Essential oils Chemotypes and synergy

Applications for aromatherapy and herbal formulation

Andrew Pengelly PhD 2017

OVERVIEW

Essential oil chemistry

Structure-effect model

Aromatherapy perspectives

What is a chemotype?

Biosynthesis of chemotypes – how do they come about?

Terpene variation in Myrtaceae

Chemotypes of selected Myrtaceae species to know

Benefits of knowing chemotypes

Identifying aromatic notes

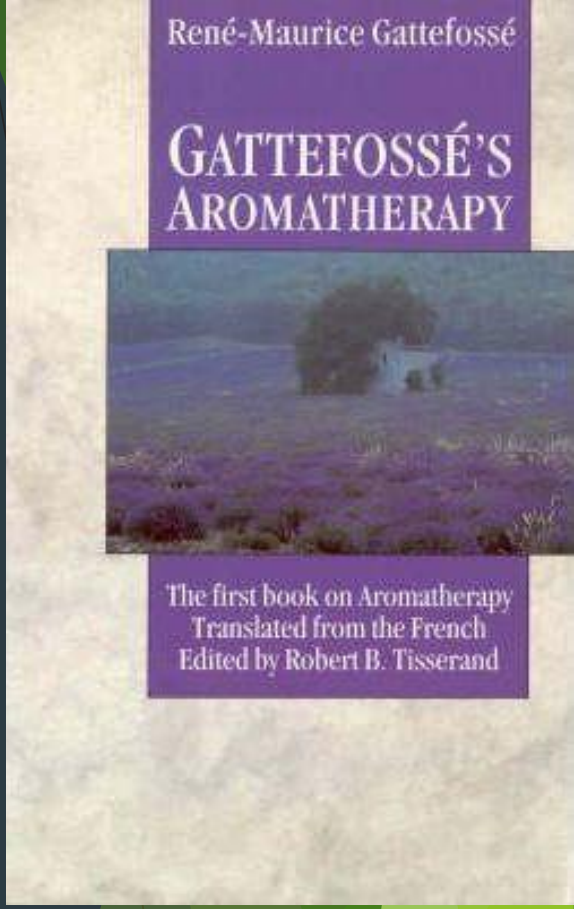
Synergistic combinations

Essential oils and MDRs

Finito

Essential oil chemistry and aromatherapy

- Gattefosse (1937) “Father of Aromatherapy”
  - Classified essential oils based on chemistry of functional groups in volatile constituents
  - eg terpene alcohols, ethers, aldehydes
  - Terpeneless essences (based on removal of non-oxidized hydrocarbons)
    - Non-selective activity of low molecular, lipophilic monoterpenes found in most essential oils
      - (Schnaubelt, 2011)



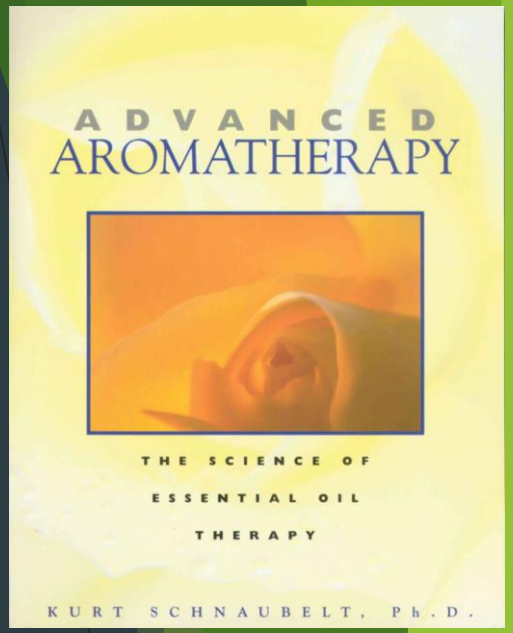
Biosynthetic approach

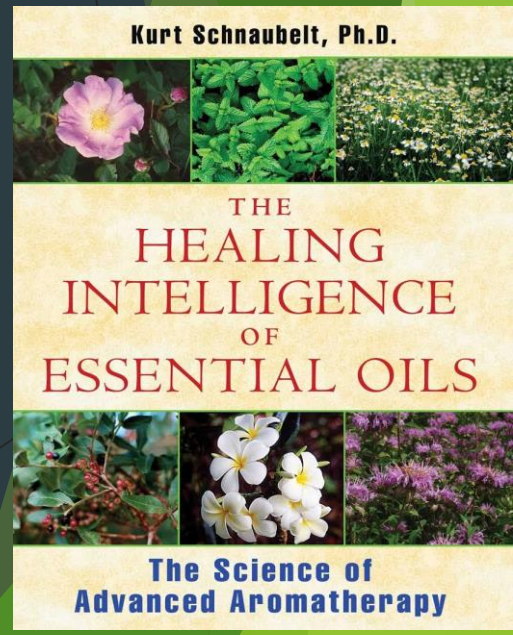
- Direct correlations between functional groups and physiological effects ie structure determines function
- More complex molecules - phenylpropanes, sesquiterpenes – less influenced by functional groups, have more specific effects

FUNCTIONAL GROUP HYPOTHESIS

- Schnaubelt's coordinate system based on Franchomme's experimental findings
  - essential oil molecules tend to accept or donate electrons
  - categorized as electrophilic or nucleophilic
  - Further categorization as hydrophilic (oxidized terpenes) or lipophilic (terpene hydrocarbons)
- Structure-effect diagrams for essential oils

STRUCTURE-EFFECT MODEL



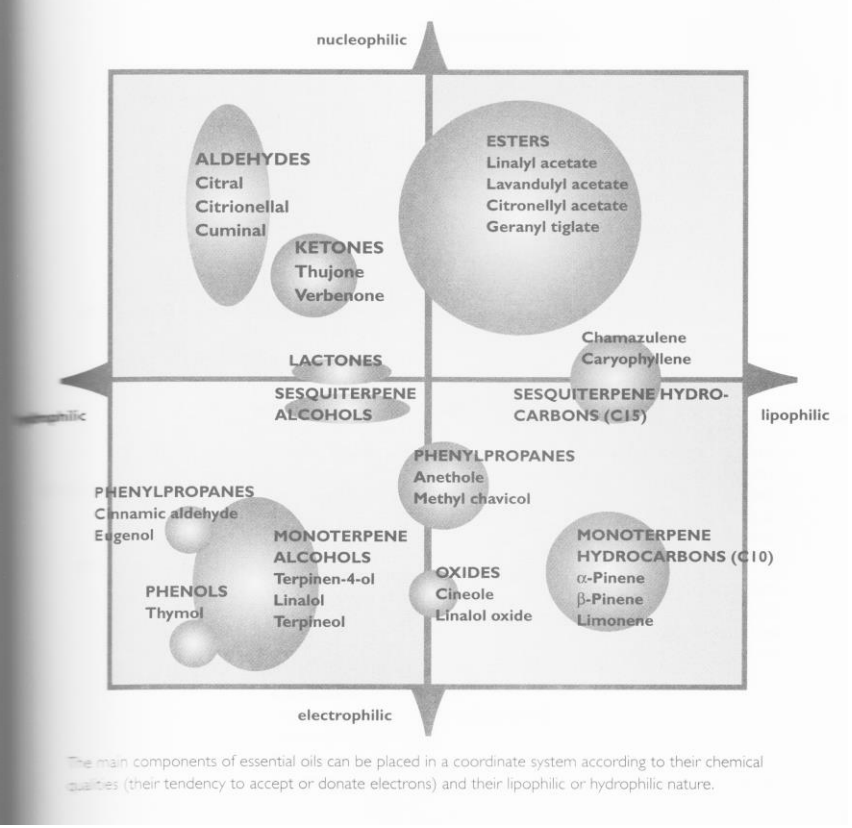


Properties of essential oil families

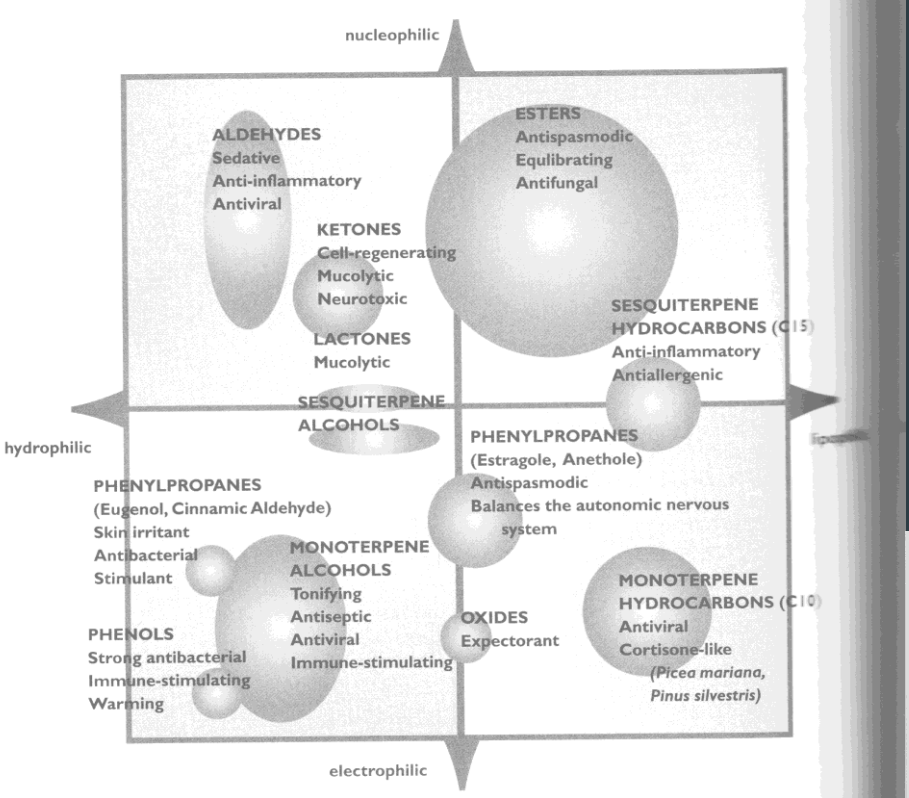
Compound	Properties
Hydrocarbons	Stimulant, decongestant, antiviral, antitumour
Alcohols	Antimicrobial, antiseptic, tonifying, spasmolytic
Sesquiterpene alcohols	Anti-inflammatory, anti-allergenic
Phenols	Antimicrobial, irritant, immune stimulating
Aldehydes	Spasmolytic, sedative, antiviral
Cyclic aldehydes	Spasmolytic,
Ketones	Mucolytic, cell-regenerating, neurotoxic
Esters	Spasmolytic, sedative, antifungal
Oxides	Expectorant, stimulant
Coumarins	UV sensitising, antimicrobial
Sesquiterpenes	Anti-inflammatory, antiviral
Phenylpropanes	Carminative, anaesthetic
Sesquiterpene Lactones	Mucolytic, immune stimulating

Structural-effect diagrams

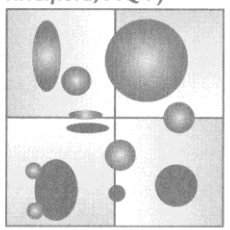
From Scnaubelt, K. (1995) Advanced Aromatherapy. Healing Arts Press



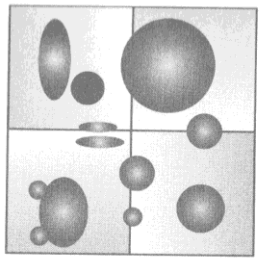
The main components of essential oils can be placed in a coordinate system according to their chemical qualities (their tendency to accept or donate electrons) and their lipophilic or hydrophilic nature.



Niaouli (*Melaleuca quinquenervia viridiflora*, MQV)

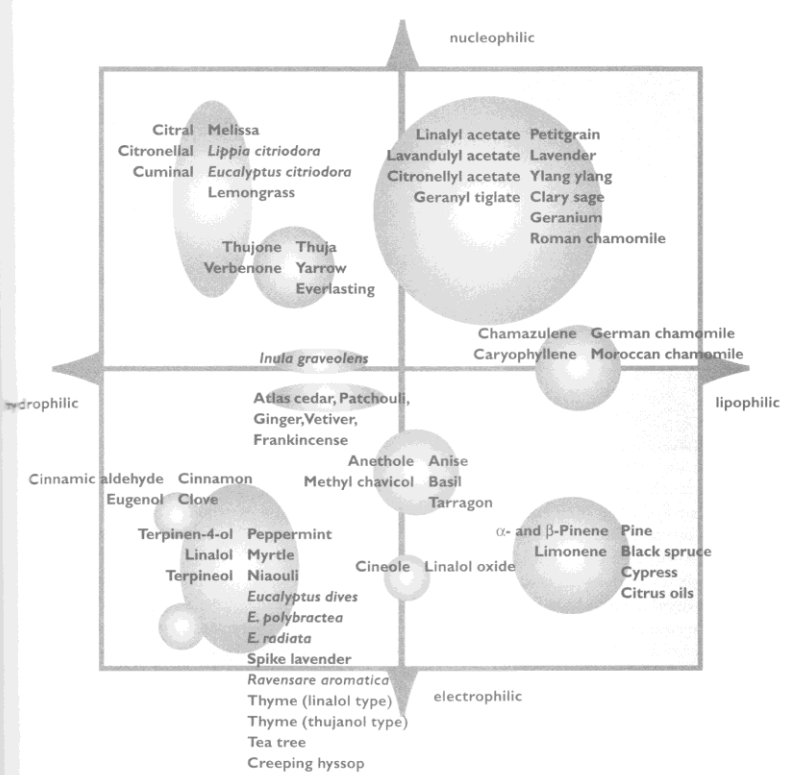


Main components: terpene hydrocarbons, terpene alcohols, sesquiterpene alcohols, terpene oxide (cineole)
Main effects: expectorant, strengthening
Contraindications: none known; hormone-like effects. Children less than 10 years old and pregnant women should *use with caution.*



b) *Eucalyptus dives*
Main components: terpene hydrocarbons (30%), piperitone (approximately 50%)
Main effects: mucolytic
Contraindications: contains ketone; not to be used by children less than 10 years old or pregnant women

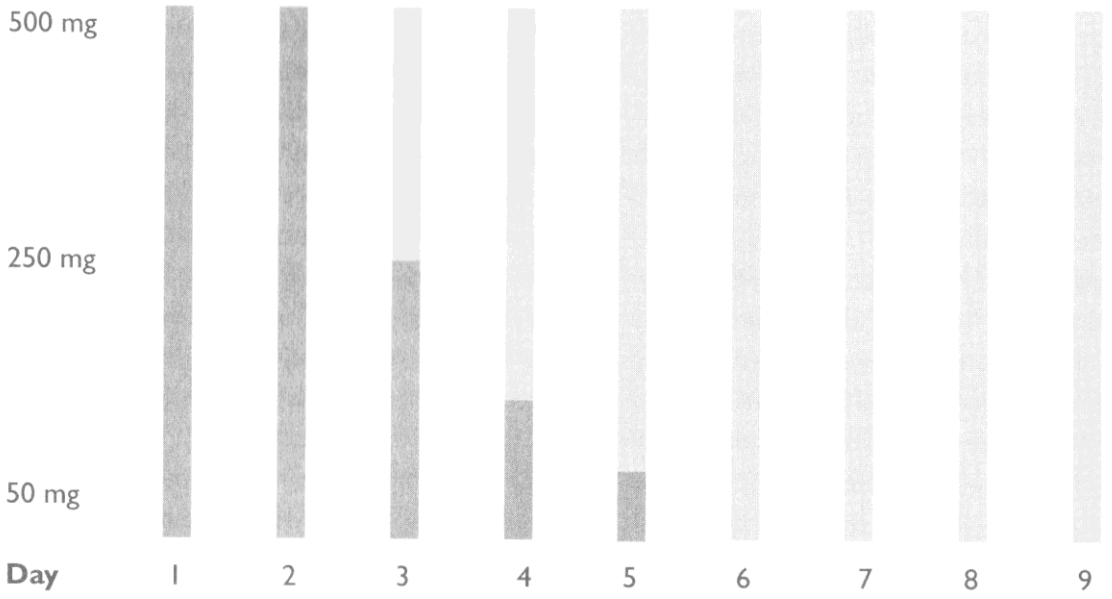
Example of Coordinate system with two Australian essential oils



Due to their particular mix of main components essential oils show specific effects. These effects can be utilized to treat specific illnesses.

Practical applications of functional group hypothesis

TREATMENT PLAN FOR WEAKENED IMMUNITY



Oils containing phenol: oregano or thyme (thymol type)

Oils containing terpene alcohol (to be applied alternately): ravensare, palmarosa, *Eucalyptus radiata,* tea tree, coriander, thyme (thymol type)

GENERAL TREATMENT PRINCIPLES FOR INFECTIOUS ILLNESSES

Days 1 to 3: Phase 1

Using oils with mucolytic and expectorant qualities to cleanse the mucous membranes.

MUCOLYTIC COMPONENTS

Ketones in:

Eucalyptus dives

Rosemary, verbenone type

Lactones in:

Inula graveolens

EXPECTORANTS

Cineole in:

Myrtle

Ravensare aromatica

Laurel

Eucalyptus globulus

Eucalyptus radiata

Days 4 to 7: Phase 2

Eliminating remaining pathogens with oils with bactericidal and fungicidal components. Phases 1 and 2 can be alternated during the first 7 days of treatment.

BACTERICIDAL COMPONENTS

Monoterpene alcohols in:

Ravensare aromatica

Niaouli

Tea tree

Eucalyptus radiata

Phenols in:

Mountain savory

Thyme

Oregano

FUNGICIDAL COMPONENTS

Esters in:

Lavender

Roman chamomile

Geranium

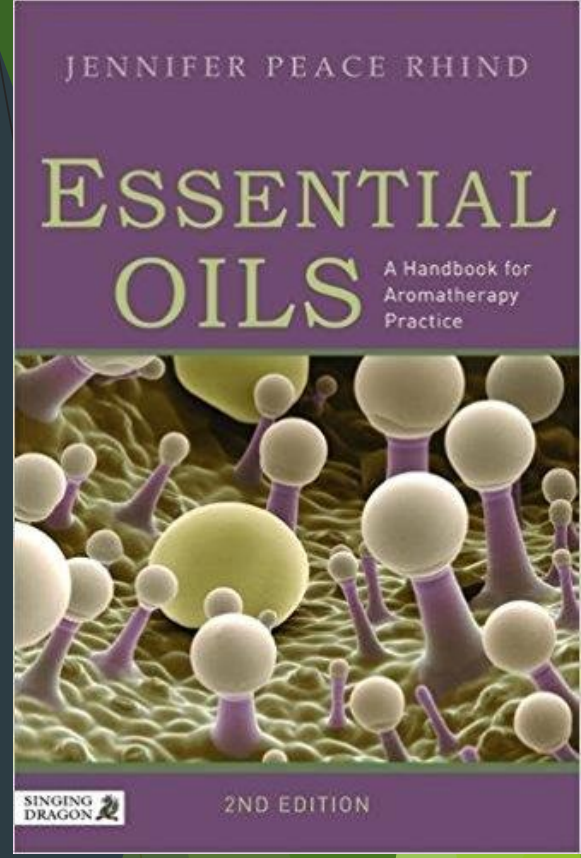
Days 8 to 21: Phase 3

Supporting convalescence. Essences with a sesquiterpene-alcohol and -ketone content are especially suited for this phase.

Beyond functional groups

- Peneol
  - Too general as global frame of reference
  - Individual constituents may have characteristics not influenced by functional group

- Rhind (2012) critiques hypothesis
  - Doesn't account for enantiomers (chirality) of molecules
    - *d*-linalool – doesn't influence mood
    - Similar variation in properties of carvone and limonene
  - Pharmacological actions of functional group largely empirical (untested or duplicated)
  - May only be valid for oral/rectal administration, not trans-dermal
  - Psychological influence of odor characteristic not considered



Phytochemical variability and essential oils

- Plant hybrids
- Sub-species
- Geographical races
- Chemotypes
- Chirality
- Authentic vs standardized oils
- Adulteration
- Distillation vs extraction
- Ontogenetic factors - leaf age, season etc

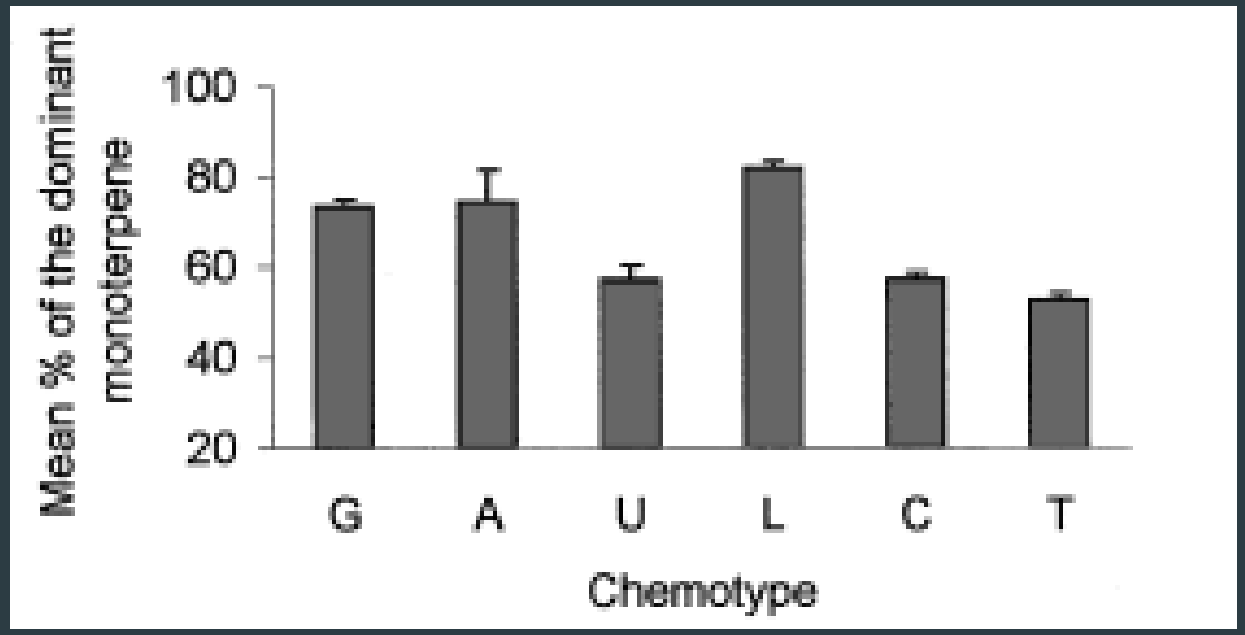
What is a chemotype (CT)?

- Previously known as chemical forms
  - Penfold and Willis definition (1953) “those plants in a naturally occurring population which cannot be separated on morphological evidence, but which are readily distinguished by marked differences in the chemical composition of their essential oils”
  - Variation within single population – sympatric polymorphism (Whiffin & Bouchier, 1992)
    - *Melaleuca alternifolia* - CTs co-exist in natural populations
    - *Backhousia citriodora*, two CTs over most of its range BUT citronellal CTs only at 25^{o}S
    - Melaleuca quinquenervia similar - CT1 found only south of 25^{o}S (Keszei, Brubaker & Foley, 2008)
- Chemical races (not chemotypes)
  - Variation within distinct populations
  - Each chemotype is genetically determined and physical features and locality are not accurate indicators.
  - Schnaubelt (2000) provides example of camphor variability in wild rosemary
  - *Melaleuca ericifolia* – linaool/cineole ratios varies according to north/south distribution (Brophy, Craven & Doran, 2013)
  - The only reliable method is to submit the leaves to chemical analysis, the most common method being GC/MS = gas chromatography coupled with a mass spectrometer.
- A characteristic of the Myrtaceae family, also occurs in some Lamiaceae, Rutaceae and in conifers.
- Not restricted to essential oils, other phytochemicals

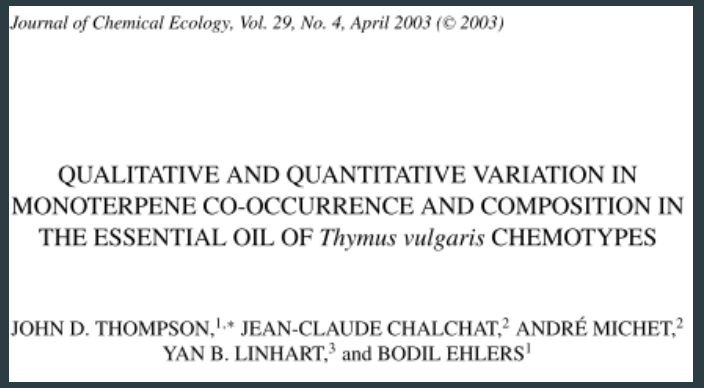
Do herbivores or pollinators induce chemotype biosynthesis?

- 1940s Ford, Haldane developed theory that disease, parasitism and herbivory can play important roles in the origin and maintenance of complex genetic polymorphisms
- Multi-species and multi-directional process
- Thyme
  - diverse parasites and herbivores respond to the biochemical polymorphism of *Thymus vulgaris* in a species-specific manner.
  - bees appear to select geraniol chemotypes for feeding
  - These results further illustrate the association between environmental heterogeneity and genetic variability(Linhart & Thompson, 1999)
- Eucalypts
  - plants with higher concentrations of terpenoids are better defended against herbivores
  - terpenoids and other volatile compounds can attract predators of herbivores eg Eucalyptus and Christmas beetles (Edwards, Manjura & Brown, 1993)

Thyme chemotypes



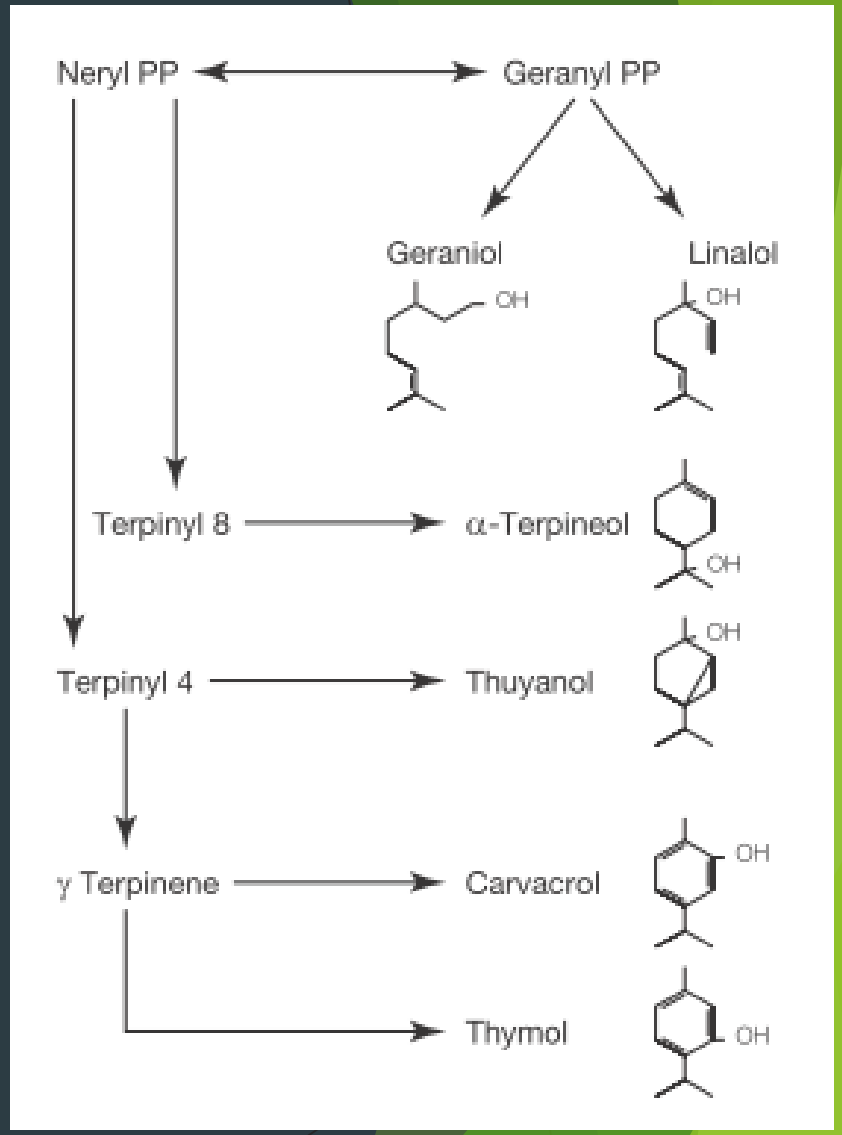
Thymus vulgaris chemotypes in southern France. Key: G, geraniol; A, a-terpineol, U, thuyanol; L, linalool; C, carvacrol; T, thymol



Journal of Chemical Ecology, Vol. 29, No. 4, April 2003 (© 2003)

QUALITATIVE AND QUANTITATIVE VARIATION IN MONOTERPENE CO-OCCURRENCE AND COMPOSITION IN THE ESSENTIAL OIL OF *Thymus vulgaris* CHEMOTYPES

JOHN D. THOMPSON,[1,*] JEAN-CLAUDE CHALCHAT,[2] ANDRÉ MICHET,[2] YAN B. LINHART,[3] and BODIL EHLERS[1]



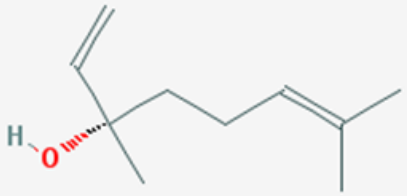
Biosynthetic pathways of thyme monoterpenes
Linhart & Thompson, 1999

Table 1. Relative deterrent values (*D*) of thyme chemotypes when exposed to herbivores, microorganisms and a competitor[a]

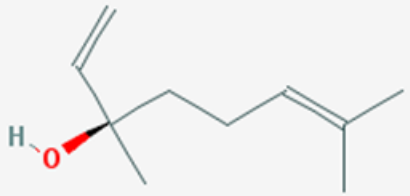
Biotic challenge		Thyme chemotype G	L	A	U	C	T	Significance
Herbivores								
Helix[b]	%	26	73	58	51	8	10	
	D	0.31	0.11	0.14	0.16	1.00	0.80	<0.001
Deroceras[c]	%	37	24	35	9	9	9	
	D	0.24	0.38	0.26	1.00	1.00	1.00	<0.001
Leptophyes[c]	%	39	32	38	36	42	52	
	D	0.82	1.00	0.84	0.89	0.76	0.62	<0.05
Arima	%	36	58	43	42	19	17	
	D	0.63	0.28	0.39	0.40	0.87	1.00	<0.001
Capra	%	32	38	52	28	40	32	
	D	0.94	0.86	0.67	1.00	0.83	0.94	<0.001
Ovis	%	45	27	51	77	33	54	
	D	0.60	1.00	0.53	0.35	0.82	0.50	<0.001
Microorganisms[d]								
Gram-positive bacteria	mg	0.8	0.6	0.9	1.8	0.5	0.4	
	D	0.49	0.73	0.42	0.12	0.77	1.00	<0.0001
Gram-negative bacteria	mg	1.1	1.6	1.9	1.8	1.5	0.6	
	D	0.64	0.29	0.09	0.14	0.36	1.00	<0.078
Fungi	mg	0.27	0.86	0.72	0.91	0.84	0.33	
	D	1.00	0.19	0.37	0.12	0.22	0.92	<0.0001
Competitor								
Brachypodium[c]	%	55	67	53	60	32	34	
	D	0.66	0.54	0.69	0.59	1.00	0.97	<0.0001
Mean deterrent	*D*	0.63	0.54	0.44	0.47	0.76	0.88	

Isomerism

The mystery surrounding organic chemical structures is partly due to the three dimensional shapes of these molecules, allowing for two or more positions of atoms on the same basic molecule



(+)-Linalool = d-linalool
Rotates light to the right
(clockwise)



(-)-Linalool = l-linalool
Rotates light to the left
(anticlockwise)

1. Structural isomers – compounds with the same molecular formula, but a different arrangement of bonded atoms.

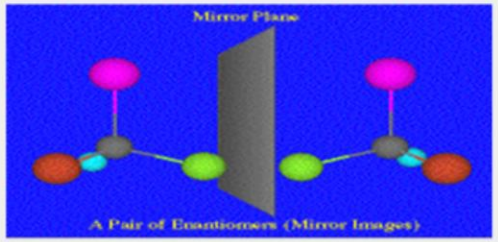
2. Positional isomers differ in the position of their functional group. They may b compounds whose side chains are attached at different locations around the carbon ring. For example the phenol **coumaric acid** may contain a hydroxyl (OH) group at any of three locations, known as *ortho* (*o*-coumaric acid), *meta* (*m*-coumaric acid) or *para* (*p*-coumaric acid). **Thymol** and **carvacrol** are positional isomers due to the different position of the hydroxyl group on the monoterpene skeleton.

3. Stereoisomers have the same bonds or connectively, but different three-dimensional orientation of atoms

   a. Geometric (*cis-trans*) isomers differ in the placement of functional groups on one side or other of the double bond.
      i. *cis-* designates the stereoisomer with like groups on the same side of the double bond
      ii. *trans-* designates the stereoisomer with like groups on opposite sides

   Cis-trans isomerism is responsible for significant differences in the properties and odours of many essential oils containing identical chemical constituents.
   The organic acids **maleic** and **fumaric acids** are also *cis-trans* isomers.

   b. Enantiomers – nonsuperimposable mirror images known as chiral* molecules. They are also known as optical isomers – molecules that rotate polarized light by identical magnitudes but different directions.
      i. dextrorotary: (*d* or +) rotates light clockwise (to the right)
      ii. laevorotary: (*l* or -) rotates light anticlockwise (to the left)
      iii. racemic mixture: (*dl* or ±) an equal amount of enantiomers



   c. Diastereomers – non-mirror image stereoisomers. These molecules have more than 1 chiral centres. The steroid structure of **cholesterol** has 256 possible stereoisomers, however only one exists in nature (ie cholesterol).

Chemical mosaicism

- Genetic mosaic hypothesis (Padovan, Keszei, Wallis & Foley 2012)
  - Leaves on certain branches of *E. melliodora* and *E. sideroxylon* are resistant to Christmas beetle attack
- "Mosaics may provide an ideal system for the analysis of molecular changes leading to ecologically significant changes in chemotype" (Keszei, Brubaker & Foley, 2008).

Monoterpenes				Sesquiterpenes		
Group A	Group B	Group C	Group D	Group E	Group F	Group G
α-thujene	α-pinene	1,8-cineole	β-myrcene	elixene	β-caryophyllene	copaene
sabinene	camphene	limonene	trans-β-ocimene	isoledene	α-caryophyllene	β-cubebene
α-terpinene	β-pinene	cis-β-terpineol	β-linalool	β-guaiene	caryophyllene oxide	cubebene
α-phellandrene	fenchol	α-terpineol		β-elemene		β-cadinene
β-phellandrene	trans-pinocarveol	thymol		α-gurjuene		calamenene
p-cymene	borneol	*p*-cymen-7-ol		aromadendrene		trans-nerolidol
γ-terpinene	pinocarvone	α-terpinyl acetate		alloaromadendrene		
terpinolene	myrtenol			viridiflorene		
terpinen-4-ol	cryptone			viridiflorol		
	myrtenal			bicyclogermacrene		
	trans-3(10)-caren-2-ol			epiglobulol		
	verbenone			globulol		
				spathulenol		

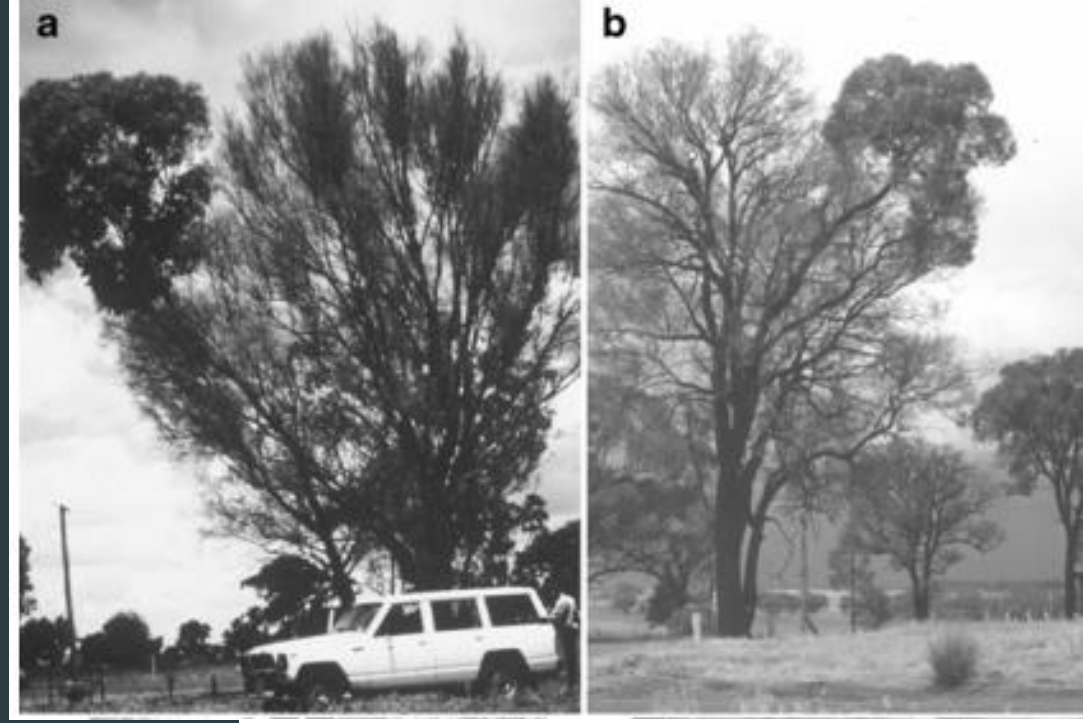
Padovan *et al. BMC Plant Biology* 2013, **13**:29
http://www.biomedcentral.com/1471-2229/13/29

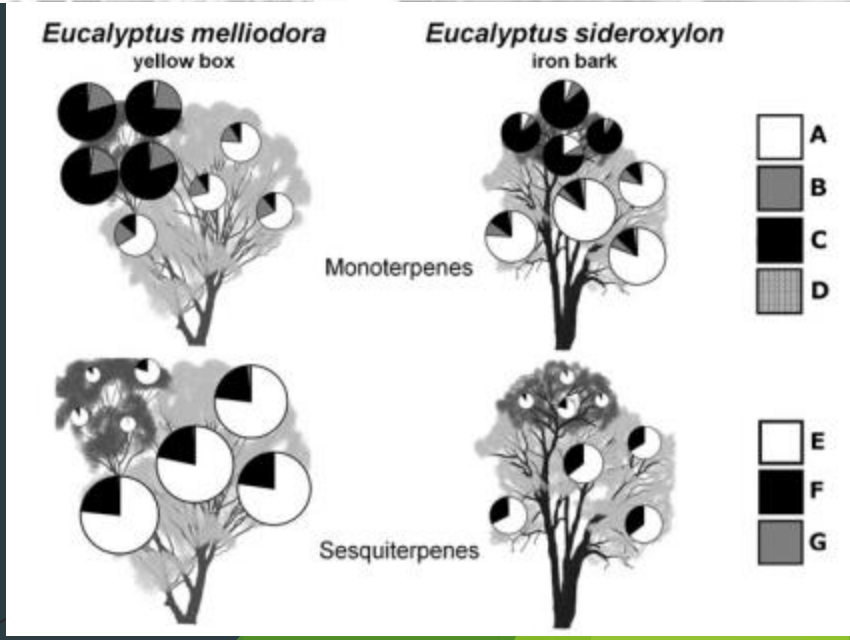
BMC Plant Biology

RESEARCH ARTICLE Open Access

Differences in gene expression within a striking phenotypic mosaic *Eucalyptus* tree that varies in susceptibility to herbivory

Amanda Padovan*, Andras Keszei, William J Foley and Carsten Külheim





MYRTACEAE family terpene variation

- Family contains highly variable taxa with respect to terpene profiles
- Leaf oil composition highly heritable – under strong genetic control (Keszei et al, 2010)
- Terpene synthesis
  - Deoxyxylulose pathway (SXP) – monoterpenes
  - Mevalonate pathway (MVA) – sesquiterpenes
  - Single family of enzymes: terpene synthases (TPS)
    - TPSa – angiosperm sesquiterpene synthases
    - TPSb – angiosperm monoterpene synthases
  - Variations in availability of enzymes affects concentration and ratio of oil components
  - Myrtaceae - largest gene family of terpene synthases on record (Kulheim et al, 2015)

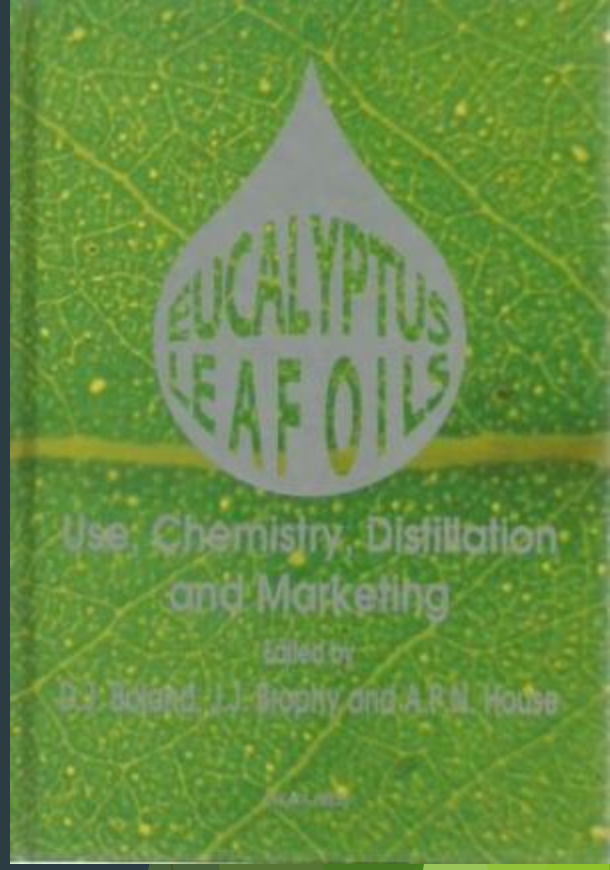
- Similar chemotypes can occur in different species
  - Common ancestry, gene flow between species
  - Similar TPS enzymes (Keszei, Brubaker & Foley, 2008)

Significance of terpene variability

- "Variation in total concentration of terpenes is the most important type of variation relevant to the essential oil industry"
  - Selection of species for land restoration
  - Correct chemical forms relative to local fauna
  - Genetic markers to optimize oil yields and quality
  - Chemical variation can now be correlated to gene sequencing
    - Keszei, Brubaker & Foley, 2008

- Kulheim et al (2015) found that nearly ½ the terpene synthase gene pool in *E. grandis and E. globulus* was located in woody tissue
  - Therefore rich terpene composition not simply for defense against herbivores and pathogens

Hybrids, sub-species and chemotypes: Variation in *Eucalyptus*



- Despite the fact that Australia is known as the "oldest continent" the *Eucalyptus* genus has evolved relatively recently, and the vast number of sub-species and hybrids suggests the evolutionary process is still quite active.
- Hence any particular wild *Eucalyptus* specimen may be a true species, or maybe hybrids of two species - making correct identification a difficult matter.
- Eucalyptus chemotypes:

Species	c/type 1	c/type 2
E. dives	52% pipertone	70-80% cineole
E. radiata	65-70% cineole	18% phellandrene 12% piperitone

Boland, Brophy & House, 1991.

Myrtaceae - chemotypes to know

Eucalyptus

Eucalyptus dives

- The broad-leaf peppermint, is common across much of south-eastern New South Wales and Victoria.
- This tree yields a high level of essential oils in its leaves (up to 4%), the main constituent being the ketone piperitone giving a fresh peppermint-like Eucalyptus aroma.
- CT1 (type)
  - Piperitone 52%, a-phellandrene 20%, globulol 6%, terpinin-4-ol 4%
- CT2
  - Cineole 70%, terpineol and citral,
- CT3
  - The fragrant phellandrene is the main constituent.
- The first 2 chemotypes are good for lower respiratory tract infections such as bronchitis, and may have mild broncho-dilating effects (Webb, 2000).
- Inhalations are beneficial for unproductive coughs, colds and respiratory tract infections

https://en.wikipedia.org/wiki/Eucalyptus_dives

E. radiata, E. australiana

- The narrow-leaf peppermint, has a similar geographic range to *E. dives*.
- One chemotype, often referred to as *E. Australiana* or var. *Australiana*, contains 65-72% cineole, α-terpineol, α-pinene, geraniol and citral.
- The latter constituents impart a refreshing aroma to the oil. It is described as fresh, fruity, probably the most pleasant, child friendly Eucalyptus (Trevena, 2016)
- Research shows it to be a potent antiviral, inhibiting both herpes and influenza viruses, while its gentle action reflects the harmonious balance of constituents.
- This oil has been used with great success for topical treatment of cold sores and shingles. Application of the oil diluted with vegetable oil has been found to help prevent the progression of colds and flu if applied in the early stages.
- A high cineole variety was shown to inhibit gram positive multi-drug resistant pathogens (Mulyaningsih et al. 2011).

Whiffin & Bouchier, 1992

http://www.downunderenterprises.com/Shop/E-K

Table 1. Results from the analyses of variance (ANOVA) for the major volatile oil compounds in the three chemical groups, with mean for each chemical group (as percentage of total oil) and variance ratio (*F*-value)

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s., not significant

Compound	Chemical group			*F*-value		Identification
	A	B	C			
1	2·97	3·19	2·87	0·95	n.s.	α-thujene
3	1·31	1·32	1·27	0·63	n.s.	myrcene
4	15·37	13·01	13·97	0·75	n.s.	α-phellandrene
5	2·97	1·95	0·95	109·45	***	α-terpinene
7	17·16	9·41	3·88	123·90	***	β-phellandrene
7A	1·81	0·34	1·45	0·59	n.s.	γ-terpinene
8	0·58	0·94	0·65	1·11	n.s.	1, 8 cineole
9	19·81	18·22	11·88	5·93	**	p-cymene
10	0·57	0·73	1·14	4·30	*	terpinolene
17	0·97	1·06	0·75	0·70	n.s.	linalool
18	12·21	7·53	2·78	49·46	***	trans-menth-2-en-1-ol
19	4·78	6·10	4·01	1·09	n.s.	terpinen-4-ol
20	8·15	5·50	1·86	45·51	***	cis-menth-2-en-1-ol
21	2·65	1·68	0·61	55·48	***	cis piperitol
22	1·38	1·19	1·31	0·51	n.s.	α-terpineol
24A	4·17	2·59	1·01	49·64	***	trans piperitol
25	1·05	23·98	48·09	770·02	***	piperitone

Leptospermum chemotypes

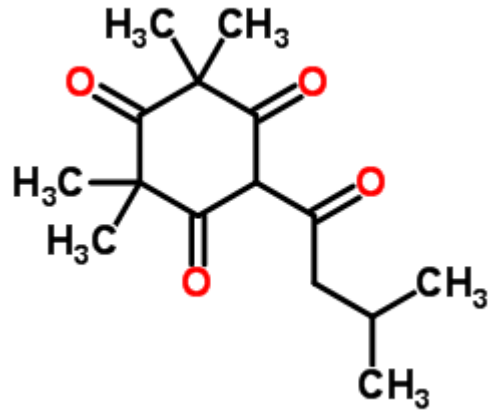
FLAVOUR AND FRAGRANCE JOURNAL
Flavour Fragr. J., 13, 353–358 (1998)

Leaf essential oils of the genus *Leptospermum* (Myrtaceae) in eastern Australia. Part 2.† *Leptospermum blakelyi* and allies

Joseph J. Brophy,[1]* Robert J. Goldsack,[1] Anthony R. Bean,[2] Paul I. Forster[2] and Brendan J. Lepschi[3]

Species	Common name	Major constituent	Minor constituents
L. petersonii	lemon-scented tea tree	citral	citronellal
L. scoparium	New Zealand tea tree; manuka	leptospermone	triterpene acids
L. polygalifolium	tantoon	eudesmol	pinene; terpenin-4-ol
L. liversidgeii	“mozzie blocker”	citronellal	citral (chemotype)

leptospermone



Leptospermum petersonii

Leptospermum petersonii - lemon-scented tea tree

- Brophy et al (2000). Note the variation between the 5 chemotypes.

Constituent	CT1	CT2	CT3	CT4	CT5
Neral	31.3	13.5		0.5	
Geranial	45.4	22.8		0.3	
Citronellal	6.8	46.2			
ð-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
ß-caryophyllene			25		

CT1 – common lemon-scented form ("type")
- variable citronellal/citral ratio
CT2 – citronella type
CT3 - sesquiterpene type
CT4 – terpinene/cajuput type
CT5 – rose-scented type

Observation:
Change in leaf chemistry between 5th and 6th node in greenhouse seedlings. All sesquiterpenes in nodes 1-5 and cotyledons. Mostly monoterpenes from node 6 upwards.

L. polygalifolium – tantoon, jellybush

- The original tea tree found at Port Jackson, used by early settlers for making tea
- Previously known as *L. flavescens*

Essential oil profile variation in subspecies (Brophy et al. 2000)

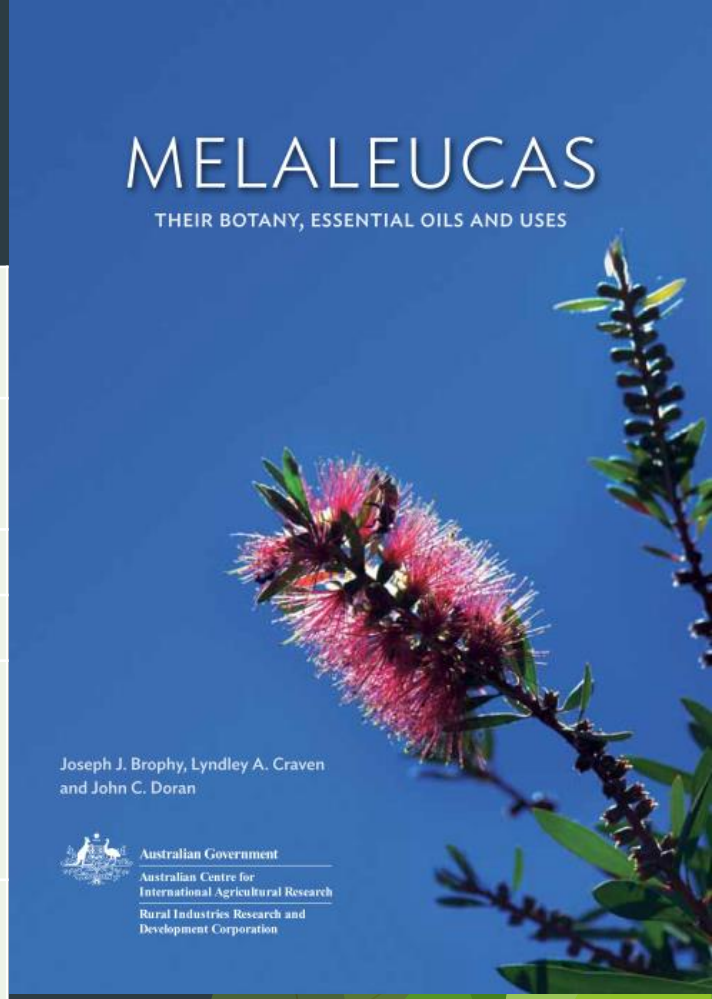
L. polygalifolium subspecies	Essential oil components
polygalifolium	*a*-, *B*- *pinene* *a*-, *B*, and *y*-eudesmol.
montanum	
howense	
cismontanum	1,8 cineole *a*- pinene
transmontanum	
tropicum	spathulenol
wallum	

https://www.flickr.com/photos/31031835@N08/6273629928/in/photostream/

Brazilian cultivated plants recorded high levels of the sesquiterpene nerolidol (Demuner et al, 2011)

Melaleucas chemotypes to know

	Melaleuca	alternifolia	tea tree	terpenin-4-ol >30%	cineole <15%
		linariifolia	snow in summer	terpinen-4-ol >30%	Cineole <15%
		ericifolia	‘rosalina’	linalool	1,8 cineole
		cajuputi	cajuput tree	1,8-cineole	α-terpineol
		quinquenervia	coastal paperbark ‘nerolina’ ‘niaouli’	nerolidol 1,8-cineole	linalool limonene
		bracteata	black tea tree	CT III. E-methyl isoeugenol	isoeugenol
		teretifolia	honey myrtle	CT II. neral	geranial
		fascicularis	Clustered scent-myrtle	geraniol 75%	geranyl acetate



Tea tree chemotypes *Melaleuca alternifolia*

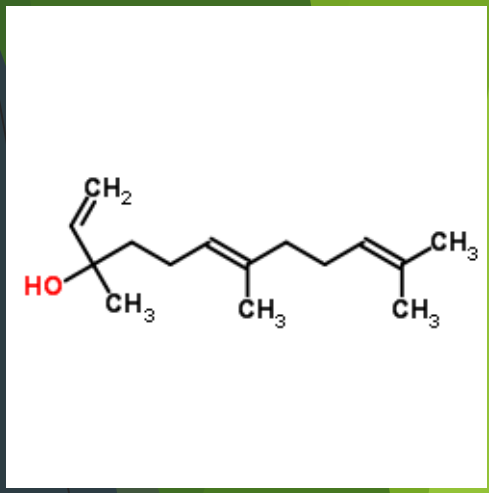
- Seven chemotypes
  - CT1: terpinen-4-ol, α-thujene, α-terpinene, γ-terpinene
  - CT2: 1,8-cineole,α-pinene, ß-pinene, myrcene, limonene, α-terpineol
  - CT3: α- phellandrene, terpinolene, linalool.
  - CT3 3,4,6,7 likely intermediate CTs
    - Keszei, Hassan & Foley, 2010.

- The Australian standard for any oil traded under that name contains less than 15% cineole and over 30% terpinen-4-ol (ie CT1).

- For anyone intent on establishing a tea tree oil plantation, it is paramount the propagation material used is derived from plants with the right chemotype.

Melaleuca quinquenervia

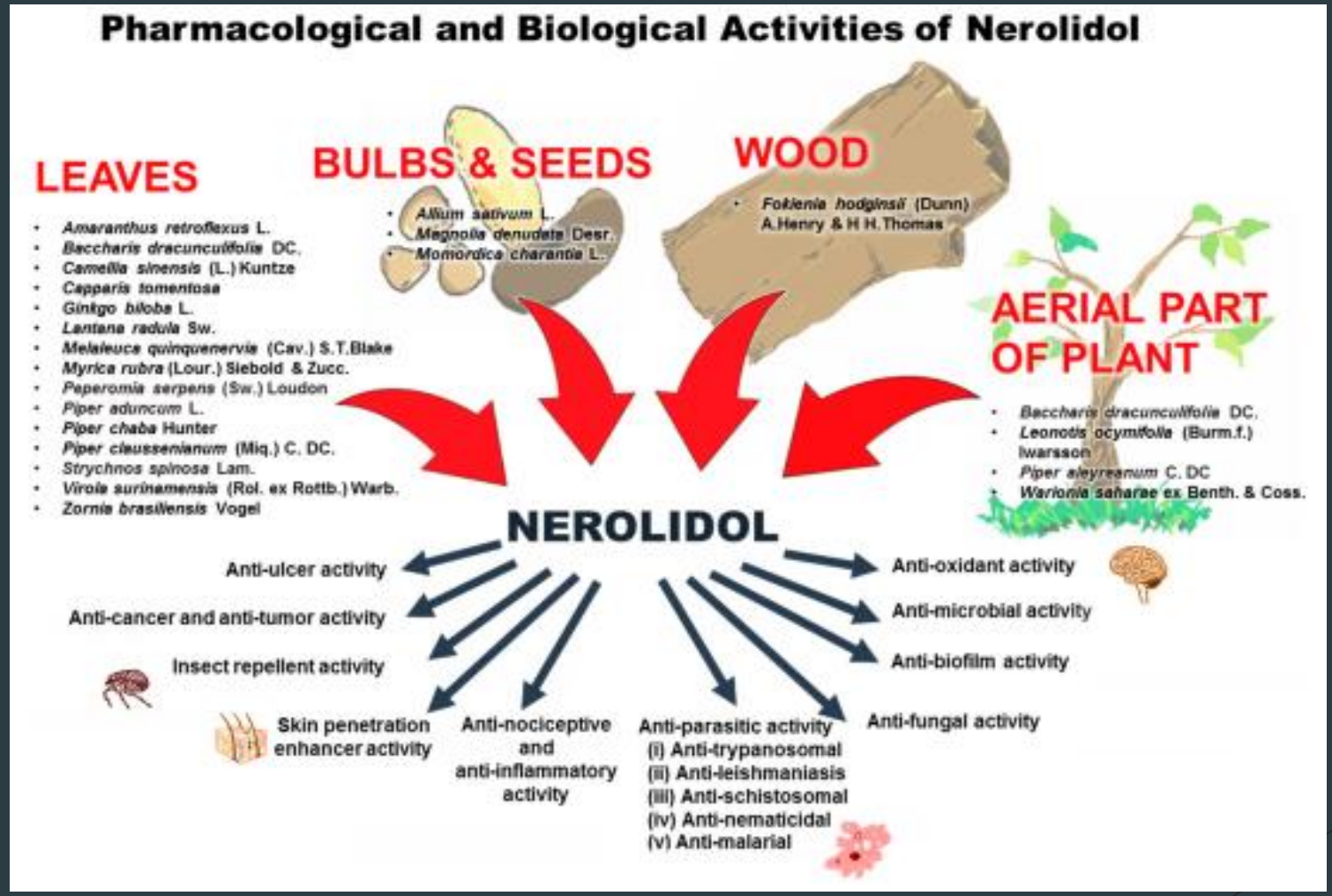
- CT1 is comprised of *E*-nerolidol (74–95%) a sesquiterpene alcohol, and linalool (14–30%). Found mainly in coastal NSW north of Sydney
  - Two divisions occur in this chemotype - based on the presence or absence of significant proportions of linalool.
- CT2 is comprised of 1,8-cineole (up to 75%), viridiflorol (13–66%), α-terpineol (0.5–14%) and ß-caryophyllene (0.5–28%). It is found in NSW, Qld, PNG New Caledonia.
  - Ireland, Hibbert et al. 2002



Trans nerolidol – image from Chemspider

Photographer: Andrew Pengelly

Melaleuca quinquenervia - nerolina



Weng-Keong C, Loh Teng-Hern T, Kok-Gan Chan, Learn-Han L and Bey-Hing G. 2016. *Molecules 21*, 529.

Naiouli – New Caledonia

- Forty-two components were identified by GC–MS analysis and the major ones were:
  - 1,8-cineole (0.1–76%), viridiflorol (0–67%), p-cymene (0–40%), γ-terpinene (0–33%), α-pinene (0–30%), α-terpineol (0–24%), terpinolene (0–19%), limonene (0.1–16%) and ledol (0–21%).
  - Viridiflorol and ledol, two sesquiterpene alcohols, were identified unambiguously by 1H- and 13C-NMR analyses. A sulphur compound, methylthiobenzoate, was characterized by gas chromatography–mass spectrometry (GC–MS).
- Niaouli essential oils from New Caledonia were classified into three chemotypes, using principal component analysis (PCA).
  - CT 2, already characterized in Madagascar, is rich in 1,8-cineole (up to 80%) and is widespread (65.4% of the overall samples);
  - CT 1 (24.8% of samples) is rich in terpinene derivatives;
  - CT 3 (9.8% of samples) is rich in α-pinene and viridiflorol
    - Ireland, Hibbert et al. 2002

Niaouli uses (based on 1,8-cineole chemotype)

- Antimicrobial – broad spectrum
- Protects skin from radiation burns
  - Breast radiation therapy – pure oil applied twice daily
- Antiallergy, venous decongestant
- “unrivalled restorative powers” for psoriasis
- Viral hepatitis (Note: referred to as hepatotoxic by Webb (2000).
- Massage oil – reduces lymphatic edemas (Schnaubelt, 2011)

Melaleuca viridiflora Sol. ex Gaertn

- This species existed in two basic chemotypes, one of which was quite variable.
- Chemotype I gave a terpenic oil, in which there seemed to be three variants.
  - Variant 1 : g-terpinene (39%), terpinolene (33%) and a-pinene (9%).
  - Variant 2: 1,8-cineole (49%), b-caryophyllene (10%), limonene (5%) and a-terpineol (6%)
  - Variant 3: a-pinene (29%), 1,8-cineole (12%) and spathulenol (16%)

- Chemotype II contained E-methyl cinnamate (81%) as its principal component, with lesser amounts of E-b-ocimene (12%) and 2,4,6-trimethoxyisobutyrophenone (5%).
  - Brophy, Craven & Doran, 2013

Cajuput oil chemotypes

- Three subspecies
  - ssp. Cajuputi
    - CT1 - 1,8-cineole (15-60%) limonene (1-5%) viridiflorene (0.5-7%) spathulenol (0.5-30%)
    - CT2 – 93-95% nerolidol
  - ssp. Cunningiana (v. low cineole)
    - Y-terpinene (19%) terpinolene (20%) caryophyllene (19%) humulene (9%)
  - ssp. Platyphylla
    - CT1 – platyphyllol (22-80%) cajuputol (3-57%) sesquiterpenes
    - CT2 – a-pinene (12-70%) cineole (0.1-10%) p-cymene (0.1*7%) + sesquiterpenes

Melaleuca teretifolia – honey myrtle

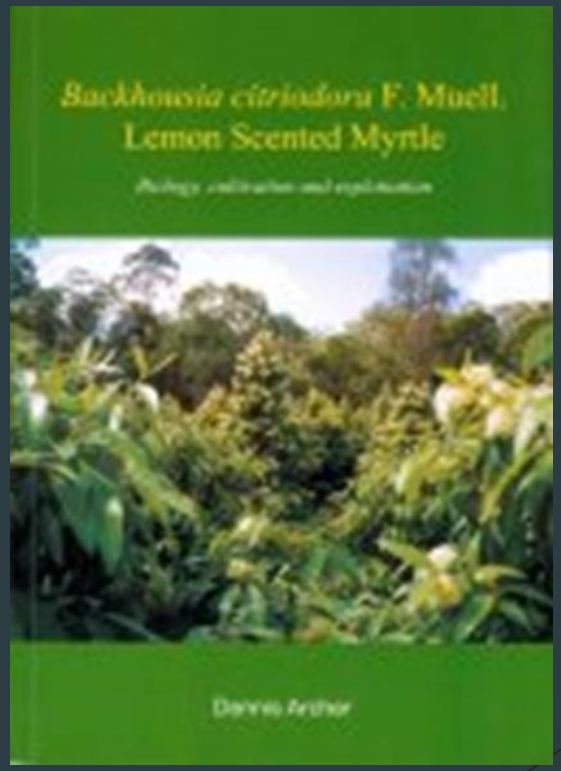
- This species occur in a limited area in SW. Western Australia.

- Chemotypes of leaf-oils (Brophy, Craven & Boland, 2013):
  - CTI was dominated by 1,8-cineole (81–88%).
    - a-pinene (1–3%), limonene (3–4%), terpinen-4-ol (1–3%) and a-terpineol (1–6%).
    - Sesquiterpenes globulol, spathulenol and aromadendrene (all <0.3%).
  - CT2 dominated by citral: neral (29.1%) and geranial (38.8%)
    - myrcene (9.8%), terpinen-4-ol (3.4%), E-isocitral (2.4%) and geraniol (2.1%). Sesquiterpenes were absent. Oil yield: (The Paperbark Co. 2013).

http://paperbarkoils.com.au/honeymyrtleoil

Backhousia citriodora - lemon myrtle

- CT1 citral (Aust. Standard min 85% citral)
  - Citral to 95%, linalool, citronellal, methyl heptanone
- CT2 citronellal
  - “Zest myrtle”



Chemotypes – some benefits

- Scientific Accuracy
  - Botanical name (binomial), variety/sub-species, chemotype
  - Creation of standards eg ISO, Australian standard
- Commercial cultivation
  - Grow correct species with desired chemical profiles
- Credibility
  - Reproducibility of clinical effects, research findings
- Quality assessment
  - Identity, chemical profile
  - Quality/purity
- Versatility
  - Select optimal oils for different stages of treatment
    - Eg acute vs. chronic respiratory infections

Chemotypes – more benefits

- Synergies – embracing complexity
  - Optimize possibilities of molecular combinations
  - Confuse superbugs
- Value adding
  - Impress customers with accurate profiles
- Safety
  - eg select CT higher in terpene alcohols than aldehydes, reduce skin irritancy
- Adventures in sensory experiences!

Aromatherapy - Identifying aromatic notes

Blending of top, middle and base notes into aromatic blends

- Top note
  - The more volatile materials in the blend give the first impression of odor
    - Green, citrus
- Middle note
  - Bears the main theme of the fragrance
    - Spicy, floral, fruity
- Bottom/end note
  - Fixes the fragrance - long lasting
    - Woody, musky

https://info.achs.edu/blog/blending-101-the-art-of-pairing-essential-oils-drop-by-drop

Synergistic essential oil combinations

- "Synergism occurs when two or more compounds interact in ways that mutually enhance, amplify or potentiate each other's effect more significantly than the simple sum of these ingredients" (Rakholya, Kaneria & Chanda, 2013).

- Synergistic blending (Rhind 2012)
  - Horizonal synergy (combining similar functional groups for single purpose)
  - Vertical synergy (combining different functional groups for multi-purpose)

- Antimicrobial blend: teatree/lemon myrtle oil 4:1
    - Increases antimicrobial effect of teatree oil and reduces sensitization of lemon myrtle (Hayes & Markovich 2002)
- Insect repellent blend: *Melaleuca ericifolia* /lemon myrtle oil 4:1 (Grieve et al. 2010).

The major EO constituents with their different functional groups, when applied in combination, are capable to achieve stronger activity; this phenomena is known as ‘vertical synergy’.

EOs combinations created in our study proved positive synergism in antimicrobial activity and substantial reduction in the MIC values, when applied as a single EOs, against both tested oral microorganisms; such valuable results certainly lead to implementation in oral clinical treatments.

Antimicrobial synergism and cytotoxic properties of *Citrus limon* L., *Piper nigrum* L. and *Melaleuca alternifolia* (Maiden and Betche) Cheel essential oils

Miloš M. Nikolić[a], Katarina K. Jovanović[b], Tatjana Lj. Marković[c], Dejan Lj. Marković[d], Nevenka N. Gligorijević[b], Siniša S. Radulović[b], Marina Kostić[a], Jasmina M. Glamočlija[a] and Marina D. Soković[a]

[a]Institute for Biological Research ‘Siniša Stanković’, University of Belgrade, Belgrade, Serbia, [b]Institute of Oncology and Radiology of Serbia, Belgrade, Serbia, [c]Institute for Medicinal Plant Research ‘Josif Pančić’, Belgrade, Serbia and [d]Department of Pediatric and Preventive Dentistry, Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia

Keywords
antimicrobial synergism; *Citrus limon*; cytotoxic activity; essential oil; *Melaleuca alternifolia*; *Piper nigrum*

Correspondence
Marina Soković, PhD, Institute for Biological Research ‘Siniša Stanković’, Boulevard Despota Stefana 142, Belgrade 11000, Serbia.
E-mail: mris@ibiss.bg.ac.rs

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Abstract

Objectives The chemical composition, antimicrobial and synergistic effect, and cytotoxic activity of *Citrus limon* (lemon), *Piper nigrum* (green pepper) and *Melaleuca alternifoila* (tea tree) essential oils (EOs) were investigated.

Methods Chemical analyses of essential oils were tested by GC-FID and GC-MS spectroscopy. The antimicrobial activity assay was conducted using microdilution method against several oral bacteria and *Candida* spp. originating from the humans with oral disorders. The synergistic antimicrobial activity was evaluated using checkerboard method. The cytotoxicity evaluation of EOs was assessed using MTT test.

Key findings Limonene (37.5%) and β-pinene (17.9%) were the major compounds in *C. limon* oil, β-pinene (34.4%), δ-3-carene (19.7%), limonene (18.7%) and α-pinene (10.4%) in *P. nigrum* oil and terpinen-4-ol (38.6%) and γ-terpinene (21.7%) in *M. alternifolia* oil. The broad-spectrum antimicrobial activity was achieved by tested three EOs, with *C. limon* oil being the strongest against bacteria and *M. alternifolia* oil strongest against fungi. The EOs demonstrated synergism; their combined application revealed an increase in antimicrobial activity. All tested essential oils showed lower cytotoxic activity in comparison with the positive control, and the obtained results confirmed a dose-dependent activity.

Conclusions The results of this study encourage use of tested EOs in development of a novel agent intended for prevention or therapy of corresponding oral disorders.

Antiviral tea tree oil – synergism

Essential oils from eucalyptus, tea tree and 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%.

The mode of antiviral action has been determined, only moderate antiviral effects were revealed by essential oils and monoterpenes when these drugs were added to host cells prior to infection or after entry of HSV into cells. However, **both 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 analyzed 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).

Essential oils and multi-drug resistant organisms

Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20170411091159

Original Research

eJM

Bactericidal activity of herbal volatile oil extracts against multidrug-resistant *Acinetobacter baumannii*

Amornrat Intorasoot[1], Piyaorn Chornchoem[1], Siriwoot Sookkhee[1], Sorasak Intorasoot[2]

6 *The Open Microbiology Journal*, 2014, *8*, 6-14

Open Access

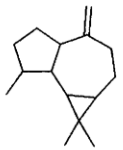
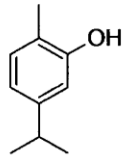
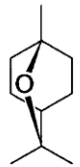
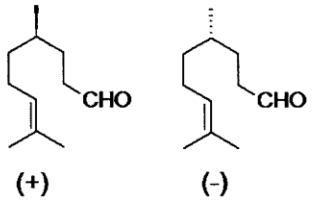
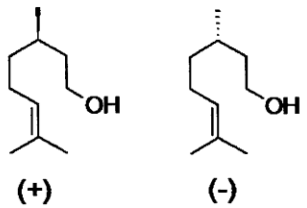
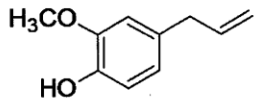
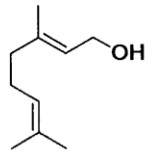
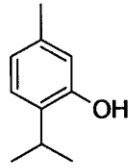
Essential Oils, A New Horizon in Combating Bacterial Antibiotic Resistance

Polly Soo Xi Yap[1], Beow Chin Yiap[2], Hu Cai Ping[3] and Swee Hua Erin Lim[2,*]

Selection of essential oils active against MDR bacteria (From Faleiro & Miguel 2013)

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	
	germacrene D 12.51			
Melaleuca alternifolia (Tea tree)	Terpinen-4-ol 40.1, γ-terpinene 23.0, α-terpinene 10.4, 1,8-cineol 5.1	MRSA	0.25—2 % (v/v)	Coagulation and leakage of cellular contents, production of extracellular vesicles, inhibition of respiration, leakage of K^+
Mentha piperita (Peppermint)	Isomenthone 50.08, Menthol 21.77, ρ-menthone 4.19, 1,8-cineol 3.83	ESBL *K. pneumoniae*	0.008—0.064 mg/mL	N/A
Mentha spicata (Spearmint)	Carvone 75.07, limonene 7.84, cis-dihydrocarvone 4.08, 1,8-cineol 2.07	ESBL *K. pneumoniae*	0.008—0.064 mg/mL	NA
Ocimum basilicum (Basil)	Linalool 54.94, methyl chavicol 11.97, methylcinnamat 7.24	MRSA; Multiresistant *S. epidermidis*; multiresistant *Enterococcus faecalis*; multiresistant *P. aeruginosa*	0.0015—0.0030% v/v; 0.0015—0.0030% v/v; 0.0015—0.0030% v/v; 0.0030% v/v	Membrane permeabilization
	Linalool 75.94, 1,8-cineol 7.73, geraniol 2.40	ESBL *K. pneumoniae*	0.008—0.064 mg/mL	

Essential oil components acting against MDRs
(From Faleiro & Miguel 2013)

Component (Origin)	Chemical group	Chemical structure	Target multidrug bacteria (MIC)	Bacterial cell target and/or mode of action[a]
Aromadendrene (Eucalyptus)	Sesquiterpene hydrocarbon		MRSA (0.5–1 mg/mL); VRE (1 mg/mL); *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (> 8 mg/mL); *Acinetobacter baumannii* (2 mg/mL)	Disturbance of cellular membranes; incorrect protein conformation
Carvacrol (Oregano, savory, thyme)	Monoterpenoid phenol		MRSA(0.05–0.03% v/v); MRSA ATCC 25923 (15.25 mg/mL); methicillin-resistant *S. epidermidis* (0.03% v/v); *K. pneumoniae* (0.008–0.064 mg/mL)	Membrane damage, pH homeostasis disturbance, induction of heat shock proteins, and inhibition of flagellin synthesis
1,8-cineol (Basil, camphor tree coriander, eucalyptus, sage, rosemary)	Monoterpenoid cyclic ether		MRSA, VRE, *E. coli*; *Pseudomonas aeruginosa*; *K. pneumoniae* (> 8 mg/mL), *Acinetobacter baumannii* (8 mg/mL)	Increase in permeability; contraction of protoplasm, and loss of cytoplasmic material
Citronellal (Eucalyptus, lemon, lemongrass)	Aliphatic monoterpenoid		MRSA (0,5–8 mg/mL), VRE (8-> 8 mg/mL), *E. coli*, *P. aeruginosa*, *K. pneumoniae* (0.008–> 8 mg/mL), *Acinetobacter baumannii* (2–4 mg/mL)	Tumescence of cell wall, damage of cellular membrane, and leakage of cellular constituents
(+)-Citronellol, (-)-citronellol (Lemongrass, geranium)	Aliphatic monoterpenoid		MRSA (0.125–8 mg/mL); MRSA ATCC 25923 (not inhibited); VRE (2–8 mg/mL); *E. coli* (4 mg/mL), *P. aeruginosa* and *K. pneumoniae* (0.008-> 8 mg/mL); *Acinetobacter baumannii* (0.125–0.25 mg/mL)	Tumescence of cell wall, damage of cellular membrane, and leakage of cellular constituents
Eugenol (Clove)	Phenylpropanoid		MRSA ATCC 25923 (133.75 mg/mL); *K. pneumoniae* (0.008–0.064 mg/mL)	Alterations to membrane permeability and inhibition of uptake and utilization of glucose
Geraniol (Lemongrass)	Monoterpenoid alcohol		Methicillin-resistant *S. aureus* ATCC 25923 (55 mg/mL)	Disruption of the cellular membrane and leakage of cell constituents
Thymol (Thyme)	Monoterpenoid phenol		Methicillin-resistant *S. aureus* (0.06% v/v); MRSA ATCC 25923 (30.15 mg/mL); methicillin-resistant *S. epidermidis* (0.06% v/v); *K. pneumoniae* (0.008–0.064 mg/mL)	Increase in permeability and leakage of cell constituents

Combining essential oils and antibiotics

- Essential oils “may not necessarily have strong antimicrobial activities themselves but synergize with classic antibiotics through known or novel modes of action” (Rakholya, Kaneria & Chanda, 2013).
- Essential oils may not kill bacteria but may modify them to produce a phenotype that is more susceptible to antibiotics
- “Treatment with essential oils leads to increased bacterial cell permeability, resulting in a loss of cellular material” (Faleiro & Miguel 2013)

Synergistic combinations

- Antimicrobial efficacy of eucalyptus oil and 1,8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures
  - Hendry, Worthington, Conway & Lambert (2009)
- Synergy between oxacillin and manuka honey sensitizes methicillin-resistant Staphylococcus aureus to oxacillin.
  - Jenkins & Cooper, (2012a).

Oregano/ fluoroquinolones **Oregano/ doxycycline** **Oregano/ lincomycin** **Oregano/ maquindox**	*E. coli*	Broth microdilution Checkerboard assay	Synergistic

  - Yap et al, 2014

Antagonistic combinations

- The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials
  - Thyme, rosemary, peppermint, thyme oils with amphotericin B
    - S.F. van Vuuren, S. Suliman and A.M. Viljoen (2009)

Synergistic aromatic blends

Using the information in this presentation, what synergistic combinations can you devise for personal and clinical use?

The end

Thank you

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