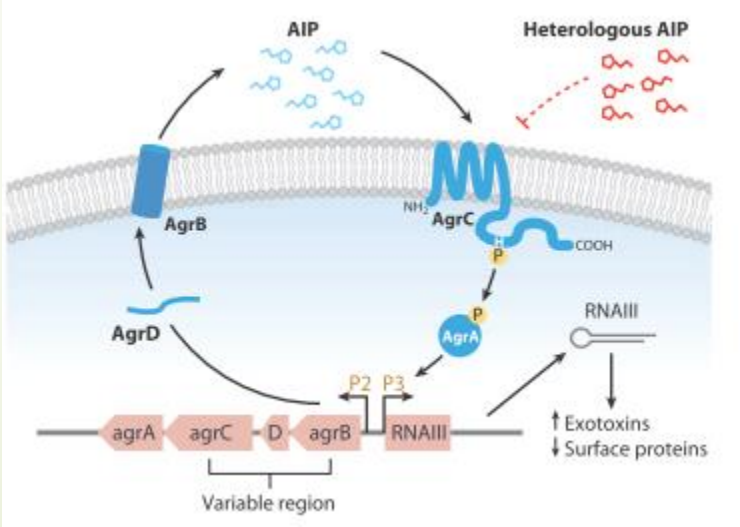
- Quorum sensing is a cell-cell communication process used to monitor cell number and species complexity in a population.
- Bacteria and fungi monitor their cell-population density by measuring the concentration of small signaling molecules, called autoinducers.
- The most common autoinducers are *N*-acyl homoserine lactones (AHLs)
- Those used by gm+ve bacteria are usually peptides (AIPs)
- Quorum sensing-processes include;
  - bioluminescence
  - virulence factor production
  - biofilm formation, sporulation and motility (Kearney & Kearney, n/d).

- "It is now clear that cell-cell communication is the norm in the bacterial world and that understanding this process is fundamental to all of microbiology, including industrial and clinical microbiology." (Waters & Bassler, 2005)

- "For every class of quorum-sensing signal thus far identified, a mechanism has been discovered that inhibits, destroys, or removes it" (Federle & Bassler, 2003).



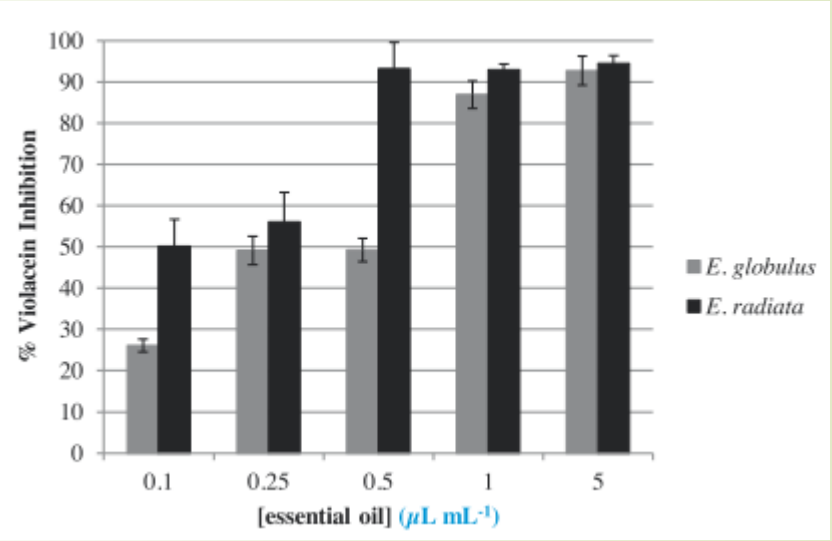
Bacterial auto-inducers in gm – ve bacteria (Waters & Bassler, 2005)



Auto-activation circuit typical of *Staphylococci* bacteria (Novick and Geisinger, 2008)

Quorum sensing and Essential oils

- Szabo et al (2010)demonstrated inhibition of quorum sensing signaling molecules in vitro, by essential oils of geranium, rose, lavender, rosemary, Eucalyptus and citrus.
- Several essential oils including Eucalyptus and TTO, with minimum anti QS potential, were emulsified with oleic acid glycolipids to improve solubility.
- The resultant EOSLs (essential oil sophorolipids) showed significant anti-QS activity against *Candida bombicola and Vibrio cholerae* (Mukherji & Prabhune, 2014)
- Anti-QS activity of two eucalypt EOs (*E. globulus: E. radiata*) was evaluated using the biomonitor strain, *Chromobacterium violaceum,* a gm–ve bacterium which synthesizes the purple pigment violacein (Luis et al, 2016)
  - *E. radiata* demonstrated the highest potency of the two EOs, especially at the lower doses. Notably this sample was high in limonene, which is not a constituent found in the main *E. radiata* chemotypes.
  - Hence these results cannot be extrapolated to *E. radiata* in general, only to a high limonene chemotype.
- Other essential oils with anti-QS activity include clove oil (Khan et al., 2009), cinnamonaldehyde (Nui, Afre & Gilbert, 2006), peppermint and menthol (Husain et al., 2016).



(Image from Luis et al., 2016)

Medicinal Honey

- Manuka honey
  - *Leptospermum scoparium*
- Kanuka honey
  - *Kunzea ericoides*
- Jellybush honey
  - *L. polygalifolium*
- New Zealand Medihoney
  - Combination of manuka and kanuka
- Australian Medihoney
  - combination of manuka and jellybush

Mechanisms for antimicrobial action

- Hydrogen peroxide H_2O_2 – product of glucose breakdown by glucose oxidase
  - Pro-oxidant – required for initial stages of wound healing
- Osmotic pressure from concentration of sugars
- Low pH (3.5-4.5)
- Phytochemicals: polyphenols; peptide – bee defencin 1; methylgloxal (MGO); methyl syriginate

Resistant strains of *Staphylcoccos aureus* and other species depend on biofilm formation for protection from antibiotics. Honey can penetrate biofilms and reduce the viability of these strains. MGO appears to be the main component of honey responsible for this effect (Carter et al. 2016)

Survey of Australian honey flora based on UMF values – *L. polygalifolium* honey has equivalent antimicrobial power to Manuka honey (Irish, Blair & Carter, 2011).

Throat soothers and gargles

- Aromatic honey
  - 90 parts unfiltered honey or “medi-honey”
  - 10 parts essential oil (eg lemon-scented tea tree, niaouli, tea tree oil)
  - Mix well, take a small teaspoon every 15-20 minutes

- Alternative
  - 80 parts hydrosol of above essential oils
  - 20 parts honey
  - Mix well, use freely as throat gargle

Essential oil evidence-based blends

- Klonemax is a custom-made mixture consisting of Eucalyptus oil (EO)136 mg/mL, TTO 131 mg/mL, lemongrass oil (LGO) 86 mg/mL, lemon oil (LO) 71 mg/mL, clove oil (CO) 73mg/mL, and thyme oil (TO) 26 mg/m dissolved in ethanol.

- TTO, LGO, and EO are effective against multi-resistant bacteria like vancomycin-resistant *Enterococcus,* methicillin-resistant *Staphylococcus aureus,* multi-resistant *Pseudomanas aeroginosa*,

- The results indicate that some essential oils have better anti-infective properties than standard oral antiseptics. (Karbach et al., 2015)

- KMPT Mix is a mixture of eucalyptus oil, tea tree oil, lemongrass oil, lemon oil, clove bud oil and thyme oil dissolved in 30 % ethanol (Warnke et al., 2009)

Home-made blends

Ear oil – for mild external ear infections or mild ear aches

- 90% vegetable oil base eg. Mullein, Hypericum or Calendula infused oils. Alternatively sweet almond oil
- 10% essential oil (Eucalyptus, tea tree etc.)
- Place 2-4 drops in ear, cover with cotton wool. Remove after 15 minutes
- This oil can also be applied to the lymphatic glands around the ear

Note: for blocked ears an aromatic diffuser can be used, to which 5 drops of *Eucalyptus dives* or *E. radiata* essential oils are added.

Nose drops

- Essential oils diluted with vegetable oils for instillation into the nasal cavities. From 5 -10% essential oil
  - *Eucalyptus dives, E. radiata, Melaleuca* species essential oils
- Squirt 2 to 4 drops of a blend up each nostril and inhale.
- Take this regularly for relief of blocked or stuffy nose and sinusitis.

Aromatic respiratory blend

- Macadamia nut oil 40mL
  - *Eucalyptus polybractea* 8mL *E. Australiana* 2mL

Home-made blends

Antimicrobial EO blend

4 pts. Tea tree oil

1 pt. lemon myrtle

Antiviral essential oil (50mL)

Infused oil Hypericum flowering tops 30mL

Tea tree oil 14mL

Honey myrtle oil 2mL

Zest myrtle oil 2mL

Blue cypress oil 2mL

Plaque fighting mouth-rinse (100mL)

Eucalyptus extract 5mL

Hopbush tincture 10mL

Essential oils (Eucalyptus, lemon myrtle, tea tree) 5mL

Hydrosol 80mL

Dose: 5mL, dilute as required

Household cleanser

- White vinegar 40mL
- Blend of essential oils e.g. Eucalyptus, tea tree, lemon myrtle, lemon tea tree, Rosalina etc. 5mL
- Hydrosol 300mL

Antimicrobial plant extracts

Dodonaea viscosa – sticky hop bush

- Seven subspecies.
- Subsp. *angustifolia* is widely distributed in eastern Australia and in other countries.
- Methanol extract found to inhibit *Streptococcus mutans* biofilm formation, implicated in dental plaque, dental abscesses and periodontal disease.
- Also inhibits *Candida albicans* adherence to epithelial cells
- The authors conclude that *D. viscosa* ssp. *angustifolia* extracts may improve oral hygiene by reducing plaque formation and reducing the chances of oral thrush (Naidoo et al, 2012).

Subspecies *angustifolia*

Medicinal uses for *D. viscosa:*

- inflammatory skin diseases
- Arthritis, joint pain
- Type 2 diabetes
- Antimicrobial

Persoonia spp. - geebung

- Geebung fruit (most likely from *P. linearis*) is highly revered by the Wiradjuri Language people of the Hunter Valley.

- One of its' reported uses is by the application of the juice derived from the fruit, for local treatment of skin infections due to infection by *Staphylococcus* bacteria, and for other skin disorders including psoriasis.

- Extracts prepared from the ripening fruit of a hybrid of *Persoonia linearis* and *P. pinifolia* was found to inhibit the growth of pathogenic bacteria (gm+ve and -ve) and a fungus (*Phytophthora cinnamomi*).

- Further investigations revealed the presence of a single antimicrobial compound, a previously unknown phenolic glycoside ester (MacLeod, Rasmussen & Willis, 1997).

Persoonia linearis - narrow leaf geebung

Persoonia pinifolius - pine-leaf geebung

Indigenous Plants for Health Association (Inc) (IPHA)

- **IPHA** is an incorporated association formed with the objectives of raising awareness, sourcing grants and sponsorship for sustainable production of indigenous plant-based products.

- We have created a list of significant indigenous species that meet health-promoting criteria, including but not restricted to plants with medicinal, aromatic and nutritional benefits. By promoting rural and Aboriginal community engagement, we aim to create opportunities for employment in the areas of sustainable land management, plant propagation, processing and sale of indigenous plant products.

- The Association will ensure opportunities and any rewards from such activities flow through to Aboriginal communities.

www.indigenousplantsforhealth.com

IPHA members newsletters

IPH Newsletter October 2018

"A Community Not for Profit Association"

Welcome to the October edition of our Indigenous Plants for Health newsletter. We hope you will find the topics in each of our newsletters helpful and of interest to you. The plan is for newsletter to gradually expand into a leading publication focused on Australian native medicinal and edible plants. We welcome comments and contributions from members, please forward them to trueunicorn11@gmail.com.

Plant of the month

Smilax glyciphylla Sm. Family: Smilacaceae

Common name ... *Native Sarsaparilla, sweet tea*

Description

This is a slender evergreen vine with alternately arranged dark green ovate leaves with entire margins, and black berries appearing in umbel formation.
Leaves have sweet, liquorice-like flavour with slight bitterness

Distribution

Common in bushland throughout eastern Australia – from coast to rainforest

Part used

Fresh or dried leaf

IPH A Newsletter Summer edition

January 2019

Health-promoting plant of the month

By Andrew Pengelly PhD.

***Melia azedarach* L. var. *australasica* Fam.** Meliaceae

Common names: White cedar, Chinaberry, Persian lilac.

Description

Medium sized deciduous tree with furrowed grey bark. Leaves are compound and bipinnate, glabrous and bright green. Lilac, sweetly scented flowers are arranged in axillary panicles, followed by drupes which turn from green to orange, and are persistent through winter when the tree is otherwise dormant.

Image from Wikipedia commons

Distribution

M. azedarach is native to southern Asia, including southern China and India, while the variety "australasica" is found from Arnhem Land in the north of Australia through to coastal and sub-coastal regions of southern New South Wales. It has been introduced to North and South America, Africa and elsewhere, and it readily naturalises. It is widely cultivated as a shade tree, and survives very dry conditions.

Part used

Stem and root bark, leaves, flowers, fruit, oil from seeds

Constituents

The main active constituents are terpenes (tetranortriterpenes to be precise) known as secoliminoids - salannal, meliacarpinin E, salannin, azedarachin, nimbolinin B, nimbolidin B (root bark), meldenin and meliatoxins in fruits, trichilin-type limonoids, sendanin-type limonoids - sendanal, amoorastatin

Triterpenoids and steroids (in fruits and root) – cyclolanastane derivatives including methylene cycloartanone

Anthraquinones (stem bark) – dihydroxy methylanthraquinone glycosides

Alkaloid - margosine, Protease – melain from fruit, Glycopeptide - meliacine

Traditional uses

There is scare evidence of indigenous use of this species in Australia, most information available derives from a long history of use in Asia, particularly India and Pakinstan. Here is used to treat fevers, diarrhoea, malaria, rheumatic pain, intestinal parasites, head lice, scabies, bacterial skin conditions and much more (Sharma & Paul, 2013; Abid Askari, 2010). It also has a long tradition of insecticidal uses, many of which resemble uses for the more well-known and closely related neem tree (*Azadirachta indica*).

Actions

Antipyretic, anthelmintic, bitter tonic, antidiarrheal, emmenagogue, deobstruent, astringent, antiparasitic, antiviral, anodyne, emetic

Antimicrobial activity

The traditional use of *M. azedarach* for treatment of bacterial and fungal disease in humans has been substantiated by in vitro studies using extracts prepared from both leaves (Sen & Batra, 2012) and seeds (Khan et al., 2011).

Therapeutic uses (Indications)

Based on traditional uses and research findings:

Fevers, viral infections
Intestinal worms, tropical parasites
Protozoas— *Trichmonas vaginalis*
Head lice, scabies, ringworm
Externally for wounds, boils, , pustular eruptions, cellulitis
Fungal diseases, incl. *Candida albicans*
Diarrhoea, dysentery
Stomach irritation, vomiting
Renal calculi (kidney/bladder stones)
Cystitis
Nervous headaches, dyspepsia
Rheumatic pain, glandular swellings
Inflamed eyes, cataracts
Haemorrhoids

Preparations:

Dried bark decoction, 15-20 ml every 2 hrs as anthelmintic.
Root bark, powdered, 0.5g TDS
Tincture leaf 1:5, 0.5-1mL TDS
Paste of the flowers for skin and head
Leaf fomentations

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Indigenous Plants for Health Association Inc.

IPH Newsletter November 2018

"A Community Not for Profit Association"

Welcome to the November edition of our **Indigenous Plants for Health** newsletter. We plan for the newsletter to gradually expand into a leading publication focused on Australian native medicinal and edible plants. Comments and contributions from members are welcome, please forward them to the editor at trueunicorn11@gmail.com.

Plant of the month By Andrew Pengelly PhD.

Melaleuca – paperbarks

Melaleucas are sometimes referred to as tea tree (tea tree oil), but they are more correctly named paperbarks. There are approximately 300 species nationwide, if we count the *Callistemon* genus, which some authorities include in the *Melaleuca* genus. Whatever their name, this group of plants consist of evergreen trees and shrubs, with aromatic foliage and distinctive bottlebrush shaped flower spikes.

They are distributed mainly throughout Australia, however a few species are found in SE Asia and Pacific Islands. Botanical features of 140 Melaleuca species are described in Ivan Holliday's Field Guide to Melaleucas (1989) while essential oil profiles for the genus (including *Callistemon*) are documented in a classy book titled *Melaleucas. Their Botany, Essential oils and Uses* (Brophy, Craven & Doran, 2013). Both texts feature excellent photographs and line drawings for most species.

Melaleuca alternifolia

M. alternifolia – tea tree oil

This is one of the top selling essential oils in the world. Unlike Eucalyptus, most of the oil is grown and produced in Australia – notably in the natural area of natural distribution in northern NSW.

Chemotypes: The Australian standard (ISO 4730:2017.E) for any oil traded as tea tree oil contains 30-48% terpinen-4-ol, 14-28% α-terpinene and less than 10% 1,8-cineole. For anyone intent on establishing a tea tree oil plantation, it is paramount the propagation material used is derived from plants with the this chemotype. In aromatherapy terms, the odour of tea tree oil is regarded as fresh, sharp, and somewhat medicinal. It was once promoted as "The first aid kit in a bottle".

Tea tree oil is clinically proven for treatment of acne, scabies, resistant Staph. aureus and fungal infections.

"First-aid kit in a bottle"

M. quinquenervia – coastal paperbark

This is the common coastal paperbark found in eastern NSW north from Sydney and Queensland. The common chemotype is comprised of *E*-nerolidol (74–95%) a sesquiterpene alcohol, and linalool (14–30%). Another chemotype, known as niaouli oil, is high in 1,8-cineole.

Niaouli also occurs in New Caledonia, the main commercial source of

How to use Melaleuca

While this article focuses on essential oil distillation, since most people don't have stills at home, the benefits of these species may be obtained by alternative methods. The leaves are pleasant to drink as teas, or infused in a vegetable oil for topical use. Herbalists may prefer to make up tinctures, using an aqueous-ethanol solvent.

IPHA Newsletter #6

June 2019

https://indigenousplantsforhealth.com/

Health-promoting plant of the month

By Andrew Pengelly PhD.

Podocarpus elatus R.Br. Ex Endl. Family: Podocarpaceae

Common names: Illawarra plum, plum pine.

Podocarpus literally means foot fruit, which doesn't seem to make sense, however it refers to the distinctive foot-stalk of the fruit. Elatus simply means tall, this being a rainforest tree than can attain heights in excess of 30 metres. It has a natural distribution from Kiama on the south coast and adjacent ranges of NSW, right up to Cairns in tropical Queensland (Floyd, 1989).

The Podocarpus family or Podocarps are one of the three families of conifers found in Australia, the others being the Araucariaceae (eg bunya pines) and Cupressaceae (cypress pines). They are distinguished from other conifers by the structure of the female cones, in which the scales unite to form a fleshy receptacle, ie the plum-like fruit. In the Flora of NSW the fruiting receptacle of *P. elatus* is described as being blue-black, glaucous and about 20mm in diameter, ripening between March and July (Harden, 1990). The tree has a straight trunk with brown fissured bark, with oblong to linear leaves, up to 14cm in length. The tree is often planted in parks and other public places.

Illawarra plum is considered a health-promoting plant both for it's highly nutritious fruit, a traditional bushfood, and for it's potential as a chemopreventative agent that may treat or prevent cancer.

Constituents

Nutritional

Illawarra plum fruit are quite low in fat and protein, but they do provide energy to the consumer of over 700kj/100g, a level much high than for most common household fruits and vegetables. The fruits also contain vitamin C, but at lower levels than for oranges (see table) (Low 1991).

Illawarra plums are also a good source of mineral salts, particularly potassium and magnesium. On the other hand they are relatively low in iron (Cherikoff, 2017).

Phenolics

Phenolic compounds in food and beverages are a major source of antioxidant phytochemicals in human diets. The traditional method for assessing phenolic levels in plants is the Folin–Ciocalteu assay, which provides the total phenolic content expressed in gallic acid equivalents per gram of fresh weight. Using this method Illawarra plum registers a total phenolic content of 68.2, which is one of the higher levels found in Australian bush fruits, and far higher than blueberry (26) which is an international reference standard for total phenolic content and antioxidant capacity (Netzel et al., 2007).

Image from Wikipedia Commons

Selective nutrient levels in *P. elatus*

Nutrient	Content
Vitamin C	11
Calcium	156
Magnesium	161
Potassium	2730
Sodium	179
Sulphur	102
Energy (kj.)	728

Source: Cherikoff, 2017, Low 1991

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Indigenous Plants for Health Association Inc.

IPH A Newsletter #5

March 2019

Health-promoting plant of the month

By Andrew Pengelly PhD.

Eucalyptus camaldulensis Dehnh.— River red gum

This eucalypt is often described as Australia's most iconic tree, and it is certainly the most widely distributed, found across every state bar Tasmania. The species was first described by Dehnhardt in 1832, named after the Count of Camalduli, in whose gardens in Naples the species was first cultivated outside Australia. The common name refers to the category of eucalypt (red gums) that the species belongs to (see below) and the fact it always grows along rivers and watercourses.

The red gums are a group of smooth barked eucalypts with occasional patches of rough bark, especially at the base of the trunk. Bark is regularly shed (referred to as decorticating) to producing a mottled surface of grey, white and/or bluish colours. The main botanical distinguishing feature of this group is the exserted fruit, in which the tips of the valves inside the woody capsule are visible above the rim. Flower buds are also distinctive, the cap being quite long and pointed—often referred to as "beaked". Some common red gums in eastern Australia are the forest red gum (*E. tereticornis*), Blakely's red gum (*E. blakelyi*), cabbage gum (*E. amplifolia*) and—found only in a site near the Hunter Valley wineries— the Pokolbin mallee (*E. pumila*).

Lithograph by Rosa Catherine Fiveash (1882) from *Eucalyptus Flowers* by John Rigby 2013. National Library of Australia.

E. camaldulensis is a spreading eucalypt of medium height (approx. 30m) which can grow taller in a forest situation. Bark often decorticates across the whole trunk, sometimes producing a pinkish appearance. Juvenile leaves start out opposite in arrangement, gradually turning alternate, the mature leaves are lanceolate in shape, dull green on both sides. Flower buds are spherical and beaked on long thin pedicels (stalks), arranged in umbels of 7-11. White flowers appear during summer, maturing to form spherical capsules with exserted valves. Crushed leaves give off a strong, typical Eucalyptus odour.

Windswept red gums in WA. Photo courtesy of Geraldton Visitor's Centre.

One of the strongholds for this species is along the Murray River, and the Barmah forest in northern Victoria contains the largest of all red gum communities, dominated by forest trees reaching 40m in height. By contrast, I was astounded to see the same species growing near Geraldton in WA, where the prevailing westerly wind has caused this population to take on a prostrate form, as seen in the photo opposite.

The river red is not usually found along eastern coastal rivers, however the exception is the Hunter River, where a small remnant community can be found just east of Singleton. Larger populations occur around Scone and Aberdeen in the Upper Hunter. The Hunter River red gum community has been declared an endangered population, and groups such as Landcare are encouraging plantings along the watercourses of the Hunter. Given its' potential as a medicinal and aromatic species, as well as a haven for wildlife, the IPHA also supports the cultivation of *E. camaldulensis* west of the Great Dividing Range and in the Hunter Valley.

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IPHA Newsletter #7

September 2019

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Health-promoting plant of the month

Wattles (*Acacia* spp.)

By Andrew Pengelly PhD.

September 1st is wattle day in Australia, so while that date is still in the memory and some wattles are still in bloom, I decided that this editions feature will be the *Acacia* genus, which I believe to be the largest genus (ie most number of species) in Australia, and one which has a long history of use for food, medicines and many other purposes. The distinctive green and gold colour of the wattle are readily recognized as the official Australian colours, as may be observed at any sporting event at which our nation is represented.

The first wattle day on record was held in Hobart as far back as 1838, and the event was championed by the first curator of Sydney's Royal Botanical Gardens, Joseph Maiden, in 1906, declaring the wattle be regarded as an emblem of peace. Wattle day was held sporadically at different times in different states, and it wasn't until 1992 that it was gazetted as a national event to be held on the 1st September. We can thank the so-called wattle lady, Maria Hitchcock of Armidale NSW, who campaigned to get wattle day gazetted, following her success in having the golden wattle (*Acacia pycnantha*) gazetted as the Australian floral emblem in 1988. Her book, "Wattle" (later updated under the name of "A Celebration of Wattle" tells the story of the history of wattle day, whilst also containing botanical and horticultural tips along with an anthology of wattle poems, songs and plays. For more on wattle day, and events that are held round the country, check out the Wattle Day Association at http://www.wattleday.asn.au/ .

Acacia nigricans—true leaves (bipinnate shape)

Botany and classification

According to worldwidewattle.com, there are 1068 species of *Acacia*, worldwide, of which a whopping 1058 come from Australia, with well over half of these found in Western Australia. Acacias are classified within the sub-family Mimosoideae of the super family Fabaceae (legumes). Along with most other leguminous plants they are nitrogen fixers, and hence play a role in maintaining soil fertility. Plants of the Mimosoideae are distinguished from other divisions of the Fabaceae by having regular flower shapes, and with more than 10 stamens. All members of the Fabaceae are related by the distinctive fruit type, the legume or pod.

Acacia longifolia—phyllodes replace leaves

Another characteristic of Acacias are the bipinnate leaves that emerge in the seedlings, but which often develop into phyllodes or false leaves in maturing plants. These may be flat, cylindrical or thorny. Acacias can then be readily classified according to whether they have bipinnate leaves or phyllodes (see images opposite), however there is often a stage in young plants in which they have both characteristics.

Along with variations in the fruiting pod, the other main method of characterising wattles is by their inflorescence (flower arrangement). Flowers are typically arranged in either globular heads (*A. nigricans* opposite) or extended spikes as in *A. longifolia*.

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Thank you

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