# Chemical composition and cytotoxicity of oils and eremophilanes derived from various parts of *Eremophila mitchellii* Benth. (Myoporaceae)

**Karren D. Beattie,[a*1] Peter G. Waterman,[a] Paul I. Forster,[b] Dion R. Thompson[a], David N. Leach[a]**

[a] Centre for Phytochemistry and Pharmacology, Southern Cross University, PO Box 157, Lismore NSW 2480, Australia

[b] Queensland Herbarium, Environmental Protection Agency, Brisbane Botanic Gardens, Mt Coot-tha Road, Toowong QLD 4266, Australia

*Corresponding author:

Dr Karren Beattie

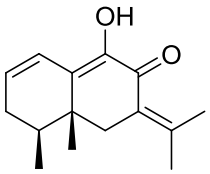
karren.beattie@hotmail.com

phone: + 61 2 6622 3211

fax: + 61 2 6622 3459

## Graphical Abstract

The biologically active wood, leaf, branch and root oils of *Eremophila mitchellii* (Benth.) were investigated in detail. 9-Hydroxy-1,7(11),9-eremophilatrien-8-one (**9**), and five previously identified eremophilane sesquiterpenes and the zizaene sesquithuriferone were isolated and elucidated.




**9**

[1] Present address: School of Pharmacy, Griffith University, Gold Coast campus, QLD, 4222, Australia.

***Research Highlights**

- *E. mitchellii* contains eremophilanes; a class of rare bicyclic sesquiterpenes
- The chemical composition of the wood, leaf, root and branch oils of *E. mitchellii*
- Isolation and elucidation of sesquithuriferone and six eremophilanes
- The eremophilanes inhibit P388$D_1$ cancer cell growth *in vitro*
- A revision of the NMR and mass spectroscopic data for the eremophilanes

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*Corresponding author:

Dr Karren Beattie

karren.beattie@hotmail.com

phone: + 61 2 6622 3211

fax: + 61 2 6622 3459

## Abstract

A detailed investigation of the wood, leaf, branch and root oil of *Eremophila mitchellii* (Benth.) was carried out by a combination of GC-FID, GC-MS and NMR. The wood oil was composed predominantly of eremophilanes, a rare class of biologically active, bicyclic sesquiterpenoids. The root oil was also found to contain the eremophilanes together with the zizaene sesquiterpene, sesquithuriferone . 9-Hydroxy-1,7(11),9-eremophilatrien-8-one and the known 1(10),11-eremophiladien-9-one (eremophilone), 9-hydroxy-7(11),9-eremophiladien-8-one (2-hydroxyeremophilone), 8-hydroxy-11-eremophilen-9-one (santalcamphor), 8-hydroxy-10,11-eremophiladien-9-one, sesquithuriferone and 8-hydroxy-

[1] Present address: School of Pharmacy, Griffith University, Gold Coast campus, QLD, 4222, Australia.

1,11-eremophiladien-9-one were purified and elucidated by NMR. Three approaches to the purification of the major eremophilanes from the wood oil are described. (+) Spathulenol, *α*-pinene, globulol, viridiflorene were the major constituents of the leaf oil. All of the essential oils and the eremophilanes exhibited cytotoxicity against $P388D_1$ mouse lymphoblast cells *in vitro*.

**Keyword Index**

*Eremophila mitchellii*; Myoporaceae; cytotoxic; sesquiterpene; eremophilane; essential oil; eremophilone; santalcamphor; wood oil.

## 1.0 Introduction

*Eremophila mitchellii* is a shrub or small tree that occurs in arid inland areas of New South Wales and Queensland. "*E. mitchellii* is drought resistant, capable of regenerating from the roots, recovers well after burning, ring barking, or cutting, and is an classified as an invasive species in some parts of Australia" (Cunningham et al., 1992). The tree is known colloquially as bastard sandalwood, buddah, budtha, or native sandalwood and bears a profusion of white flowers in spring (Cunningham et al., 1992). Its timber is widely described as possessing a very strong scent that is reminiscent to that of sandalwood (*Santalum* spp.)and historically its essential oil has been exploited commercially by the perfume industry (Bradfield et al., 1932a; Low, 1990). Currently there is a resurgence of interest into the commercial production of the wood oil of *E. mitchellii* owing to its insecticidal and termiticidal properties (Leach et al., 2004). The oil has shown promise for remedial applications as a fumigant, in chemical soil barriers and for the treatment and preservation of timber (Maupin et al., 2002; Beattie, 2009).

The eremophilanes were first reported from the wood oil of *E. mitchellii* in 1932 by Bradfield and co-workers (Bradfield et al., 1932a, 1932b). The elucidation of eremophilone (**1**) predated NMR spectroscopy and confirmation of the structure by chemical methods took almost 30 years to accomplish (Zalkow et al., 1959, 1960). In 1955 Robinson suggested that the eremophilones were in fact the first of a new class of sesquiterpenes, the eremophilanes, and were not based on the more common eudesmane skeleton as first thought (Figure 1) (Robinson, 1955). This was controversial at the time because it challenged Ruzicka's isoprene rule (Ruzicka, 1959) and demonstrated that methyl migrations were naturally possible in the biosynthesis of terpenoids. Since the discovery of eremophilone (**1**) and its oxygenated derivatives (**2**, **3**, **4** and **5**) only three additional eremophilanes, the keto aldehyde **6** and the dimers **7** and **8** have been reported from *Eremophila* despite intensive chemical investigation of the genus by Ghisalberti and co-workers (Figure 2) (Ghisalberti, 1994). The supposed rarity of the eremophilanes may be overstated given that most investigations have focused on leaf extracts and not on the constituents of the wood or roots.

There are several reports on the biological activity and ethnopharmacology of *E. mitchellii* in the literature. It is reported that the plant has been used by the aboriginal people as a treatment for rheumatism (Low, 1990). Kerr (1951) demonstrated that the wood oil was virtually non-toxic as a fly spray but when incorporated with the pyrethrins it had an adjuvant action, producing a marked increase in fly mortality in comparison to the pyrethrins alone. More recently Wilkinson and Cavanagh (2005) have reported on the antimicrobial activity of the wood oil against *Candida albicans* and five different bacteria. The undiluted oil showed inhibition against *Escherichia coli*, *Staphylococcus aureus, Salmonella typhimurium*, *Alcaligenes faecalis* and *Candida albicans*, no inhibition was observed against *Pseudomonas aeruginosa*.

A comprehensive chemical characterisation of the plant oil is necessary in order to establish yields and best practises for harvesting and oil production. Oil chemistry is fundamental to the quantitative and qualitative analysis of the oil for quality control as well as the requisite studies needed to ascertain the efficacy, stability, pharmacology and toxicology of such a chemically-complex bioproduct. At present, the published NMR data for the eremophilanes is only very low resolution (60 MHz) and incomplete. Consequently, a comprehensive revision of the NMR data has been reported here. There are no published reports on the chemical composition of extracts of the leaf, root or branchlets. This paper reports the investigation of the distribution, yield, and variation in the chemical composition of the oil in the whole plant and also describes the purification of the major constituents of the steam distilled wood and root oil of *E. mitchellii*. All of the essential oils together with the eremophilanes **1**, **3** — **5** were evaluated for cytotoxicity against $P388D_1$ mouse lymphoblast cells *in vitro.*

## 2.0 Results and Discussion

### *2.1 Purification of the eremophilanes from E. mitchellii*

Isolation of the eremophilanes from *E. mitchellii* was achieved using both reverse phase (RP, C18) and NP phase (silica) preparative chromatography techniques. Large scale NP low pressure column chromatography was invaluable for enriching several grams of the major constituents and/or obtaining enriched fractions containing the minor constituents. RP chromatography was efficient for one-step purification of several milligrams of the major constituents; **1**, **3**—**5**.

### *2.2 Purification and elucidation of 9-hydroxy-1,7(11),9-eremophilatrien-8-one*

Compound **9** co-eluted as a minor constituent with **1** from NP preparative HPLC. It became apparent that **9** and was highly unstable since only trace levels of this compound were present after 24 hours in solution and not present in some commercial oils. The stability of **9** was monitored in multiple solvents to determine the optimal solvents for purification work. It was observed that the order of increasing stability was; $CH_3CN$, acetone, $CH_3CN$/0.5 % TFA, EtOAc, $CHCl_3$, DMSO, EtOH, MeOH, and hexane. No discernible breakdown products or increases in relative peak intensities were observed by GC-MS.

Purification of **9** from the minor constituents was achieved by subjecting a freshly prepared, enriched NP preparative HPLC fraction immediately to RP preparative HPLC. An isocratic gradient and 2 preparative columns connected in tandem were required to achieve adequate resolution of **9** from **1**. Compound **9** was obtained as a yellow gum in a yield of approximately 0.4 % (w/w) of the crude oil. The EI mass spectrum for this compound gave a molecular ion $[M^+]$ at *m/z* 232. HRAPCIMS established the molecular weight $[M+H]^+$ 233.1514 that was consistent with the molecular formula $C_{15}H_{20}O_2$ and 6 DBE.

Compound **5** and **9** exhibited similar $^{13}C$ JMOD spectra with the main difference being the absence of two methylenes ($\delta_C$ 23.8 and 25.8 ppm) and the presence of two additional methines ($\delta_C$ 122.0 and 134.3 ppm). Like **5**, **9** possessed a highly deshielded carbonyl group ($\delta_C$ 185.5 ppm) situated in an *α,β,α′,β′* dieneone system. Compared to **5** the $^1H$ NMR spectrum of **9** showed two additional, highly deshielded, peaks at $\delta$ 6.63 and at $\delta$ 6.10 ppm as part of an $ABX_2$ system consisting of two vinylic protons ($\delta_H$ 6.63, dd, $J$ = ~2.7, ~9.9 Hz; 6.10 (ddd, $J$ = 2.6, 5.7, 9.8 Hz) adjacent to a methylene group ($\delta_H$ 2.20, m; 2.03, m). HSQC and HMBC correlations confirmed the presence of a C-1–C-2 double bond and was in agreement with the novel compound 9-hydroxy-1,7(11),9-eremophilatrien-8-one (**9**). Owing to the instability of the compound in solution a crystal structure could not be obtained,

consequently the stereochemistry of C-4 and C-5 has been assumed on the basis of its affinity to the preceding eremophilanes **1**, **3**, **4** and **5**.

### *2.3 Purification and elucidation of sesquithuriferone from the root oil of E. mitchellii*

Compound **10** was isolated using a combination of low-pressure NP chromatography and RP preparative HPLC and was elucidated as the known zizaene sesquiterpene, sesquithuriferone on the basis of HSQC, HMBC, TOCSY, COSY and nOe experiments. The relative stereochemistry was confirmed on the basis of nOe difference experiments.

Sesquithuriferone (**10**) has previously been isolated from *E. georgei* (Ghisalberti, 1976), *E. metallicorum*, (Ghisalberti, 1994) and *E. subteritifolia* (Carrol et al., 1976). The published $^{1}$H NMR data for sesquithuriferone is limited (Barrero et al., 2000), and due to lack of material **10** was first characterised as its *p*-bromobenzoate derivative (Carrol et al., 1976). The structure was confirmed by X-ray diffraction studies (Ghisalberti et al., 1976). At the time of compiling this article Adams (2007) reports, on the basis of mass spectral data, that **10** is a component of *E. mitchellii* wood oil. Throughout the course of this research we have observed that sesquithuriferone is prevalent in the root-heartwood and that no traces of sesquithuriferone were observed in carefully prepared heartwood oil.

### *2.4 Yield, distribution and chemical variation of the essential oils*

To assist with commercialisation of the oil, studies were undertaken to investigate the distribution and chemical variation of the oil in the plant. A whole specimen was collected and each of the plant parts were steam distilled separately, the yields and descriptions are detailed in Table 1. A cross section of the tree trunk revealed a pale yellow timber with a distinctive red-brown heartwood and coarse bark. No oil was obtained from distillations of the bark or outer lightly coloured wood.

The essential oils of the leaves, branchlets, wood and roots were analysed by GC-MS and GC-FID, more than thirty components have been identified in the essential oils (Table 2). It is apparent from these results that the yield and chemical composition varies greatly depending on the plant part.

### *2.4.1 Chemical composition of the heartwood oil*

The heartwood yielded an aromatic oil (2.2 % w/w) that was also predominantly composed of sesquiterpenes. Three of the major constituents identified in the heartwood oil were eremophilone, santalcamphor, and 9-hydroxy-7(11),9-eremophiladien-8-one and their occurrence in *E. mitchellii* has been well documented (Bradfield 1932a, 1932b; Massy-Westropp and Reynolds 1966; Adams 2007). A fourth major constituent, the novel 9-hydroxy-1,7(11),9-eremophilatrien-9-one proved to be very unstable. These four major constituents account for 80 % of the wood oil.

Several attempts to isolate the minor constituents of the heartwood oil met with limited success. Their isolation and characterisation is complicated because they share similar molecular weights and many were found to co-elute with the major compounds throughout HPLC (both NP and RP) and GC-MS (both polar and apolar systems). Several structural analogues of the eremophilones have been reported in the literature including the aldehyde **2** (Abel and Massy-Westropp, 1985), isoeremophilone (**11**) (Chetty and Zalkow, 1969), alloeremophilone (**12**) (Bates and Paknikar., 1966) and the dimers **7** and **8** (Lewis et al., 1979, 1982). The analogues, if present, appear to be very minor constituents. The sesquiterpene ketones have the capacity to tautomerise however it is apparent that the major keto-tautomers are thermodynamically favoured. Interestingly, it is reported that eremophilone (**1**) does not readily convert to its $\Delta^{1(2)}$ isomer, isoeremophilone (**11**) under mild conditions (Zalkow and Chetty, 1975). The interconversion of the eremophilanes via isomerisation, dehydration and

hydrogenation has been reported by chemical methods (Zalkow and Chetty, 1975; Djerassi et al., 1959).

The assignment of *α*- and *β*-selinene (synonymous with eudesmenes) in *E. mitchellii* oil was based on comparison with authentic *α*- and *β*-selinene from commercial celery seed oil (*Apium graveolens*). This finding is also in accord with the observation that eudesmene is a biosynthetic precursor of the eremophilane sesquiterpenes (Cane et al., 1990).

### *2.4.2 Chemical composition of the root oil*

The roots yielded an aromatic oil (0.3 % w/w) that was composed of two major constituents, eremophilone (**1**) and sesquithuriferone (**10**) together with very low concentrations (most <1 %) of the sesquiterpenes **3** — **6** and **9** prevalent in the wood oil.

### *2.4.3 Chemical composition of the leaf oil*

Distillation of the leaves yielded a fragrant, green-black oil (1.4 % w/w) that was chemically complex and distinct from the wood and root oils. Twenty seven compounds, predominantly sesquiterpenes, have been identified by CG–MS from the leaf oil of *E. mitchellii*. The three major compounds identified in the leaf oil were *α*-pinene, spathulenol and an unidentified sesquiterpene alcohol which together account for 45 % of the oil.

With the support of spectral data and the corresponding reference compounds it was possible to confirm the presence of the monoterpenes; *α*- and *β*-pinene, *α*- and *β*-phellandrene, *p*-cymene, limonene, *α*-terpinolene, linalool, *α*-terpineol and eugenol on the basis of their prevalence in essential oils. Harborne (1998) reports that *α*- and *β*-pinene, limonene, $\Delta^3$-carene, *α*-phellandrene and myrcene are ubiquitous in leaf oils. In addition, Ghisalberti (1994) reports that bisabolene, eudesmane, eremophilane, aromadendrane, cadinane, zizaene, spathulenol and elemol classes of sesquiterpenes have all been isolated

from the leaf material of *Eremophila* species. With the support of spectral data, reference compounds and inference from metabolites reported from *Eremophila* species it was possible to confirm the presence of aromadendrene, *trans*-*β*-caryophyllene, *δ*-cadinene, *α*- and *β*-selinene, elemene, epiglubulol, globulol, *α*- and *β*-eudesmol, bisabolol, viridiflorene and viridiflorol.

It is apparent that different biosynthetic pathways are operating in the leaf compared to the wood. Inspection of the selective-ion chromatogram of the sesquiterpene-diene ion, $C_{15}H_{24}$, *m/z* 204 (retention time; 40–47 minutes) for the wood oil indicates that only six peaks are apparent (see Supporting Information). In contrast, the selective ion chromatogram for the ion *m/z* 204 for the leaf oil exhibits a multitude of peaks in this region. Unlike ions of similar molecular weights, *m/z* 204, in this context, gives rise to a single rational molecular formula, $C_{15}H_{24}$ which, within selected retention indices, corresponds to a sesquiterpene hydrocarbon parent or fragment ion that posesses four double bond equivalents. Inspection of the fragmentation patters for the *m/z* 204 peaks indicates that fewer sesquiterpene classes are represented in the wood oil compared to the leaf oil, and conceivably the eremophilene or selinene analogues could account for these. To date, aside from the selinenes, the eleven sesquiterpenes isolated from *E. mitchellii* have been of the eremophilene type.

### *2.4.4 Chemical composition of the branchlet oil*

The branchlets (twigs) yielded only minor quantities of oil (0.04 % w/w) that exhibited a chemical composition that was intermediate between the leaf and the wood oil.

## *2.5 Regarding the qualitative and quantitative analysis of E. mitchellii oils*

Currently, only a handful of the eremophilane sesquiterpenes are represented in commercial mass spectral libraries. A rudimentary structural assignment on the basis of the

mass spectra and retention indices inevitably indicates (with excellent correlation) the presence of selinenes, humulenes, bisabolols, gurjunenes, maalienes and patchoulenes in the oils. However, given the tendency for *Eremophila* species to exhibit unusual stereochemistry (Ghisalberti, 1994) and the structural similarity between the eremophilanes and other bicyclic sesquiterpenes the identity of many of the minor components can only be confidently assigned with the support of NMR data.

Qualification of the oils on the basis of mass spectra (see Supporting Information) is also difficult since many components share the same molecular weight, may co-elute and/or possess similar fragmentation patterns. (Adams (2007) has erroneously ascribed the mass spectrum of **5** as being that of compound **6**). Notably santalcamphor (**4**) and 8-hydroxy-1,11-eremophiladien-9-one (**3**) were found to co-elute by GC-MS (both polar and apolar systems) but may be differentiated by inspection of their molecular ions [$M^+$] at *m/z* 236 and 234 respectively. Similarly, 9-hydroxy-7(11),9-eremophiladien-8-one (**5**) co-elutes with 8-hydroxy-10,11-eremophiladien-9-one (**6**) (synonymous with 8-hydroxyeremophilone). The presence of the minor constituent **6** can be distinguished by the existence of its' fragment ion *m/z* 205 eluting slightly later than the major molecular ion [$M^+$] at *m/z* 234 for compound **5**. Additionally for eremophilones bearing an $\alpha$-hydroxyl group, inspection of the fragmentation pattern revealed a pronounced fragment ion $[M-29]^+$ corresponding to loss of CHO.

### *2.6 In-vitro cytotoxic activity of the essential oils and eremophilanes*

The cytotoxic activity of the oils and pure compounds was tested *in-vitro* against P388D1 mouse lymphoblast cells (Table 3, Figs. 3 and 4). All of the samples tested effected potent dose-dependent growth inhibition of this cell line. The crude essential oils exhibited half-maximal inhibitory concentrations ($IC_{50}$) between 51—110 μg/mL, whilst the pure compounds **1**, **3**, **4** and **5** exhibited $IC_{50}$ values between 42 —105 μg/mL. Eremophilone was

the most active isolate in this study, and was comparable in potency to the curcumin control ($IC_{50}$ 19 μg/mL). It is also interesting to note that the leaf oil which is chemically distinct from the wood or root oils also exhibited significant cytotoxic effects.

## 3.0 Experimental

### *3.1 General experimental procedures*

NMR spectra were obtained on a Bruker Avance DRX-500. XWin NMR software was used to analyze the spectral data. COSY, NOESY, HSQC, HMBC, TOCSY and nOe diff experiments were acquired using the standard Bruker pulse programs. The chemical shifts were calibrated relative to the $CDCl_3$ solvent peak ($^1H$, $\delta$7.27 and $^{13}C$, $\delta$77.23 ppm). Optical rotations $[\alpha]_D$ were determined using a Jasco P-1010 polarimeter fitted with a sodium lamp (589 nm). Solvents, concentrations and temperatures are as specified in the text. Concentration c is in g/l00 mL; the units of the specific rotation are $° \cdot mL \cdot g^{-1} \cdot dm^{-1}$. High resolution-mass spectrometric analyses were performed by Chemical Analysis Laboratories (Bulleen VIC, Australia) using an LC-MS (Agilent G1313A) interfaced to a TOF-MS (Agilent G1969A) fitted with an APCI source (Agilent G1978A) scanning at a mass range of 50–1100 a.m.u. The mobile phase consisted of $H_2O$-MeOH (1:1) with 0.1% formic acid at a flow rate of 0.3 mL/min operating at ambient temperature. Nitrogen was used as the nebulising gas at a pressure of 10 psi and vaporiser temperature, drying gas flow and capillary voltage were set to 350°C, 4 mL/min, 3000 V respectively. Melting points were determined by a Gallenkamp melting point apparatus and are uncorrected. MilliQ (MQ) water and HPLC grade solvents were employed throughout the course of this research.

### *3.2 Plant materials and steam distillation*

An entire specimen of *E. mitchellii* was collected near Eidsvold, South-East Queensland in December 2005, and dried at 40°C prior to processing. A voucher specimen was deposited at the Queensland Herbarium (PIF 30486). A sample of the commercially available steam distilled wood oil (1000 mL) was obtained from Cavanagh and Sons (Port Macquarie, NSW, Australia) and utilized for the isolation of the eremophilanes. Wood, root, bark and branchlet materials were ground using a Retsch cutting mill (Retsch GmbH, Haan, Germany: SM 100). The intact leaf material and ground wood, root, bark and branchlet materials (ca. 500 g) were steam distilled for 48 h in a distillation flask (5 L) fitted with a modified Cocking and Middleton trap.

### *3.3 Gas chromatography*

GC-MS analyses were performed using a GC-MSD system (Agilent Technologies 6890/5973) with helium as the carrier gas at a constant linear velocity of 28 cm/s. The transfer, source and quadrupole temperatures were 280°C, 230°C and 150°C respectively, operating at 70 eV ionisation energy. The columns used were an SGE Ltd. BPX5 capillary column (50.0 m × 0.22 mm ID × 1$\mu$m film thickness) programmed from 50˚C to 300˚C at 4˚/min and a BP-20 column (50.0 m × 0.22 mm ID × 1$\mu$m film thickness) programmed from 50˚C to 260˚C at 4˚/min. Oil samples (20 µL) were diluted with acetone (1000 µL). The injection volume was 0.2 µL, the split ratio was 1:40 and the injector temperature was 280˚C. The analytical GC system used was a Hewlett Packard GC system (HP6890) fitted with an Agilent 7683 injector using the same instrument parameters as above and an FID detector temperature of 300˚C. The column used was an SGE Ltd. BPX5 capillary column (50.0 m × 0.22 mm ID × 1$\mu$m film thickness).

### *3.4 Characterisation of the oil by gas chromatography*

Composition values were calculated as percentage area based on the FID chromatogram. Identification of the individual components was based on: (i) comparison with the mass fragmentation spectra of authentic reference compounds where possible and by reference to WILEY275, NIST 98, and Adams terpene library (Adams, 2007); (ii) comparison of their Kovats/log retention indices (RI) on a BPX-5 (polar, 5 % phenyl polysilphenylene-siloxane) and a BP-20 (polar, polyethylene glycol) column, calculated relative to the retention times of a series of C-8 to C-22 *n*-alkanes, with linear interpolation, with those of authentic compounds or literature data; (iii) by comparison of the MS, NMR and retention time data of the pure compounds isolated in this study.

Linalool, *α*-pinene, *β*-pinene, *p*-cymene, limonene, *α*-terpineol and eugenol were obtained from Aldrich chemical Co. Inc. (Milwaukee, WI); *α*-phellandrene, *α*-terpinolene, viridiflorene (syn. ledene), globulol and *α*-bisabolol were obtained from Fluka Chemie (Buchs, Switzerland); aromadendrene was obtained from Sigma-Aldrich (Castle Hill, NSW Australia). Epiglobulol, viridiflorol and *δ*-cadinene were identified by comparison with these compounds in authentic tea tree oil (*Melaleuca alternifolia*, FPI Oils, Melbourne, Australia). Elemol, *α*- and *β*-eudesmol and *α*- and *β*-selinene were identified by comparison with these compounds from celery seed oil (*Apium graveolens*, Auroma, Melbourne, Australia). Cadina-1,4-diene, *β*-phellandrene and *trans-β*-caryophyllene were identified by comparison with these compounds from betel leaf oil (*Piper betle*, FPI Oils, Melbourne, Australia). *β*-Elemene was identified by comparison with this compound from myrrh oil (*Commiphora myrrha*, FPI Oils, Melbourne, Australia). Spathulenol was identified by comparison with this compound from angelica root oil (*Angelica archangelica*, FPI Oils, Melbourne, Australia).

### *3.5 Preparative HPLC*

Fractionation of the oils was performed on a Gilson preparative HPLC system employing a Gilson 322 binary pump system, a Gilson 156 UV-Vis dual wavelength detector set at 210 nm and 280 nm, and Gilson fraction collector (FC204). A C18 column (Phenomenex Luna C18, 5 μm, 50 mm × 21.2 mm) was used for RP separations and solvent A MQ water (100%) and solvent B, $CH_3CN$ (100%) both containing 0.05 % TFA were used as the mobile phase. NP separations were achieved using a silica column (Phenomenex Luna 5 μm Silica (2), 50 mm × 21.20 mm) utilizing a hexane/EtOAc gradient operating at ambient temperatures. The sample loading was between 50-100 mg/injection (RP) or 300-500 mg/injection (NP). The preparative HPLC was interfaced with Gilson Unipoint v.3.0 software. Unless specified the fractions were dried using a rotation vacuum centrifuge (RVC) (Martin Christ, Germany) and combined as appropriate after verification by LC-MS or GC-MS.

### *3.6 RP fractionation of E. mitchellii wood oil*

Pure compounds **3**, **4**, **1** and **5** (in order of elution) were obtained directly from the crude oil using RP preparative HPLC. The eluent was a gradient of $H_2O$-ACN (3:2 to 3:7) with 0.05%TFA over 15 min, followed by an isocratic gradient of $H_2O$-ACN (3:7) with 0.05 % TFA for 5 min, then a gradient of $H_2O$-ACN (3:7 to 1:9) with 0.05% TFA over 5 min, at a flow rate of 15 mL/min. Compounds were selectively cut at the appropriate intervals and recovered from the eluent by rotary evaporation.

### *3.7 NP fractionation of E. mitchellii wood oil*

Semi-purified compounds; **5**, **9**, **1**, **4**, **6** and **3** (in order of elution) were obtained in yields of approximately 14, 1, 19, 20, 1 and 4 % (w/w) of the oil respectively, using NP preparative HPLC. The eluent gradient was EtOAc-hexane (1:20 to 2:3) over 20 min, at a flow rate of 20 mL/min. Compound **4** readily crystallised as colourless needles and could be purified by

recrystallisation from MeOH. Further purification of the remaining compounds (with the exception of **9**) was achieved using RP preparative HPLC under the conditions described above. Compound **9** co-eluted with **1** under NP conditions and was purified by RP preparative HPLC utilizing two preparative columns connected in tandem and an isocratic gradient of MeOH-$H_2O$ (7:3) over 55 min at a flow rate of 15 mL/min.

### *3.8 Large scale fractionation of the wood oil*

A rapid silica column chromatographic method was developed for large scale fractionation of the wood oil. The column (0.063–0.200 mm silica, Merck, Kilsyth VIC, Australia; 3.5 cm diam., 270 mL bed volume (BV), dry packed, ca. 6.5 mL sample loading) was eluted with hexane (100 %, 1 × 250 mL) followed by EtOAc-hexane (1:20, 4 × 125 mL), (1:10, 4 × 125 mL), (3:20, 4 × 125 mL) and EtOAc (100 %, 1 × 250 mL) to obtain semi-purified compounds **5**, **1**, **4**, **6** and **3** (in order of elution).

### *3.9 Compounds from E. mitchellii wood oil*

1(10),11-Eremophiladien-9-one (eremophilone) (**1): ** colourless oil (90.9 mg, 15.2 %); $[\alpha]_D^{20}$ -78° (c 0.49, MeOH); $[\alpha]_{Hg}$ -207 (MeOH) Lit. (Bradfield et al., 1932a); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.96 (d, 3, $J$ = 6.8 Hz, H-14), 0.97 (s, 3, H-15), 1.51 (m, 1, H-$6_b$), 1.51 (m, 2, H-3), 1.63 (m, 1, H-4), 1.75 (s, 3, H-13), 1.97 (m, 1, H-$6_a$), 2.23 (m, 2, H-2), 2.36 (m, 1, H-7), 2.41 (m, 2, H-8), 4.74 (s, 1, H-$12_a$), 4.77 (t, 1, $J$ = 1.4 Hz, H-$12_b$), 6.60 (t, 1, $J$ = 3.8 Hz, H-1); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 16.2 (q, C-14), 20.8 (q, C-13), 25.0 (q, C-15), 25.8 (t, C-2), 26.7 (t, C-3), 36.2 (s, C-5), 39.0 (d, C-4), 39.3 (d, C-7), 41.6 (t, C-6), 43.4 (t, C-8), 110.2 (t, C-12), 135.5 (d, C-1), 144.5 (s, C-10), 147.8 (s, C-11), 204.0 (s, C-9); EIMS (70eV) *m/z* (rel. int.): 218 ($M^{\cdot}$, 67), 203 (30), 185 (7), 176 (100), 161 (57), 147 (39), 133 (73), 119 (58), 107 (82), 91 (89), 79 (88), 67 (38), 53 (33), 41 (69).

8-Hydroxy-1,11-eremophiladien-9-one (**3**): yellow oil (5.7 mg, 0.95 %); $[\alpha]_D^{20}$ +167° (c 0.14, $CHCl_3$); $[\alpha]_D^{19}$ +59.3 (c 1.1) Lit. (Massy-Westropp and Reynolds, 1966); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.82 (d, 3, $J$ = 6.7 Hz, H-14), 1.00 (s, 3, H-15), 1.61 (m, 1, H-4), 1.66 (d, 1, $J$ = 13.8 Hz, H-$6_b$), 1.76 (m, 1, H-$3_a$), 1.84 (s, 3, H-13), 1.93 (dd, 1, $J$ = 14.6, 3.0 Hz, H-$6_a$), 2.10 (td, 1, $J$ = 5.0, 18.2 Hz, H-$3_b$), 2.33 (dt, 1, $J$ = 3.0, 12.1 Hz, H-7), 2.78 (d, 1, $J$ = 5.1 Hz, H-10), 4.12 (dd, 1, $J$ = 1.3, 11.4 Hz, H-8), 4.90 (s, 1, H-$12_a$), 4.93 (t, 1, $J$ = 1.4 Hz, H-$12_b$), 5.67 (m, 1, H-1), 5.88 (m, 1, H-2), consistent with lit. (Massy-Westropp and Reynolds, 1966); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 14.4 (q, C-14), 19.4 (q, C-13), 21.0 (q, C-15), 30.4 (d, C-4), 32.2 (t, C-3), 39.1 (t, C-6), 40.0 (s, C-5), 48.4 (d, C-7), 56.3 (d, C-10), 77.0 (d, C-8), 112.6 (t, C-12), 122.5 (d, C-1), 129.9 (d, C-2), 145.0 (s, C-11), c.a 212 (s, C-9); EIMS (70eV) *m/z* (rel. int.): 234 ($M^{\cdot}$, 9 %), 216 (5), 207 (15), 191 (10), 173 (14), 159 (6), 150 (16), 137 (14), 121 (24), 107 (93), 93 (100), 77 (52), 67 (40), 55 (38).

8-Hydroxy-11-eremophilen-9-one (santalcamphor) (**4**): colourless needless (95.9 mg, 16.0 %); mp 99–100°C; 102–103°C Lit. (Bradfield et al., 1932a); $[\alpha]_D^{20}$ +112° (c 0.26, $CHCl_3$); $[\alpha]_D$ +90.6° ($CHCl_3$) Lit. (Bradfield et al., 1932a) and assumes the same absolute stereochemical assignments published by Zalkow and co-workers (Zalkow et al., 1959); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.79 (d, 3, $J$ = 6.5 Hz, H-14), 1.05 (s, 3, H-15), 1.32 (m, 1, H-$3_a$), 1.43 (m, 1, H-4), 1.43 (m, 1, H-$3_b$), 1.55 (m, 1, H-$2_a$), 1.55 (m, 1, H-$6_a$), 1.55 (m, 1, H-$1_b$), 1.65 (m, 1, H-$2_b$), 1.82 (s, 3, H-13), 1.91 (dd, 1, $J$ = 3.1, 14.5 Hz, H-$6_b$), 2.08 (m, 1, H-$1_a$), 2.31 (s, 1, H-9), 2.42 (dt, 1, $J$ = 12.2, 3.1 Hz, H-7), 4.00 (d, 1, $J$ = 11.3 Hz, H-8), 4.89 (s, 1, H-$12_a$), 4.91 (t, 1, $J$ = 1.4 Hz, H-$12_b$); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 15.4 (q, C-14), 19.6 (q, C-13), 21.0 (t, C-1), 21.5 (q, C-15), 22.4 (t, C-2), 30.3 (t, C-3), 33.9 (d, C-4), 40.1 (t, C-6), 41.3 (s, C-5), 48.1 (d, C-7), 54.2 (s, C-10), 76.7 (d, C-8), 112.3 (t, C-12), 145.3 (s, C-11), 211.9 (d, C-9); EIMS (70eV) *m/z* (rel. int.): 236 ($M^{\cdot}$, 29 %), 221 (3), 207 (100), 189 (15), 179 (5), 167 (7), 149 (8), 135 (10), 123 (40), 109 (56), 95 (39), 81 (35), 69 (74), 55 (44), 41 (50).

9-Hydroxy-7(11),9-eremophiladien-8-one (2-hydroxyeremophilone) (**5**): yellow oil (267.6 mg, 44.6 %); $[\alpha]_D^{20}$ +63° (c 2.13, MeOH); $[\alpha]_D^{25}$ +138° (c 2.59, MeOH); Lit. (Pinder and Torrence, 1971); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.94 (d, 3, $J$ = 6.5 Hz, H-14), 0.96 (s, 3, H-15), 1.42 (m, 1, H-$2_a$), 1.42 (m, 1, H-$3_a$), 1.49 (m, 1, H-4), 1.54 (m, 1, H-$3_b$), 1.86 (m, 1, H-$2_b$), 1.90 (s, 3, H-13), 1.95 (m, 1, H-$1_b$), 2.11 (m, 1, H-$6_a$), 2.18 (d, 3, $J$ = 2.0 Hz, H-12), 2.88 (d, 1, $J$ = 13.7 Hz, H-$6_b$), 2.98 (m, 1, H-$1_a$), consistent with lit. (Pinder and Torrence, 1971); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 15.7 (q, C-15), 16.4 (q, C-14), 23.0 (q, C-13), 23.2 (q, C-12), 23.8 (t, C-1), 25.8 (t, C-2), 30.8 (t, C-3), 39.8 (s, C-5), 40.7 (t, C-6), 43.1 (d, C-4), 125.9 (s, C-7), 137.4 (s, C-10), 142.7 (s, C-9), 146.7 (s, C-11), 185.7 (s, C-8); EIMS (70eV) *m/z* (rel. int.): 234 ($M^{\cdot}$,73 %), 219 (48), 201 (25), 191 (57), 177 (43), 163 (100), 153 (91), 137 (42), 124 (12), 115 (19), 105 (24), 91 (55), 77 (38), 67 (37), 55 (39), 41 (81).

8-Hydroxy-10,11-eremophiladien-9-one (**6**): yellow oil (47 mg, 0.7 %, not optimised); $[\alpha]_D^{20}$ –36° (c 0.22, MeOH); $[\alpha]_D^{20}$–33.6° (c 0.84, MeOH) Lit. (Massy-Westropp and Reynolds, 1966); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.94 (d, 3, $J$ = 6.7 Hz, H-14), 1.08 (s, 3, H-15), 1.48 (m, 2, H-3), 1.60 (m, 1, H-4), 1.80 (m, 2, H-6), 1.85 (s, 3, H-13), 2.31 (m, 2, H-2), 2.35 (m, 1, H-7), 4.38 (d, 1, $J$ = 12.8 Hz, H-8), 4.85 (s, 1, H-$12_a$), 4.90 (t, 1, $J$ = 1.5 Hz, H-$12_b$), 7.11 (t, 1, $J$ = 3.9 Hz, H-1), consistent with lit. (Massy-Westropp and Reynolds, 1966); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 15.7 (q, C-14), 18.4 (q, C-15), 25.7 (q, C-13), 26.4 (t, C-2), 26.4 (t, C-3), 34.9 (s, C-5), 40.6 (d, C-4), 40.8 (t, C-6), 47.7 (d, C-7), 72.9 (d, C-8), 113.2 (t, C-12), 139.8 (s, C-10), 140.4 (d, C-1), 145.1 (s, C-11), 200.9 (s, C-9); EIMS (70eV) *m/z* (rel. int.): 234 ($M^{\cdot}$, 16 %), 219 (10), 205 (91), 191 (28), 177 (16), 163 (31), 149 (20), 137 (67), 121 (34), 109 (86), 91 (69), 79 (62), 67 (55), 55(56), 41 (100).

9-Hydroxy-1,7(11),9-eremophilatrien-8-one (**9**): unstable yellow gum (2.2 mg, 0.4 % not optimised); $[\alpha]_D^{20}$ +31° (c 0.32, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.89 (3, d, $J$ = 0.6 Hz, H-15), 0.98 (3, d, $J$ = 6.6 Hz, H-14), 1.77 (1, m, H-4), 1.93 (3, d, $J$ = 1.5 Hz, H-13),

2.03 (1, m, H-$3_b$), 2.11 (1, brd, $J$ = 13.6 Hz, H-$6_a$), 2.20 (1, m, H-$3_a$), 2.25 (3, d, $J$ = 2.3 Hz, H-12), 2.85 (1, d, $J$ = 13.6 Hz, H-$6_b$), 6.10 (1, ddd, $J$ = 2.6, 5.7, 9.8 Hz, H-2), 6.63 (1, dd, $J$ = 2.7, 9.9 Hz, H-1); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 14.6 (q, C-15), 15.9 (q, C-14), 185.5 (s, C-8), 23.4 (q, C-12), 23.5 (q, C-13), 32.7 (t, C-3), 37.0 (s, C-5), 38.4 (d, C-4), 39.6 (t, C-6), 122.0 (d, C-1), 126.2 (s, C-7), 131.9 (s, C-9), 134.3 (d, C-2), 141.9 (s, C-10), 148.1 (s, C-11); EIMS (70eV) *m/z* (rel. int.): 232 ($M^{\cdot}$, 100 %), 219 (45), 208 (3), 199 (53), 189 (36), 171 (18), 161 (19), 152 (4), 143 (12), 128 (14), 115 (19), 105 (16), 91 (39), 77 (27), 65 (15), 53 (15), 41 (36); HRAPCIMS *m/z* 233.1545 (calcd for $[C_{15}H_{21}O_2]^+$, 233.1542).

### *3.10 Isolation of sesquithuriferone*

The root oil (6.1 grams) was first fractionated by column chromatography (0.063–0.200 mm silica, Merck, Kilsyth VIC, Australia; 3.5 cm diam., 300 mL BV). The column was equilibrated with pentane (100 %, 3 BV) prior to sample loading then eluted with pentane (100%, 600 mL) to separate the unwanted hydrocarbons. The column was then eluted with pentane-diethyl ether (9:1, 2 × 400 mL) and diethyl ether (100 %, 2 × 400 mL) to generate 4 fractions. The sesquithuriferone-enriched fraction 1 (300 mg) was evaporated, then dissolved in $CH_3CN$ (ca. 2.0 mL) and subjected to RP preparative HPLC using the same method described for the wood oil. To recover eremophilone and sesquithuriferone from the aqueous fractions (ca. 30 mL), each was diluted with MQ water (150 mL) until a cloudy ppt. formed. The solutions were then passed through C18 SPE cartridges (Supelco, 6 mL, 1 g bed wt. Supelclean LC-18 SPE, Sigma-Aldrich, Castle Hill NSW, Australia), eluted with $CH_3CN$ (ca. 3 mL) and dried under nitrogen.

2,6,6,8-Tetramethyltricyclo[6.2.1.$0^{1,5}$] undecan-7-one (sesquithuriferone) (**10**): white powder (70 mg, 8.2 %, not optimised); $[\alpha]_D^{20}$ +5.9° (c 1, $CHCl_3$); $[\alpha]_D$ +5.7° (c 1, $CHCl_3$) Lit. (Barrero et al., 2000); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.88 (d, 3, $J$ = 7.2 Hz, H-12), 1.08 (s, 3,

H-13), 1.08 (s, 3, H-14), 1.12 (s, 3, H-15), 1.16-1.23 (m, 1, H-$3_a$), 1.44-1.50 (m, 1, H-$10_a$), 1.48-1.51 (m, 1, H-$11_a$), 1.56-1.64 (m, 1, H-$9_a$), 1.56-1.64 (m, 2, H-4), 1.68 (d, 1, *J* = 11.6 Hz, H-$11_b$), 1.68-1.74 (m, 1, H-$9_b$), 1.78-1.82 (m, 1, H-5), 1.86 (dq, 1, H-2), 2.0-2.1 (m, 1, H-$10_b$), 2.0-2.1 (m, 1, H-$3_b$); $^{13}$C NMR consistent with lit. (Barrero et al., 2000); EIMS (70eV) *m/z* (rel. int.): 220 ($M^{\cdot}$, 35 %), 205 (7), 192 (30), 177 (12), 159 (2), 147 (25), 136 (16), 121 (100), 108 (46), 93 (26), 81 (67), 67 (16), 55 (17), 41 (29).

### *3.11 Cytotoxicity assay*

*In vitro* cytotoxicity was measured against P388$D_1$ mouse lymphoblast cells (ATCC: CCL-46). Cells were cultured in Dulbecco's Modified Eagles Medium containing 4.5 mg/mL glucose, 4 mM glutamine, 10 % horse serum, 200 U/mL penicillin and 200 µg/mL streptomycin (all media components were purchased from Gibco-Invitrogen). Test samples were diluted in DMSO (to obtain a final concentration of 1.0 % DMSO per well) and assayed in 96 well plates ($5\times10^5$ cells/mL, 100 µL/well) in duplicate, at seven concentrations ranging between 3 — 200 µg/mL. 1.0 % DMSO, curcumin (Sigma, C-1386) and chlorambucil (Sigma, C-0253) were used as the solvent control, and two positive controls, respectively. Following 24 hours incubation (37°C, 5 % $CO_2$), cytotoxicity was assessed using a commercial ATP luminescence kit (APTlite PerkinElmer Inc, MA) with luminescence, as an indication of proliferation, recorded on a liquid scintillation and luminescence counter (MicroBeta, Wallac Oy, Finland). Inhibition of growth was calculated with respect to the relevant controls and $IC_{50}$ concentrations and confidence intervals were estimated from non-linear regression of a 4-parameter logistic equation (GraphPad Prism 4, GraphPad Software Inc, CA).

**Supporting Information Available:** The electron impact mass spectra for the major sesquiterpenes from the wood and root oils of *Eremophila mitchellii*. The selective ion chromatogram of the sesquiterpene ion, $C_{15}H_{24}$, *m/z* 204 (retention time; 40–47 minutes) for the wood and leaf oils. This material is available free of charge via the Internet.

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## Figures and Legends

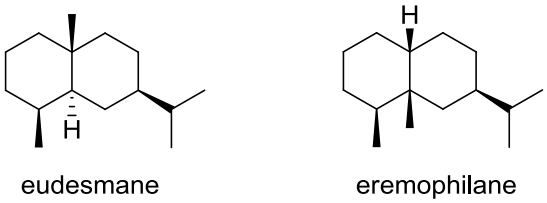


**Figure 1.**

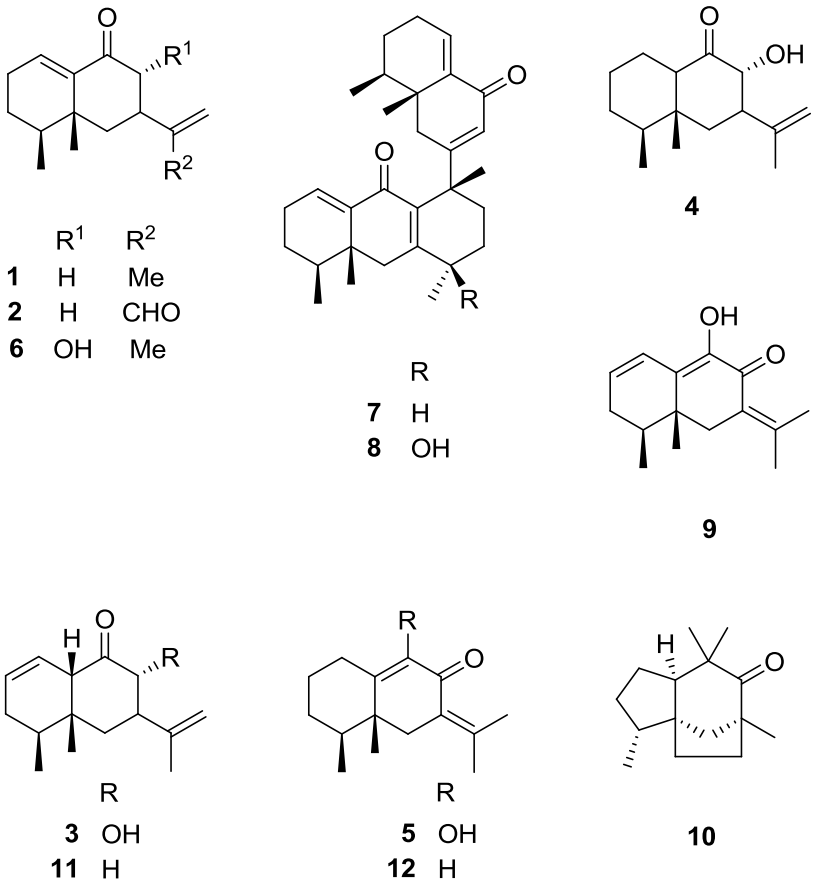


**Figure 2.**

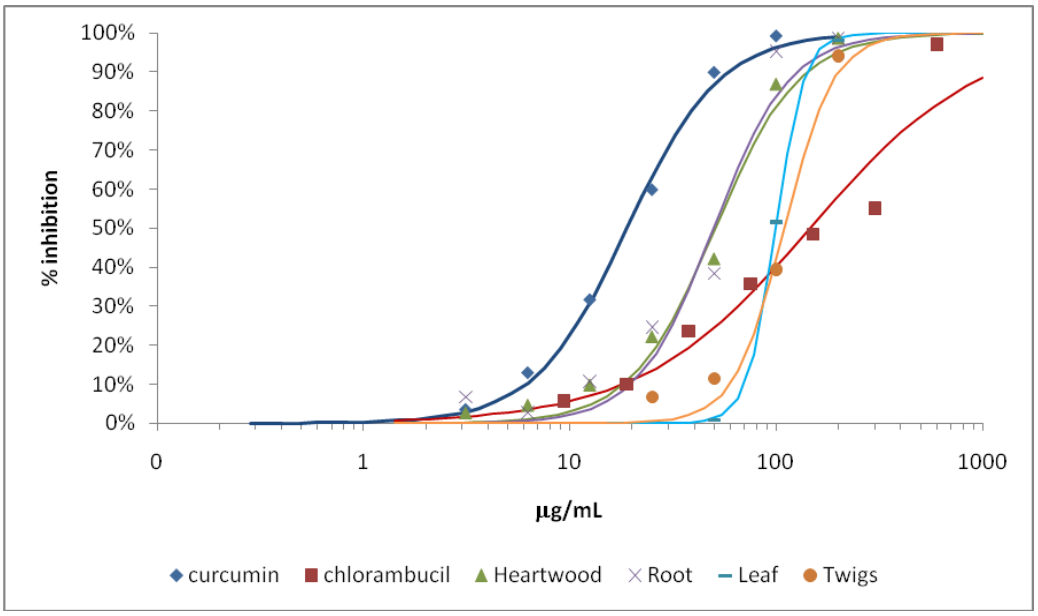


**Figure 3.** (in colour for web presentation)

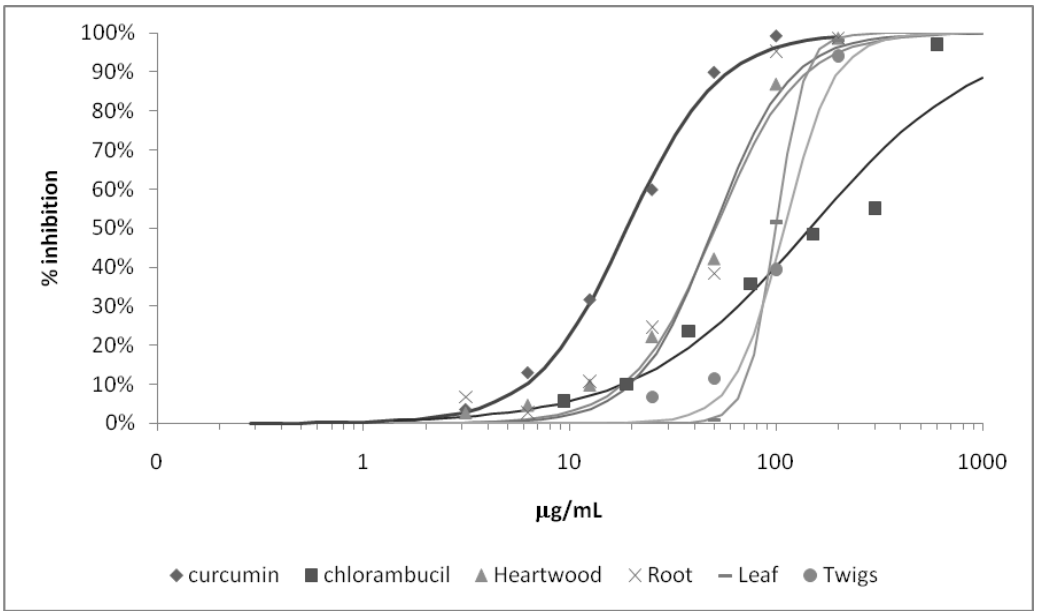


**Figure 3.** (in black and white for reproduction in print)

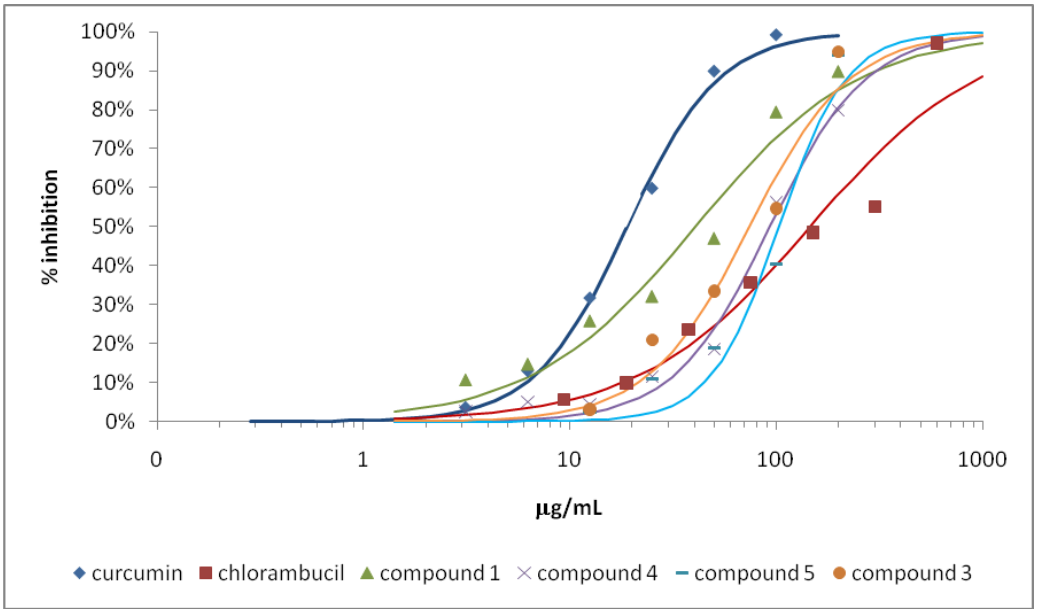


**Figure 4.** (in colour for web presentation)

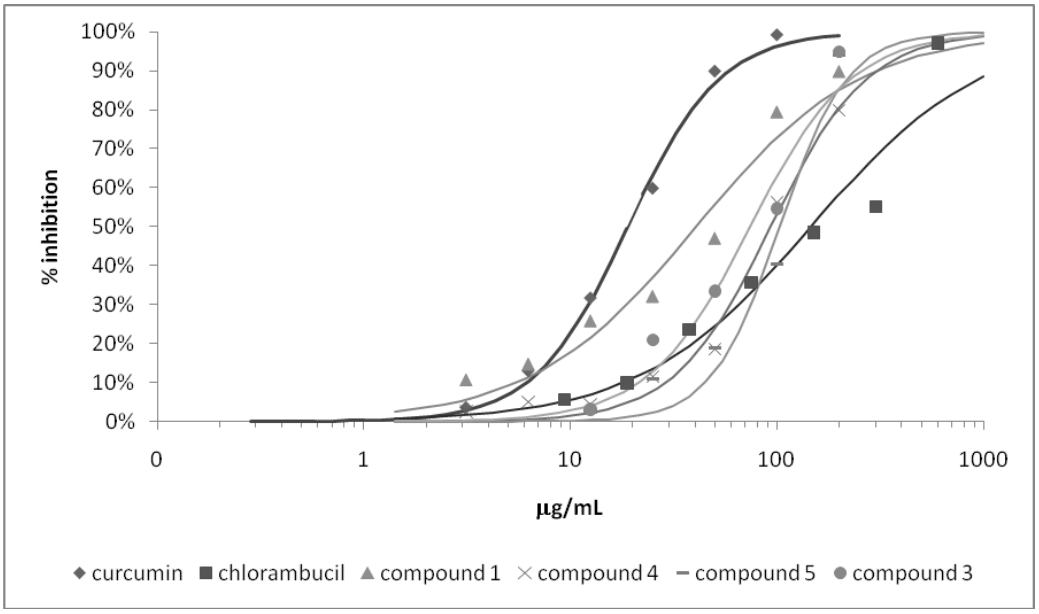


**Figure 4.** (in black and white for reproduction in print)

**Fig. 1.** Structures of the eremophilane and eudesmane (selinane) sesquiterpenes.

**Fig. 2.** Structures of the eremophilanes from *Eremophila mitchellii*.

**Fig. 3.** Cytotoxic activity of the essential oils from *E. mitchellii* against mouse lymphoblast cells.

**Fig. 4.** Cytotoxic activity of the eremophilanes **1**, **3**—**5** against mouse lymphoblast cells.

## Tables

**Table 1** Yield and descriptions of the essential oils obtained from steam distillation of *E. mitchellii* leaves, twigs, wood, bark, heartwood and roots.

| Plant part[b] | Yield (vol/wt) | Description |
|---|---|---|
| leaf | 1.4 % | black-green oil |
| branchlets | 0.04 % | dark amber oil, $d^{b} \geq 1.0$ |
| roots | 0.3 % | dark amber oil, $d \geq 1.0$ |
| bark | 0.0 % | - |
| red heartwood | 2.2 % | dark amber oil, $d \geq 1.0$ |
| white outer wood | 0.0 % | - |

[a] *d* = density; [b] *E. mitchellii* Specimen # PIF 30486

**Table 2** Percentage composition of the essential oils derived from different parts of *Eremophila mitchellii*.

| Compound | RI A | RI P | Leaf | Branchlet | Heartwood | Root | Identification |
|---|---|---|---|---|---|---|---|
| *α*-pinene | 953 | nd | 22.84 | 1.84 | - | - | RI, MS, RC, UB |
| *β*-pinene | 1000 | 1105 | 0.55 | tr | - | - | RI, MS, RC, UB |
| *α*-phellandrene | 1026 | 1166 | 1.29 | - | - | - | RI, MS, RC, UB |
| *p*-cymene | 1046 | 1273 | 0.60 | 0.55 | - | - | RI, MS, RC, UB |
| limonene | 1050 | 1197 | 0.89 | 0.42 | - | - | RI, MS, RC, UB |
| *β*-phellandrene | 1056 | 1206 | 0.23 | - | - | - | RI, MS, RC, UB |
| $C_{10}H_{16}O$ | 1097 | 1323 | - | 0.24 | - | - | |
| *α*-terpinolene | 1106 | 1283 | 0.67 | - | - | - | RI, MS, RC, UB |
| linalool | 1111 | 1606 | tr | 0.76 | - | - | RI, MS, RC, UB |
| $C_{10}H_{12}$ | 1115 | 1442 | 0.13 | 0.70 | - | - | |
| $C_{10}H_{14}O$ | 1218 | 1857 | 0.14 | tr | - | - | |
| $C_{10}H_{16}O$ | 1225 | 1519 | - | tr | - | - | |
| *α*-terpineol | 1228 | 1701 | 0.65 | 0.69 | - | - | RI, MS, RC, UB |
| $C_{10}H_{16}O$ | 1232 | nd | - | tr | - | - | |
| $C_{10}H_{18}O$ | 1265 | 1448 | - | tr | - | - | |
| $C_{10}H_{14}O$ | 1326 | 1855 | - | tr | - | - | |
| $C_{15}H_{24}$ | 1362 | 1740 | tr | - | - | - | |
| unknown | 1384 | 1773 | - | 0.61 | 0.07 | 0.10 | |
| eugenol | 1387 | 2177 | 0.35 | - | - | - | RI, MS, RC, UB |
| $C_{15}H_{24}$ | 1406 | 1469 | 0.18 | 0.17 | - | - | |
| $C_{15}H_{24}$ | 1413 | 1491 | 0.47 | 0.77 | - | - | |
| *β*-elemene | 1421 | 1592 | 0.83 | 0.88 | - | - | RI, MS, RC, LG |
| $C_{15}H_{24}$ | 1431 | 1505 | - | - | - | 0.10 | |
| $C_{15}H_{24}$ | 1436 | 1484 | - | 0.34 | - | - | |
| $C_{15}H_{24}$ | 1451 | 1531 | 0.30 | - | - | - | |
| $C_{15}H_{24}$ | 1461 | 1544 | 0.22 | tr | - | - | |
| *trans*-*β*-caryophyllene | 1469 | 1599 | 1.77 | 0.21 | - | - | RI, MS, RC, LG |
| $C_{15}H_{24}$ | 1476 | 1602 | 0.28 | 0.23 | - | - | |
| aromadendrene | 1488 | 1609 | 2.95 | 2.77 | - | - | RI, MS, RC, LG |
| unknown | 1490 | 1845 | - | - | 0.14 | 0.18 | |
| $C_{15}H_{24}$ | 1494 | 1618 | 0.34 | 0.25 | - | - | |
| $C_{15}H_{24}$ | 1503 | 1648 | 0.34 | 0.67 | - | - | |
| $C_{15}H_{24}$ | 1507 | 1673 | 0.60 | 0.27 | - | - | |
| alloaromadendrene | 1513 | 1649 | 0.52 | 0.31 | - | - | RI, MS, RC, LG |
| $C_{15}H_{24}$ | 1516 | 1678 | 0.57 | 0.55 | 0.22 | - | |
| $C_{15}H_{24}$ | 1519 | 1683 | - | - | 0.15 | - | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $C_{15}H_{24}$ | 1521 | 1666 | 0.29 | tr | - | - | |
| $C_{15}H_{24}$ | 1529 | 1671 | 0.28 | - | - | - | |
| $C_{15}H_{24}$ | 1531 | 1712 | 0.20 | - | - | - | |
| $C_{15}H_{24}$ | 1536 | 1718 | tr | - | - | - | |
| $C_{15}H_{24}$ | 1537 | 1713 | - | 0.22 | 0.40 | - | |
| viridiflorene | 1539 | 1697 | 3.59 | 0.72 | - | - | RI, MS, RC, LG |
| unknown | 1540 | 2002 | - | - | - | 0.36 | |
| *β*-selinene | 1544 | 1723 | 1.03 | 1.74 | 1.73 | - | RI, MS, RC, LG, BS |
| *α*-selinene | 1548 | 1723 | 0.90 | 2.50 | 0.77 | - | RI, MS, RC, LG, BS |
| *δ*-cadinene | 1557 | 1761 | 1.83 | 0.63 | - | - | RI, MS, RC, LG |
| $C_{15}H_{24}$ | 1560 | 1763 | 0.26 | 0.34 | - | - | |
| $C_{15}H_{22}$ | 1561 | 1727 | - | - | 0.45 | - | |
| $C_{15}H_{22}$ | 1566 | 1829 | - | - | 0.28 | 0.15 | |
| $C_{15}H_{22}$ | 1568 | 1838 | 0.26 | 0.50 | - | - | |
| cadina-1,4-diene | 1579 | 2073 | 0.15 | - | - | - | RI, MS, RC, LG |
| unknown | 1582 | 1794 | 0.12 | 0.06 | - | - | |
| elemol | 1591 | nd | 0.26 | - | - | - | RI, MS, RC, LG |
| unknown | 1593 | 1922 | - | 0.29 | - | - | |
| $C_{15}H_{22}O$ | 1612 | nd | - | - | 0.18 | - | |
| epiglobulol | 1622 | 2020 | 0.38 | 0.25 | - | - | RI, MS, RC, LG |
| unknown | 1624 | nd | - | 0.37 | - | - | |
| unknown | 1625 | nd | - | 0.46 | - | - | |
| unknown | 1629 | nd | tr | 0.96 | - | - | |
| sesquithuriferone (**10**) | 1631 | 1946 | - | - | - | 42.61 | RI, MS, $^{1}H$, $^{13}C$, 2DNMR, LG |
| $C_{15}H_{26}O$ | 1632 | 2023 | - | - | 0.23 | - | |
| $C_{15}H_{26}O$ | 1635 | nd | 0.42 sh | tr | - | - | |
| spathulenol | 1639 | 2134 | 10.50 | 15.90 | - | - | RI, MS, RC, LG |
| $C_{15}H_{24}O$ | 1645 | 2128 | 1.21 | 2.59 | - | - | |
| globulol | 1650 | 2085 | 4.45 | 6.00 | - | - | RI, MS, RC, LG |
| unknown | 1658 | nd | 0.88 | - | - | - | |
| unknown | 1659 | nd | - | 0.89 | - | - | |
| viridiflorol | 1661 | 2092 | 2.93 | 2.01 | - | - | RI, MS, RC, LG |
| unknown | 1663 | nd | - | 2.27 | - | - | |
| $C_{15}H_{24}O$ | 1664 | 2091 | - | - | | 1.50 | |
| $C_{15}H_{24}O$ | 1669 | 2114 | 1.26 | 2.00 | - | - | |
| $C_{15}H_{24}O$ | 1672 | 2114 | 0.55 | tr | - | - | |
| $C_{15}H_{26}O$ | 1674 | 2103 | - | - | - | 0.27 | |
| $C_{15}H_{26}O$ | 1676 | 2066 | - | - | 0.20 | - | |
| unknown | 1678 | nd | - | 0.54 | - | - | |
| $C_{15}H_{26}O$ | 1681 | 2146 | - | 1.76 | 1.88 | 0.98 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| unknown | 1685 | nd | 1.11 | 0.59 | - | - | |
| $C_{15}H_{24}O$ | 1690 | 2123 | 2.33 | 0.61 | - | - | |
| $C_{15}H_{24}O$ | 1694 | 2175 | 0.48 | - | - | - | |
| unknown | 1696 | nd | tr | 0.54 | - | - | |
| $C_{15}H_{26}O$ | 1699 | 2194 | 10.16 | 12.35 | - | - | |
| $C_{15}H_{26}O$ | 1713 | nd | 0.93 | 1.62 | - | - | |
| $\alpha$-eudesmol | 1723 | 2207 | ~ 1.5 | ~ 3.7 | - | - | RI, MS, RC, LG |
| $\beta$-eudesmol | 1725 | 2210 | ~ 2.8 | ~ 6.9 | | | RI, MS, RC, LG |
| $C_{15}H_{26}O$ | 1727 | 2203 | - | - | 2.25 | 1.35 | |
| $\alpha$-bisabolol | 1730 | 2206 | 2.81 | sh | - | - | RI, MS, RC, LG |
| $C_{15}H_{24}O$ | 1736 | 2167 | - | 1.57 | 1.24 | 1.56 | |
| $C_{15}H_{26}O$ | 1743 | 2216 | - | - | 0.18 | - | |
| unknown | 1744 | 2216 | - | 0.72 | - | 0.18 | |
| $C_{15}H_{22}O$ | 1748 | 2222 | - | - | 3.90 | 0.54 | |
| $C_{15}H_{26}O$ | 1756 | 2202 | - | - | - | 0.36 | |
| unknown | 1766 | nd | - | 0.20 | - | - | |
| unknown | 1797 | nd | - | 0.15 | - | - | |
| $C_{15}H_{24}O_2$ | 1802 | nd | - | 0.19 | 0.38 | | |
| $C_{15}H_{24}O$ | 1802 | 2210 | - | | | 0.15 | |
| $C_{15}H_{22}O_2$ | 1806 | nd | - | - | - | 0.16 | |
| eremophilone (**1**) | 1816 | 2238 | - | 4.90 | 42.99 | 41.18 | RI, MS, $^{1}H$, $^{13}C$, 2DNMR, LS |
| unknown | 1818 | nd | - | 0.22 | - | - | |
| unknown | 1827 | nd | - | 0.16 | - | - | |
| santalcamphor (**4**) | 1839 | 2257 | - | 0.47 | 17.54 | 4.40 | RI, MS, $^{1}H$, $^{13}C$, 2DNMR, LS |
| 8-hydroxy-1,11-eremophiladien-9-one (minor) (**3**) | 1839 | 2257 | | | minor | minor | RI, MS, $^{1}H$, $^{13}C$, 2DNMR, LS |
| $C_{15}H_{22}O_2$ | 1848 | 2259 | - | - | tr | 0.11 | |
| $C_{15}H_{22}O_2$ | 1852 | 2260 | - | - | tr | - | |
| unknown | 1864 | 2261 | - | - | 0.14 | 0.10 | |
| $C_{15}H_{22}O_2$ | 1879 | 2262 | - | - | 0.08 | 0.09 | |
| $C_{15}H_{22}O_2$ | 1884 | 2263 | - | - | 0.28 | - | |
| $C_{15}H_{22}O_2$ | 1895 | 2263 | - | - | 0.96 | 0.18 | |
| $C_{15}H_{22}O_2$ | 1900 | 2265 | - | - | 0.43 | - | |
| $C_{15}H_{22}O_2$ | 1921 | nd | - | - | - | 0.19 | |
| 8-hydroxy-10,11-eremophiladien-9-one (minor) (**6**) | 1936 | 2270 | - | - | minor | minor | RI, MS, $^{1}H$, $^{13}C$, 2DNMR, LS |
| 9-hydroxy-7(11),9-eremophiladien-8-one (**5**) | 1937 | 2270 | | | 18.00 | 0.83 | RI, MS, $^{1}H$, $^{13}C$, 2DNMR, LS |
| $C_{15}H_{20}O_2$ | 1943 | 2273 | - | - | tr | 0.20 | |
| 9-hydroxy-1,7(11),9-eremophilatrien-8-one (**9**) | 1981 | 2277 | - | - | 1.04 | 0.34 | RI, MS, $^{1}H$, $^{13}C$, 2DNMR |
| unknown | 2000 | 2278 | - | - | 0.17 | tr | |
| $C_{15}H_{20}O_2$ | 2086 | 2282 | - | - | 0.23 | 0.10 | |
| $C_{15}H_{20}O_2$ | 2112 | 2288 | - | - | 0.33 | 0.15 | |

| | | | | |
|---|---|---|---|---|
| Total (%) | 91.26 | 91.25 | 96.84 | 98.48 |

RI A: retention indices on a slightly polar (BPX-5) column; RI P retention indicies on a polar/wax (BP 20) column; tr <0.10 %. RC; identification by reference compound.
Order of elution and percentages (%) of individual component are derived from the RI A (BPX-5) data; - not present; nd: not determined; ~ approximately.
LS: previously reported from this species; LG: previously reported from this Genus; UB: Ubiquitous in plant leaf oils; BS: anticipated on the basis of biosynthetic pathway studies.

**Table 3** Half maximal cytotoxic activity of *E. mitchellii* oils and eremophilanes **1**, **3**—**5** against mouse lymphoblast cells.

| **Sample** | **$IC_{50}$ (μg/mL)** | **95 % CI (μg/mL)** |
|---|---|---|
| heartwood oil | 52 | 50—54 |
| root oil | 51 | 48—53 |
| leaf oil | 100 | 97—102 |
| branchlet oil | 110 | 107—114 |
| eremophilone (**1**) | 42 | 40—45 |
| 8-hydroxy-1,11- eremophiladien-9-one (**3**) | 76 | 71—80 |
| santalcamphor (**4**) | 95 | 91—99 |
| 9-Hydroxy-7(11),9-eremophiladien-8-one (**5**) | 105 | 101—108 |
| curcumin[a] | 19 | 18—20 |
| chlorambucil[a] | 170 | 155—185 |

**[a] Commercially available cytotoxic compounds used as positive controls.**