Australian Native Oils

Antimicrobial and Anti-inflammatory Activity

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Background

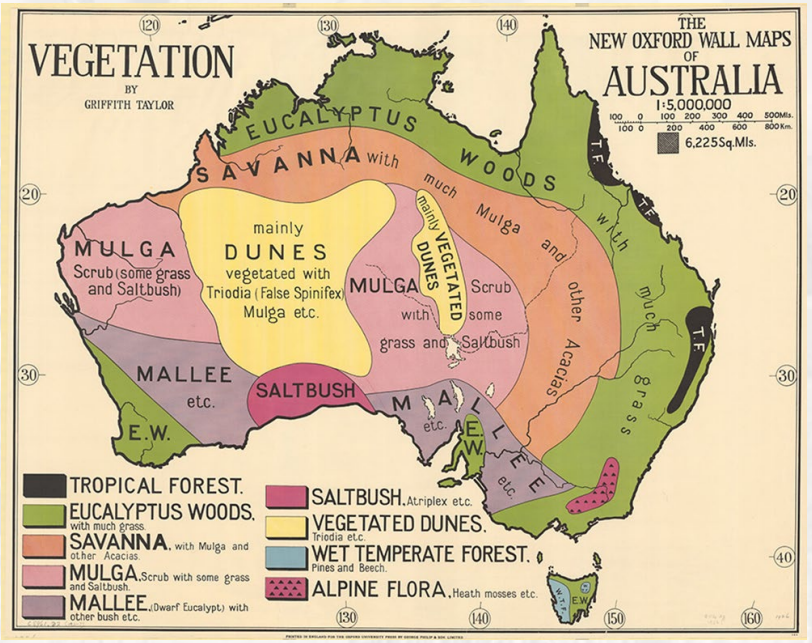
Australia has a vast range of native plants many of which produce chemical compounds which can be distilled as essential oils.

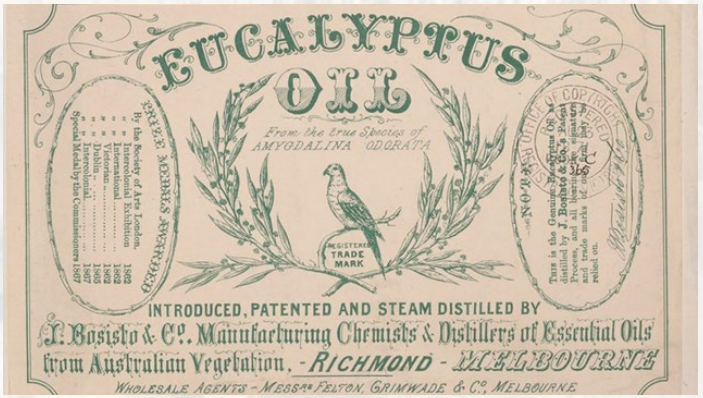
Essential oil production began soon after British settlement with distillation of E. piperita. Export of native sandalwood commenced in the 1840's and distillation of cineole type Eucalyptus oils for medicinal use began in the 1860's.

Microbiological examination of Australian essential oils, began in the 1920's by AR Penfold and more extensively by Atkinson in the 1940's.

Although the essential oil industry in Australia waned after the second world war it has had a resurgence since the1970's with renewed popularity of Tea Tree Oil and Eucalyptus oils.

Consequently, a large amount of biological activity information is available for these oils but there is a relative dearth of information regarding other Australian native oils which are less well known.





EOPAA Native Oils Project

The Essential Oil Producers Association of Australia (EOPAA) was founded in the 1990's :

- to provide a unified approach for the industry in such things as industrial matters, government **regulations and standards**
* to assist with the co-ordination of **research into essential oils** and allied natural plant extracts and to **disseminate research findings to members**.

EOPAA Agrifutures project to develop industry standards and explore the biological efficacy of selected oils included:

* Literature review of uses and efficacy

* Primary analysis - anti-microbial, cytotoxicity, anti-inflammatory

Species selected for project

Binomial Name	Common name/s	Composition
Leptospermum petersonii	Lemon Scented Tea Tree Oil	Citronellal/Citral type oil
Acacia dealbata	Silver Wattle (Mimosa concrete)	Floral concrete/ absolute
Backhousia citriodora	Lemon myrtle	Citral type oil
Melaleuca ericifolia	Rosalina/Lavender Tea Tree	Linalool/1,8-cineole
Eremophila mitchellii	Desert Rosewood, Ba-dha Wood	Eremophilones
Leptospermum scoparium	Manuka	β-triketones
Kunzea ambigua	Tick Bush	Viridiflorol/Leptospermone
Melaleuca quinquenervia	Nerolina/Broad Leaf Paperbark	Linalool/Nerolidol
Eucalyptus staigeriana	Lemon Ironbark	Citral/Citronellal
Callitris intratropica	Northern Blue Cypress	Guaiol/Guaiazulene

Anti-inflammatory Activity

Examined Inhibition of:

Nitrous oxide
TNF-a
Interleukin – 6
PGE-2

Measured as IC50 – concentration at which 50% of activity is inhibited

- Lipopolysaccharide (LPS) stimulated murine macrophages, RAW264.7 cells.
- The production of inflammatory mediators, including NO, TNF-a, IL-6, and PGE2 were measured by established methods using commercial ELISA kits.
- Each sample was tested with at least 6 concentrations (using 3 replicates, maximum concentration was 200ug/mL) (n=9), with relevant internal controls (N-(3-aminomethyl) benzylacetamide, dexamethasone, diclofenac, andrographolide).

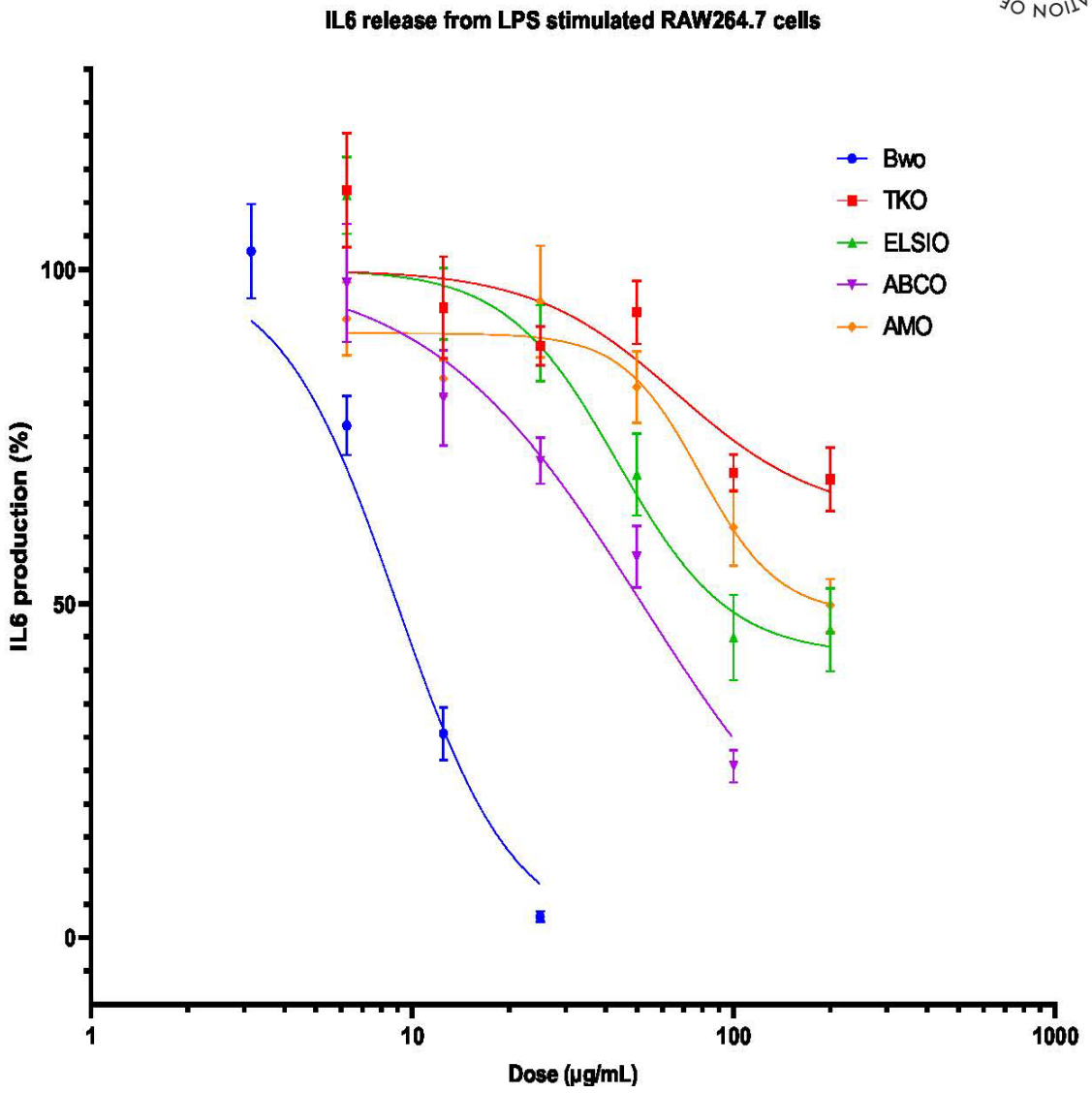


Figure 9: IL-6 assay dose response curves for essential oils

Anti-inflammatory Activity

Essential Oils and Controls Tested	IC50 (ug/mL)					
	NO	TNF-a	IL-6	PGE-2	MEAN	SDEV
Eremophila mitchellii	11.8	14	9	4.7	10	3
Leptospermum petersonii	16.3	33	27	15	23	7
Backhousia citriodora	14.5	43	61	23	35	18
Eucalyptus staigeriana	4	96	44	23	42	34
Callitris intratropica	12.8	91	52	93	62	33
Melaleuca quinquenervia (linalool)	no activity	83	62	46	64	15
Kunzea ambigua	110.9	50	67	89	79	23
Melaleuca ericifolia (cineole)	103.8	76	130	94	101	20
Melaleuca ericifolia (linalool)	no activity	142	147	31	107	54
Leptospermum scoparium (β-triketone)	28.3	417	79	range too wide	175	173
Diclofenac	na	na	na	0.025	0.03	0
Dexamethasone	2	0.6	0.3	na	0.97	1
Aspirin	na	na	na	2.2	2.2	0

Most active	Second Most Active	Control

Anti-inflammatory Activity – Active Compounds

Inflammatory Mediator	Most Active Essential Oils	IC50 %v/v	Predominant Compound Class
Nitrous oxide	E. staigeriana	4	phellandrenes, citrals
	E. mitchellii	11.8	eremophilones
	C. intratropica	12.8	azulenols, eudesmols, other sesquiterpenoids
	B. citriodora	14.5	citrals
	L. petersonnii	16.3	citrals, citronellal
Prostaglandin E2	E. mitchellii	4.7	eremophilones
	L. petersonnii	15	citrals, citronellal
	B. citriodora	23	citrals
	E. staigeriana	23	phellandrenes, citrals
	M. ericifolia (linalool)	31	monoterpenes, oxygenated monoterpenes
	M. quinquenervia	46	monoterpenes, oxygenated monoterpenes
Interleukin-6	E. mitchellii	9	eremophilones
	L. petersonnii	27	citrals, citronellal
	E. staigeriana	44	phellandrenes, citrals
	C. intratropica	52	azulenols, eudesmols, other sesquiterpenoids
	B. citriodora	61	citrals
TNF-a	E. mitchellii	14	eremophilones
	L. petersonnii	33	citrals, citronellal
	B. citriodora	43	citrals
	K. ambigua	50	monoterpenes, oxygenated monoterpenes, beta-triketone

Anti-microbial Activity

- MIC (Minimum Inhibitory Concentration) was determined using Colorimetric Broth microdilution technique.
- 10 out of 11 oils showed moderate to extreme anti-microbial efficacy (0.097 – 25 ug/mL).
- 7 showed extreme efficacy against at least one micro-organism and 3 with extreme efficacy against 3 or more micro-organisms.
- *L. scoparium* was extremely efficacious against 4 micro-organisms but the most ineffective against *P. aeruginosa.*
- *C. intratropica* was extremely efficacious against similar micro-organisms to *L. scoparium* but also highly effective against *P. aeruginosa.*
- The control sample *M. alternifolia* (Tea Tree Oil) was found to be extremely efficacious against all micro-organisms examined.

Anti-microbial Activity

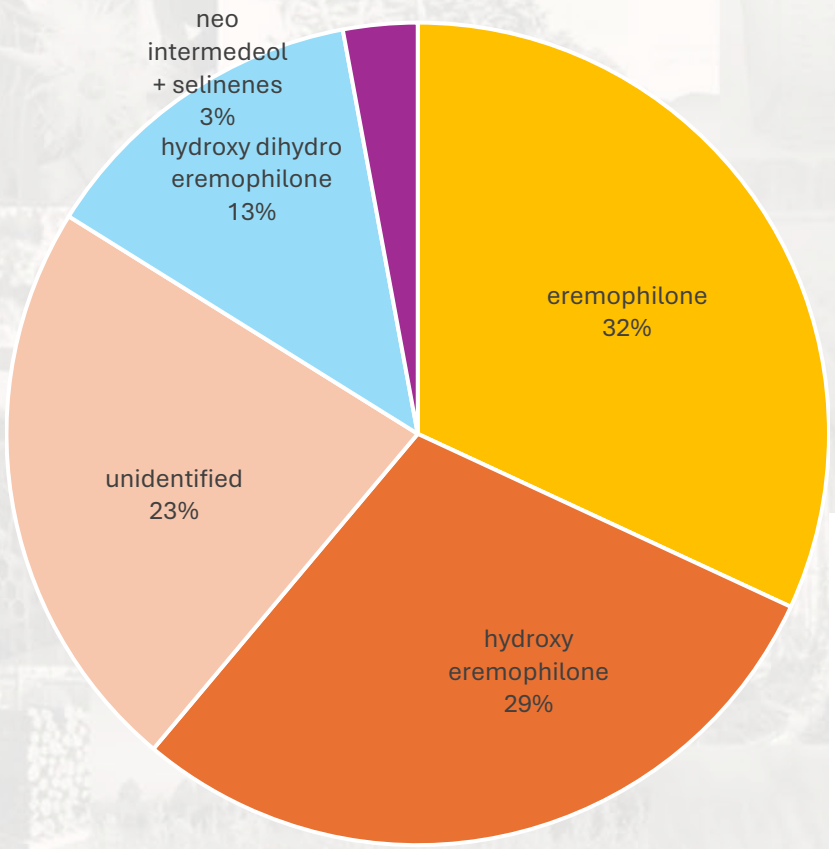
	Minimum Inhibitory Concentration (% v/v) against selected microorganisms							
Essential oil	*E. coli*	*P. aeruginosa*	*S. hofit*	*L. monocytogenes*	*S. aureus*	*P. acnes*	*C. albicans*	*A. brasiliensis*
L. scoparium	6.3	25.0	3.1	0.4	0.4	0.8	3.1	0.4
M. alternifolia	0.4	0.2	0.4	0.8	0.4	0.2	0.8	0.8
K. ambigua	0.8	3.1	3.1	1.6	0.8	6.3	3.1	0.8
L. petersonii	0.4	6.3	1.6	1.6	0.8	3.1	0.8	1.6
E. mitchellii	1.6	3.1	1.6	3.1	6.3	6.3	1.6	1.6
A. mearnsii concrete	1.5	3.1	3.1	1.6	1.6	1.6	3.1	1.6
B. citriodora	1.6	6.3	6.3	3.1	0.8	3.1	6.3	1.6
M. ericifolia - cineole	0.8	1.6	3.1	1.6	1.6	3.1	6.3	1.6
C. intratropica	1.6	0.8	0.1	0.1	0.8	1.6	1.6	3.1
M. ericifolia - linalool	0.8	12.5	3.1	1.6	1.6	1.6	3.1	3.1
E. staigeriana	6.3	0.8	3.1	1.6	3.1	0.8	1.6	6.3
M. quinquenervia	12.5	6.3	6.3	25.0	12.5	12.5	6.3	12.5

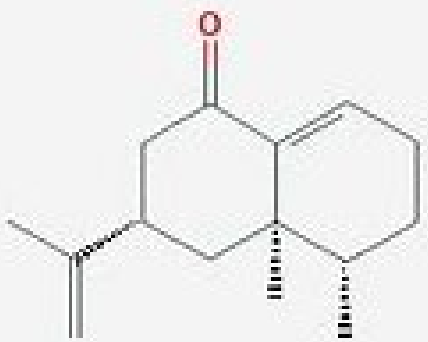
	Extremely active		Highly active		Moderately active

Anti-Microbial Activity– Active Compounds

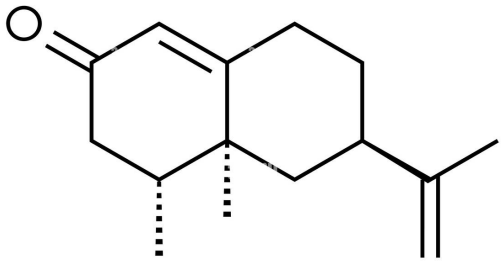
Micro-organism Type	Microbes included	Most Active Essential Oils	Average MIC %v/v	Predominant Compound Class
Gram +ve	E. coli, P. aeruginosa, S. hofit	M. alternifolia	0.3	monoterpenes, oxygenated monoterpenes
		C. intratropica	0.8	azulenes, eudesmols, other sesquiterpenes
		M. ericifolia (cineole)	1.8	monoterpenes, oxygenated monoterpenes
Gram -ve	L. monocytogenes, S. aureus, P. acnes	M. alternifolia	0.5	monoterpenes, oxygenated monoterpenes
		C. intratropica	0.8	azulenes, eudesmols, other sesquiterpenes
		M. ericifolia (linalool)	1.6	monoterpenes, oxygenated monoterpenes
Fungal	C. albicans, A. brasiliensis	L. scoparium	0.5	sesquiterpenes, beta-triketones
		K. ambigua	0.8	monoterpenes, oxygenated monoterpenes, beta-triketones
		M. alternifolia	0.8	monoterpenes, oxygenated monoterpenes

Eremophilones





eremophilone

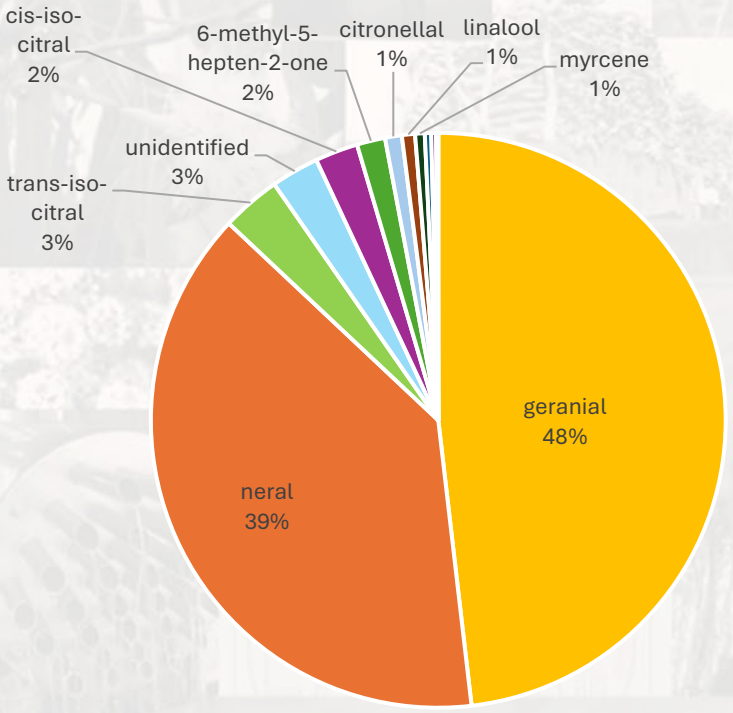


nootkatone

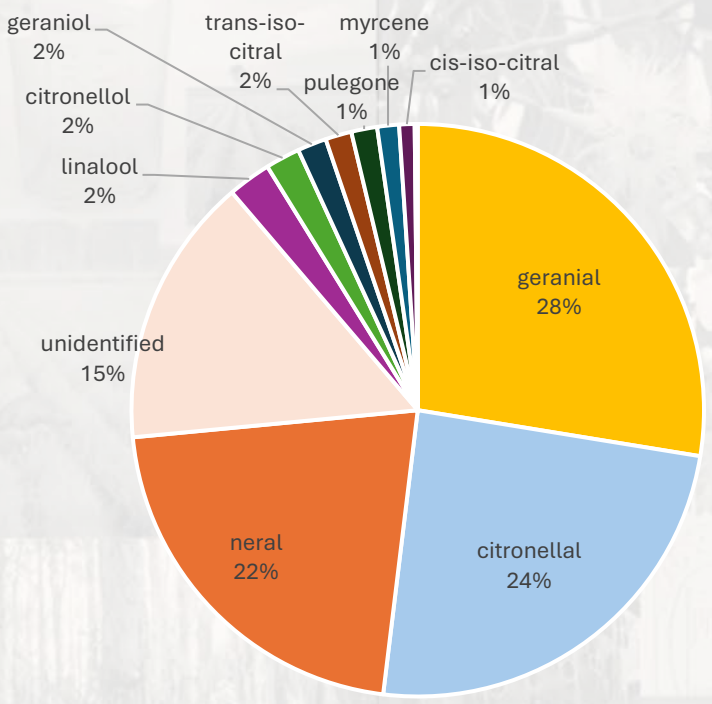
- Essential oil of *Eremophila mitchellii* is rich in Eremophilones at > 60%.
- Eremophilones belong to the Eremophilane class of sesquiterpenes also found in the genus *Callitropsis*, *Alpinia* and *Aquilaria.*
- *E. mitchellii* antimicrobial against five bacteria (*Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa and Alcaligenes faecalis*). (Wilkinson 2005)
- Saleh (2010) examined the antioxidant and ROS/ RNS radical scavenging activity of *Eremophila mitchellii* essential oil which was found to cause more than 90% inhibition of DPPH radical scavenging activity at 100, 25 and 5 mg/mL.
- Eremophilones are chemically related to Nootkatones, a class of molecules found in a variety of plants including their name-sake *Callitropsis nootkatensis.*
- Nootkatone and related eremophilanes are attributed with a range of activities including anti-inflammatory and anti-microbial (Alcantara, 2021)(Fan, 2022).

Citrals/ Citronellal

B. citriodora (Lemon myrtle)



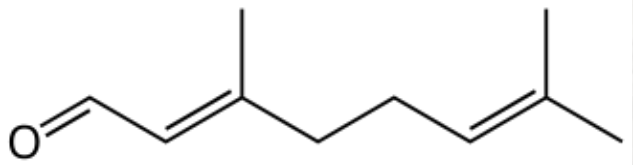
L. petersonii (Lemon Scented Tea Tree)



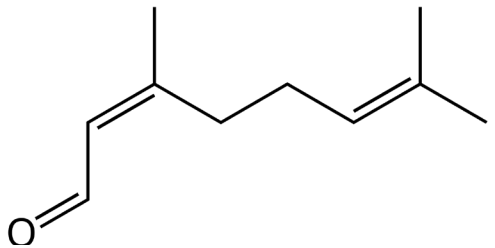
Lee, 2008 – Citral significantly inhibited

* nitric oxide (NO) production (IC50: 6.5 µg/mL).
* inhibited the transcriptional activity and expression of iNOS
* DNA binding activity and nuclear translocation of NF-κB
*IκB phosphorylation

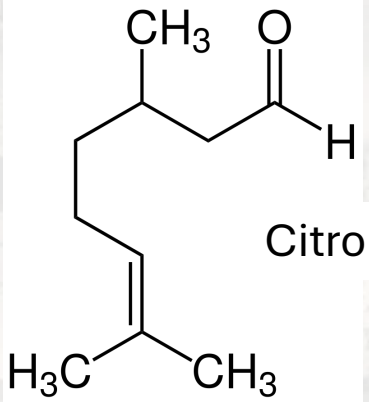
Citral is anti-inflammatory, and its effects may be due to the inhibition of NO production through the suppression of NF-κB activation.



Geranial



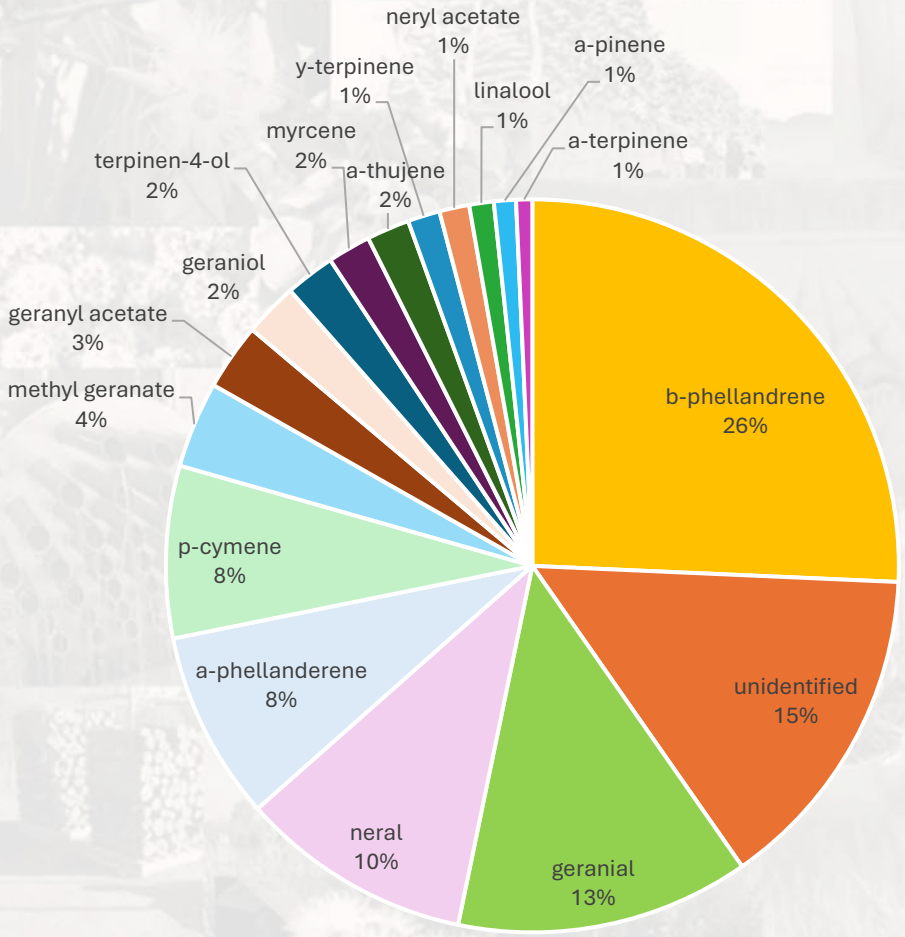
Neral

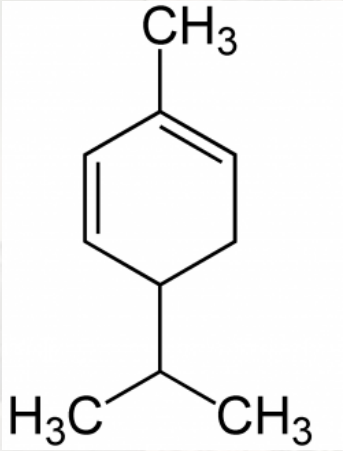


Citronellal

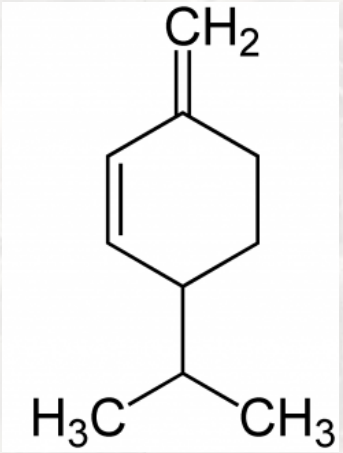
Phellandrenes

E. staigeriana (Lemon Ironbark)





α- phellandrene



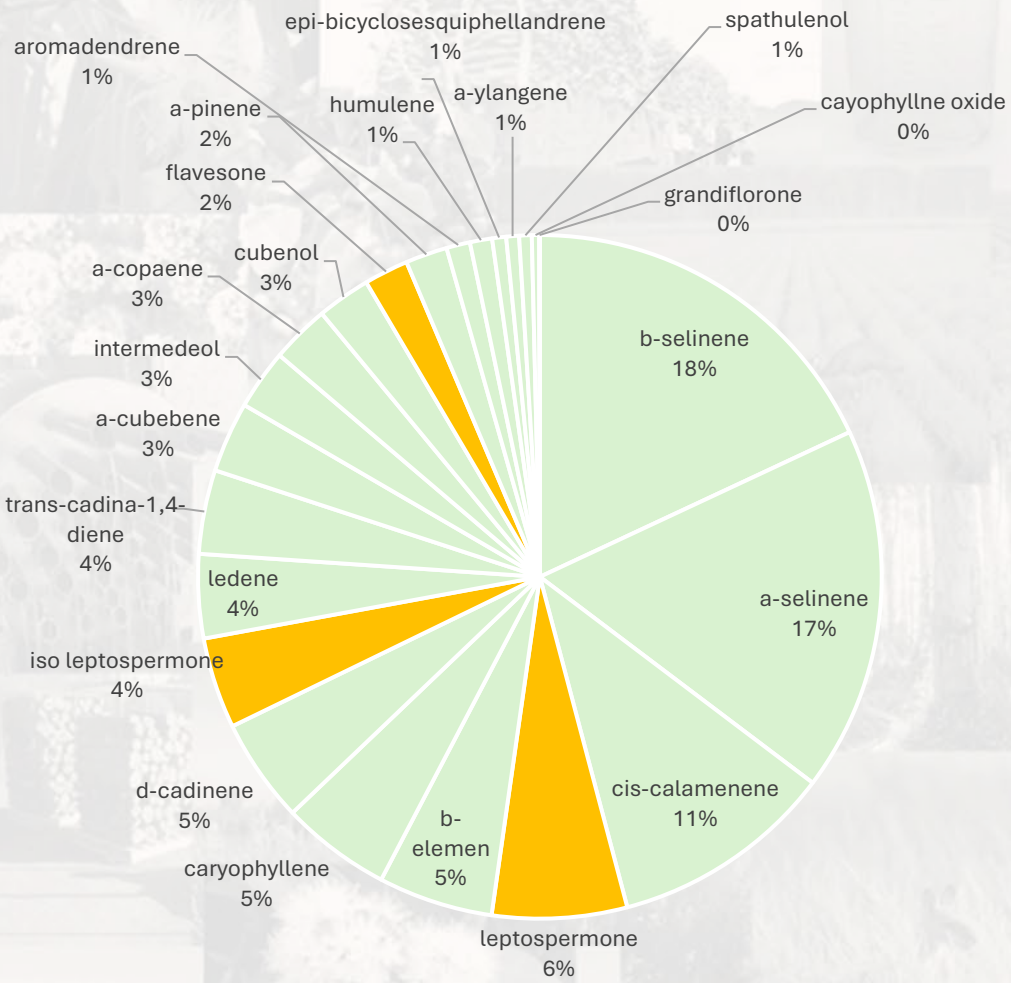
β - phellandrene

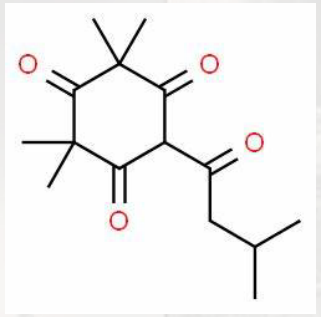
α-phellandrene significantly reduced NO production in in LPS-stimulated RAW 264.7 macrophages (de Christo Shere 2019).

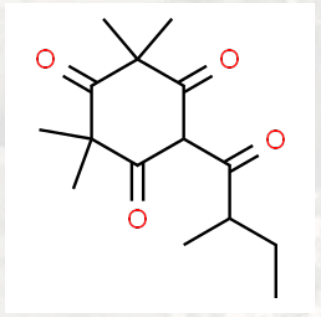
E. Staigeriana essential oil exhibited very high inhibition of NO compare with other Citral based oils. The high level of phellandrenes may be the source of this observed NO inhibition.

Beta-triketones

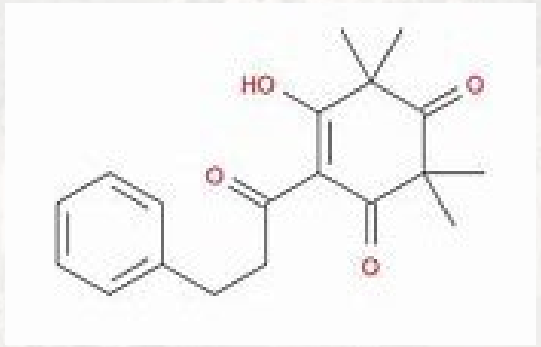
L. scoparium(Manuka)



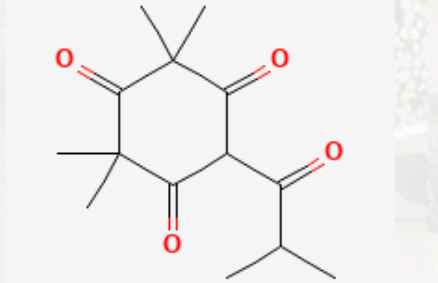




Beta – triketones are unusual compounds found in some essential oils. Derived from phloroglucinol they are structurally different to terpenes and have anti-microbial, anti-inflammatory and insect repelling properties.

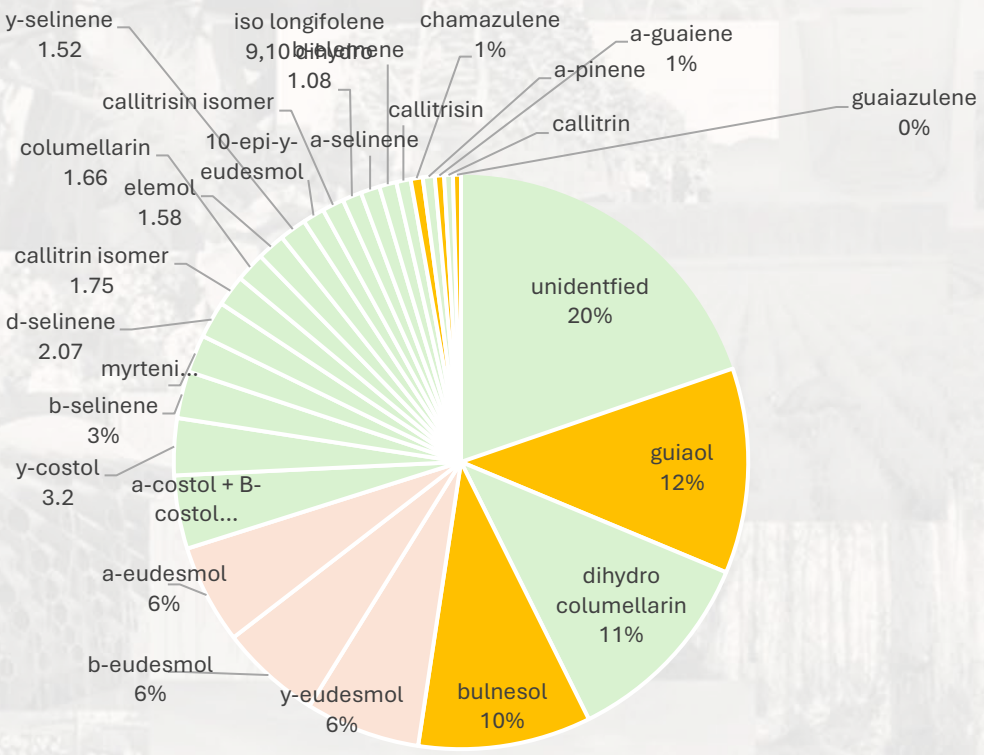


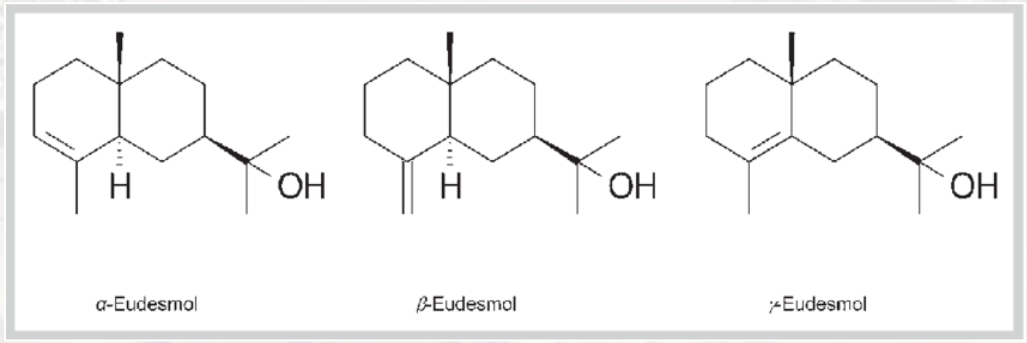
Anti-microbial activity is reported for oils of *L. scoparium* rich in beta-triketones (Jeong, 2018)(Mishra, 2018)(Porter, 2009).

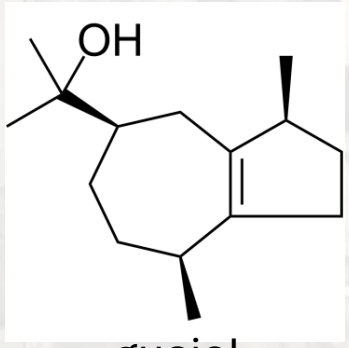


Azuleno + Eudesmol Sesquiterpenes

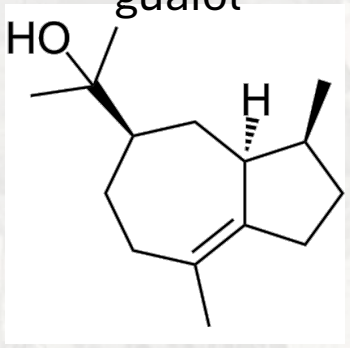
C. intratropica (Blue Cypress)



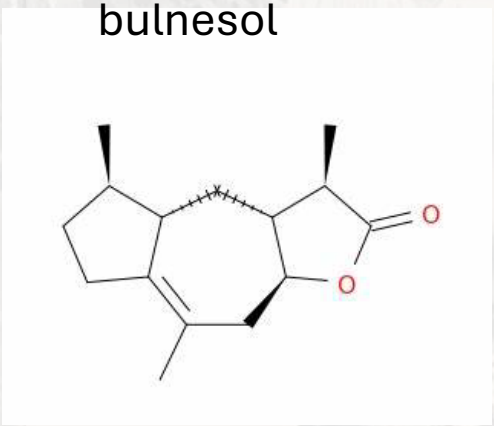




guaiol



bulnesol



dihydrocolumellarin

Essential oil of *Callitris intratropica* is rich in Azuleno and Eudesmol sesquiterpenes.

Eudesmols are a class of bicyclic sesquiterpenes with reported anti-allergic activity against mast cell-mediated allergic reactions (Han 2016). Acharya (2021) reported potential anti-tumor and anti-angiogenic activities.

C. intratropica oil was found to be anti-inflammatory (Zhao 2008) and anti-microbial(Wilkinson, 2005) (Powers, 2019)(Wanner, 2010)

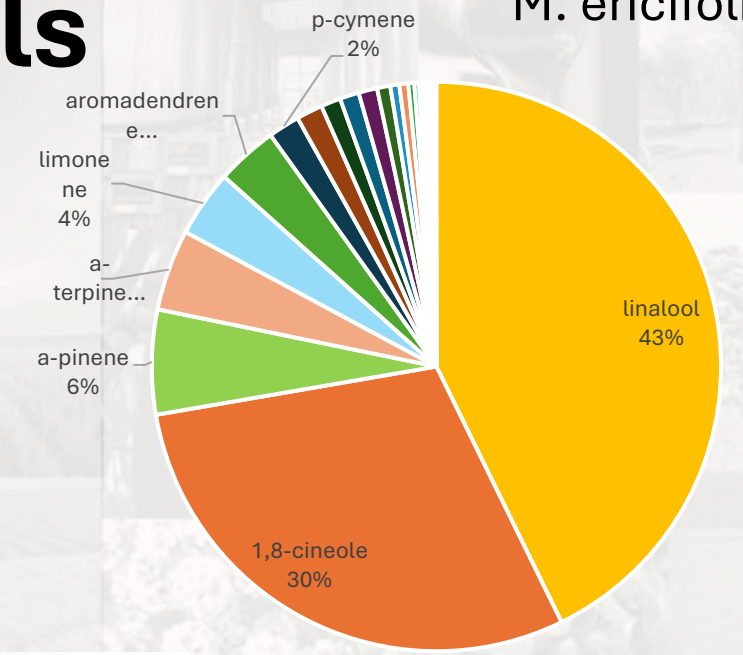
Guaiol, has reported anti-inflammatory activity (Ringrose, 1975).

Monoterpene Alcohols

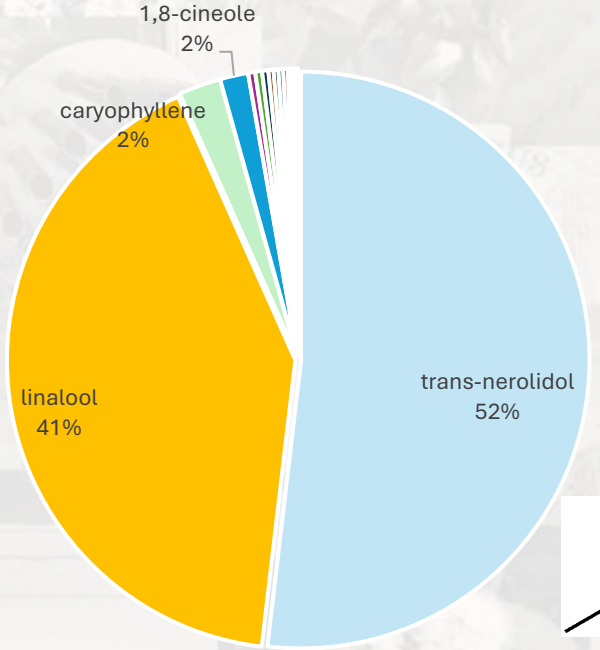
Monoterpene alcohols include linalool, and terpinen-4-ol.

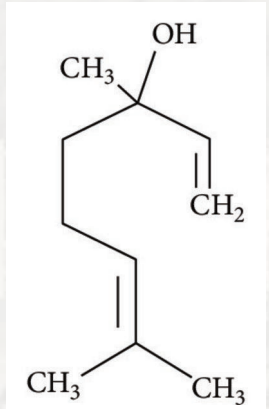
1,8-cineole is a monoterpene endoperoxide abundant in Eucalyptus oil used as decongestant.

M. ericifolia (linalool)

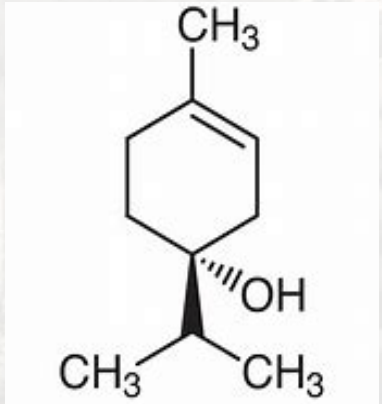


M. quinquenervia (Linalool/Nerolidol)

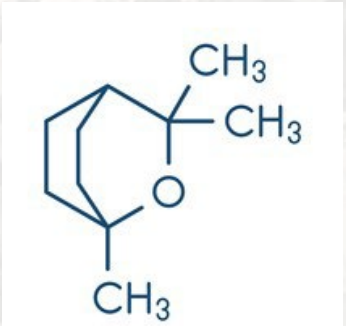




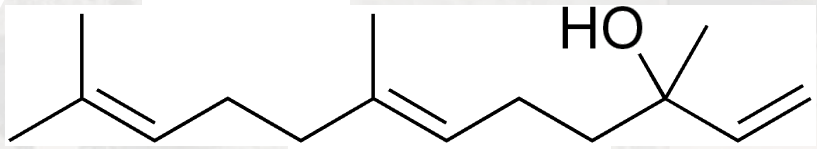
linalool



terpinen-4-ol

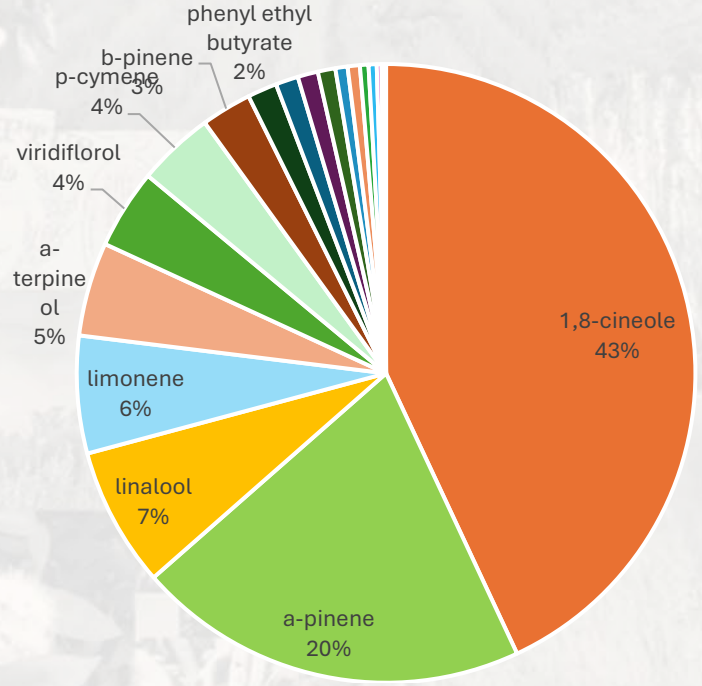


1,8-cineole



t-nerolidol

M. ericifolia (cineole)



Summary

- The range of essential oils examined, exhibited both anti-inflammatory and anti-microbial activity. In some cases, these were very significant.

- Oils containing monoterpene alcohols and aldehydes were generally effective but oils containing unusual sesquiterpenes (*Callitris intratropica*, *Eremophila mitchellii*) were notably active against anti-inflammatory assays as well as showing relatively high anti-microbial activity.

- The study highlights the range of active constituents present in various Australian native oils and presents the opportunity to selectively cultivate chemotypes to enhance efficacy and /or optimise distillation techniques to maximise the efficacy potential of essential oil products.

- The comparison of activity displayed for each essential oil supports the use of these essential oil products in therapeutic applications. Some products including essential oil of **B. citriodora** (Lemon myrtle), ***M. alternifolia*** (Tea Tree), ***K. ambigua*** (Kunzea) are already listed therapeutics, but others such as *Eremophila mitchelli* (potent anti-inflammatory activity) are not.

Acknowledgements

https://agrifutures.com.au/product/developing-industry-standards-for-australian-native-oils/