Chapter 1

Introduction:

Essential Oils and Ethnopharmacology
in Australia

1.1.0   Essential Oil Chemistry

1.1.1 Context

Throughout its long evolutionary prehistory, the Australian continent has been subject to selective pressures that have produced a range of drought tolerant plant species yielding an abundance of secondary metabolites. A large number of such species were utilised in the *materia medica* of Australian Aboriginal people, often employing volatiles derived by placing leaves over the hot embers of a fire. Indigenous informants advise that when foraging for medicines they often bias their selection to specimens that produce the strongest fragrance, believing that the medicinal principle is related to the aroma of the plant. Thus, in examining the *materia medica* of Aboriginal people, ethnopharmacologists may uncover interesting biologically active compounds if various laboratory based approaches are employed in examining the chemical character and biological activities of volatile components derived in hydrodistillation and also at higher temperatures following partial pyrolysis, more similar to the temperatures obtained in the hot embers of a fire.

Unlike the anthropogenically induced floral homogeneity caused by cultivar selection, familiar in plants of European provenance, Australian botanical diversity has been influenced to a greater extent by the Aboriginal Australian fire regime (fire stick farming). This means that recognised Australian species often have significant intraspecific variation, which can be morphological as well as chemical in nature. The unique abundance of essential oil varieties or 'chemotypes' within discrete plant species is one such example of this intraspecific variability. Earlier taxonomic methods for species delimitation utilized this chemical diversity as a means to qualify differences between specimens of apparently the same species. Improvements of molecular fingerprinting and phylogenic techniques have now largely replaced chemical variability in this context.

However, oftentimes olfactory cues are still employed by botanists in the field to prompt closer examination of morphological characters. This sometimes leads to recognition of intraspecific variability that may have been otherwise overlooked. In taking this approach further, the chemical character of essential oils may tell a story, reveal relationships and guide or facilitate taxonomic decisions. Likewise, in Aboriginal Australia the variability in the aroma of a single species as classified by contemporary botanists, would have been regarded by Aboriginal groups as indicating distinct and separate entities. This was probably because the botanical nomenclature in Aboriginal lore reflected medicinal attributes, whereas emphasis in contemporary botany lies on morphological characters.

Due to the inherent natural variability of morphological characters within a plant species, taxonomists are challenged to demonstrate that morphological traits

recruited to define new species, are not merely due to natural variability within the species. This is where molecular techniques are used and where 'chemotaxonomy' was previously widely utilised. Of course the reason we employ chemotaxonomy in the current dissertation is not because of an inadequacy of molecular techniques, but rather to bring more pieces of evidence to the puzzle and of course to 'tell a story'.

1.1.2 General characteristics of essential oils

Essential oils are a mixture of volatile lipophilic constituents, most commonly sourced from leaf, twig, wood pulp or bark tissue of higher plants, but also widely found in bryophytes, such as algae or the liverworts (Asakawa *et al.*, 2012). Although essential oils are only slightly soluble in water, the solubility of individual essential oil components varies with respect to polarity. Generally, components with more polar groups are expected to be more soluble in water, relative to other components.

Essential oils are most commonly produced using hydrodistillation; however prior to this, individual components of the whole essential oil are present within the source tissue, either in the same molecular form or as a heat labile precursor. The process of hydrodistillation involves heating in the presence of water to temperatures higher than boiling point, to produce mixed gases that expand and travel into a condenser. Subsequently mixed gases are cooled to below 30°C and condensed into two liquid phases; one phase being a hydrosol and the other an essential oil. The two condensed liquids are gravity fed into a separation funnel, where the two phases are manually separated.

Most authorities contend that if a different process is used, such as solvent extraction or mechanical pressing, the product is not regarded as an essential oil and may be more usefully termed an 'absolute' or an 'expressed oil' respectively. A host of other names can be used to describe aromatic preparations, such as 'concrete', 'tincture', 'spice oleoresin', et cetera. The term 'volatile oil' is commonly used if reference is made to the volatile fraction of any of these extracts, but this expression also encompasses essential oils. Having said this, some authorities still refer to the mechanically expressed oil from citrus peel as an essential oil and this is upheld by the International Organisation for Standardisation (Schnaubelt, 1999).

The specific definition of an essential oil may be subject to disputation among interested individuals including scientists, aromatherapists or lay people. Although a consensus has generally been agreed upon, extraction methods are still evolving, and this has the capacity for further confusion in terminology. Essential oils can now be extracted using modern microwave-assisted hydrodistillation (Fadel *et al.*, 2011; Asghari *et al.*, 2012) or microwave-assisted distillation techniques that require no additional water, other than cytosolic and vascular fluids already present in the

source tissue (Mohamadi *et al.*, 2013). These methods result in differences, both qualitative and quantitative, in the composition and yield of the subsequent essential oil (Fadel *et al.*, 2011; Mohamadi *et al.*, 2013). Strictly speaking the latter technique, requiring no additional water, is not hydrodistilled but merely distilled.

In this regard, the International Organisation for Standardization defines an essential oil as a 'product obtained from natural raw material, either by distillation with water and steam, or from the epicarp of citrus fruits by mechanical processing, or by dry distillation' (Schnaubelt, 1999). With regard to the classification of expressed oil from citrus peel, such as orange or Bergamot, these are commonly referred to as essential oils (Stewart, 2005; Kostadinovic *et al.*, 2011). However, using this terminology they may be confused with the essential oils produced using hydrodistillation. In the former case of expressed oils, the source tissue is not hydrodistilled and the subsequent oil contains dissolved lipids (waxes and sterols) that are not volatile (Markley *et al.*, 1937; Kostadinovic *et al.*, 2011).

Further implications of definition of essential oils appear when considering the medicinal applications of aromatic plants. This is particularly relevant when plant material is heated to produce an acrid steamy smoke and then either condensed onto the skin (Sadgrove & Jones, 2013b) or in the lungs through inhalation (Braithwaite *et al.*, 2008). In this context, medicinal effects may sometimes be produced via a molecular interaction between multiple compounds that could be of both lipophilic (essential oils) and hydrophilic character (not present in the essential oils). Such potentially synergistic interactions may not occur using only the pure essential oil as produced in hydrodistillation (Sadgrove & Jones, 2013b). However, more often than not there is a single active compound involved that produces the greater part of the medicinal effect (Sadgrove *et al.*, 2014), which can be of either lipophilic or hydrophilic character.

In this regard, slightly larger intact or modified compounds are evaporated, in significant quantities, when higher temperatures are involved, such as in ethnobotanical smoke fumigation practices (Sadgrove & Jones, 2014), or microwave assisted distillation (Fadel *et al.*, 2011; Mohamadi *et al.*, 2013). These slightly larger compounds can be found in smoking extracts or dissolved in either the essential oil or hydrosol, when microwave assisted distillation is used.

Some may propose that volatile oils produced using microwave-assisted distillation or hydrodistillation technology should correctly be called essential oils because of the chemical alteration of heat labile constituents that become part of an essential oil with both natural ingredients and these derived 'artefacts'. This is clearly an area of contention and the essential oil industry may need to embark upon the development of a new system for communicating information related to the distillation method used to produce essential oil products, to create consumer

awareness of potential qualitative differences. A similar approach may also need to include expressed oils from citrus peel, to avoid confusion with hydrodistilled oils, also produced from citrus peel. Furthermore, in the case of heating plant material to produce therapeutic gases in ethnomedicinal contexts, this may be recognised as a mixture of essential oils and other larger compounds, such as diterpenes, together with the more hydrophilic components that are not usually detected in significant quantities in the essential oil *per se*.

To avoid further confusion researchers and academics often use the word 'hydrodistilled' or more recently 'distilled' instead of 'extracted' if they are referring to an essential oil. This is to clarify that essential oils are being described and not the same volatile components that have been separated using other techniques. Such oils are either produced using solvent extraction or are mechanically expressed from the source material to produced volatiles dissolved in lipid oils or vice-a-versa. Systematic avoidance of the term 'extracted' can become contentious, cumbersome and impractical, so it should be avoided only when there is a possibility of confusion. As far as we can tell, using the term 'produced' is acceptable with reference to essential oils.

Disputes regarding the nomenclature of essential oils also impact on received history of essential oil usage, because volatile oils in earlier use may not fall into the modern definition of an 'essential oil', since they were not hydrodistilled in the conventional sense. For instance, there is no evidence that modern hydrodistillation technology was available in biblical times or in ancient Egypt, meaning that the medicinal applications described in these earlier references most likely used expressed or absolute oils with a mixture of volatile and fixed components and were therefore not essential oils *per se* (Guenther, 1948b), as is commonly accepted (Stewart, 2003, 2005).

The earliest authentic description of an essential oil, produced by a method resembling conventional hydrodistillation, was compiled by Arnald de Villanova sometime during the late 12th or early 13th century (1235 – 1311 AD). Prior to this, details of a primitive form of distillation, used to produce turpentine and camphor, were described by the ancient Romans and Greeks in the first century (Guenther, 1948b). However, because no other essential oil was produced in this manner it is unclear if this can be taken as evidence of essential oil production at that earlier time. Although there is clear evidence that a primitive form of distillation technology was in use from that time until the 9th century, this method was primarily used to produce distilled waters where essential oils were often produced as a by-product (Guenther, 1948).

Distillation technology was improved in the 9th century by earlier Arabic scientists (Bauer & Garbe, 1985; Burt, 2004) but again it is not clear if they used this

technology to deliberately produce essential oils or if the primal focus was for floral waters. Therefore, historians currently agree that the essential oil technology that was adopted into therapeutic use in Europe in the Middle Ages was from the 13th century work of Villanova, who provided the earliest record that can be reliably authenticated (Guenther, 1948).

Essential oil components are usually no larger than 300 Daltons (amu) in size (Sell, 2010), except in unusual cases involving larger diterpenoids such as incensole acetate from *Boswellia* spp. (Moussaieff *et al.*, 2008a; Moussaieff *et al.*, 2008b) and these require longer periods of hydrodistillation. This general observation may change with the advent of new distillation technology that produces slightly heavier molecules (approx. 350 Daltons), such as the microwave-assisted distillation method aforementioned.

With regard to the production of floral waters or hydrosols, the hydrophobic character of essential oil causes phase separation of oil and water, but trace quantities of the essential oils dissolve as mentioned before. Usually, due to a relatively low saturation point, the hydrosol dissolves only small amounts of the essential oils, but occasionally volatile components can be dissolved in hydrosols at relatively high concentrations (Sadgrove *et al.*, 2014). In such cases these constituents have greater polarity than other essential oil components, making them more soluble in water.

The essential oil phase typically floats over the hydrosol, but in fewer cases the essential oil is denser than water so it settles below the hydrosol (Baser & Demirci, 2007). For example, some phenylpropanoids, such as safrole and methyl eugenol ether, are denser than water and will settle below the hydrosol, but only if they occupy sufficiently high relative abundance in the whole essential oil. An example of this is the essential oil produced from one of the chemotypes of *Eremophila longifolia* (Scrophulariaceae) in Western Australia (Della & Jefferies, 1961), which is a mixture of safrole and methyl eugenol ether, comprising approximately 97% of the whole essential oil.

Essential oils are biologically regarded as metabolites of secondary importance to the organism because, in contrast to primary metabolites, they are not universal across the plant kingdom, nor do they constitute any of the basic building blocks of life (Sell, 2010). Although such secondary metabolites are generally regarded as metabolic by-products, it is widely acknowledged that they provide an evolutionary advantage to the plant (or liverwort), which may involve protection against grazers such as fungi, insects or herbivores. Alternatively, the essential oils may play an ecological role, such as in fire tolerance, attracting pollinators and/or herbivores for seed dispersal, drought tolerance or plant to plant biosemiosis via pheromones.

Although essential oils may contribute significantly toward the evolutionary survival of the respective organism, the noun 'essential oil' did not derive from this function. A common misconception is that essential oils are called 'essential' oils to highlight their importance in the biological survival of the organism. The term 'essential oil' has its origin from the word 'quintessence', the English rendering of *Quinta essentia*. This term means the fifth element in the earlier alchemical constellation, used for essential oils in the early 16th century by the Swiss medical pioneer, Bombastus Paracelsus von Hohenheim (Guenther, 1948). At the time von Hohenheim believed that the essential oil was the most pure and concentrated form of the medicinal principle of any plant, produced by hydrodistillation of the plant tissue.

Use of the term 'quintessence' by von Hohenheim is a reflection of the Aristolean paradigm which described matter as being composed of the five elements: earth, fire, water, air and spirit (Sell, 2010). Quintessence (literally the fifth essence) was regarded as the latter of these; the spirit or life force of the plant, which could be removed and contained by the distillation process. Use of the modern term 'spirits' to describe various liquors, specifically those produced by distillation, is again a reflection of this ancient concept (Sell, 2010).

A variety of other names are given to the essential oil. These include essence, fragrant oil, volatile oil, etheric oil, aetheroleum or aromatic oil (Baser & Demirci, 2007). The latter term 'aromatic' is another term that generates a lot of confusion and contention. Although the term 'aromatic' in modern usage describes the quality of giving off an aroma that is either pleasant or odious to the nose, an aromatic compound or moiety, in the language of chemistry, has a chemical arrangement that results in delocalisation of electrons, producing greater molecular stability. Thus, essential oils may be a mixture of aromatic and aliphatic (non-aromatic) compounds, all of which contribute to the perceived aroma. This is obvious to professional chemists but leads to confusion with other non-scientific users of essential oils.

1.1.3 Aromatic or aliphatic

In strictly chemical terms, aromatic compounds, also often called arenes, contain an aromatic group. An aromatic group is planar, cyclic with overlapping p electron orbitals and an odd number of electron pairs within the π bond formation ($(4n+2)/2$). Although the benzene moiety is the most commonly cited example (Bruice, 2004); other aromatic groups include the heterocycles pyrrole, pyrans, furans and thiophenes.

The term aromatic (or arene) first entered the language of chemistry when Augustus W. Hofmann (1855) used it in reference to a series of volatile mono- and 'bibasic [sic] acids' , including the provisionally named insolinic acid. Because all of the compounds in Hofmann's series contained a benzene moiety, the term aromatic

came to be associated with arene compounds (Sainsbury, 1992). Because all of the compounds in Hofmann's aromatic series contained a benzene moiety and have odour, the term aromatic came to be associated with essential oils and other odour causing molecules. When the advancement of chemistry eventually demonstrated that odour causing compounds were mostly terpenes and other non-benzenoid chemical groups, use of the term aromatic to describe these respective compounds persisted. Thus, although the term 'aromatic plants' is now widely used to described essential oil yielding varieties, most essential oil compounds are aliphatic in the strict chemical sense.

1.1.4 Biosynthesis and classification of essential oils

Essential oils are classed as secondary metabolites derived from the lipids, which are one of the four primary metabolites (Sell, 2010). Of these secondary metabolites the most significant with regard to essential oils are terpenoids and shikimates, although polyketides and alkaloids also occur in essential oils (Sell, 2010). The biosynthetic origin of phenylpropanoids is from the *shikimic acid* pathway and terpenoid essential oils are via the *mevalonate pathway*. The latter involves the derivatisation and polymerisation of 5-membered isoprene alkenes from isoprenyldiphosphate (IPP) and dimethylallyldiphosphate (DMAPP). Isoprene units are therefore the building blocks of terpenes.

There are currently more than 30,000 known terpenoids, isolated from plants, microorganisms and animals, many of which occur in essential oils. Within this array of known terpenoids are multiple chemical classes divided into groups of size and elemental/structural composition. The monoterpenes are known to comprise 25 different classes of terpenoids, 147 classes exist for sesquiterpenes, and diterpenes occur in 118 classes (Baser & Demirci, 2007).

The term 'terpene' was coined by Kekulé in 1880 (of benzene structure fame (1865, 1866)), because terpenes were first discovered in turpentine oil, as the main constituents (Baser & Demirci, 2007). A single terpene, called a monoterpene, is formed from two isoprene units, typically connected head to tail. Hemi- (1 isoprene), mono- (2 isoprenes), sesqui- (3 isoprenes) and di- (4 isoprenes) terpenes are the most common essential oil components, followed by the non-terpenoid group, phenylpropanoids. Although in the earlier literature the term 'terpene' was often used to describe terpenoid compounds, in modern terminology 'terpene' only describes monoterpene hydrocarbons (Sell, 2010).

With regard to the conventions for describing the qualitative character of whole essential oils, they can be described as terpenoid if they are predominantly composed of components of terpenic character. An essential oil is of monoterpene character if it is dominated by monoterpene hydrocarbons and of monoterpenol

character if components are predominantly monoterpene alcohols. The same convention is used for sesquiterpene or sesquiterpenol rich essential oils (Bowles, 2003). This convention is not commonly used to denote other chemical classes, such as ketones or ethers, as the suffix may be confused with coumarins/lactones or ether/oxides respectively.

During the late 1800's when essential oil chemistry was in its infancy, the chemical character of essential oils was communicated in broad generic categories, such as terpenoid or phenylpropanoid, or otherwise specialist chemical nomenclature was used. Terminology to express essential oil character was later expanded upon and improved following the proposition by Belaiche to assign chemical classes that could be used to predict the biological activity of the oils themselves (Schnaubelt, 1999, p. 177). This took place when the well-known French authors Pierre Franchomme and Daniel Penoel published *L'Aromathérapie éxactement* (Schnaubelt, 1999), which provided a framework for essential oil classification that continues to be used to this day. Franchomme and Penoel provided a list of these types, which are included in Table 1 (Schnaubelt, 1999).

**Table 1 Essential oil pharmacological types (Schnaubelt, 1999).**

Essential Oil Types Described by Franchomme and Penoel		
Alcohols and Phenols (hydroxyl group)	Coumarins	Ether-Oxides
Methoxycoumarins	Acetophenones	Hydroquinones
Non-Terpenoid Hydrocarbons	Acids	Oxides
Terpenoid and Non-Terpenoid esters	Ketones;	Lactones
Phenol and Methyl-Ether	Phthalides	Aldehydes
Bi- or Multifunctional Compositions	Acids and Esters	Terpenes (hydrocarbons)
Nitrogen Compositions	Sulfur Compounds	-

1.1.5 Pharmacology of well-known essential oil components

Pharmacologically significant essential oil groups are of both terpenoid and non-terpenoid origin unless specified. With regard to commercially significant essential oils, monoterpene hydrocarbons, such as limonene, α-pinene (**1**), myrcene (**2**) or *p*-cymene (**3**) are major components in grapefruit, Pine (*Pinus pinaster*: Pinaceae), Juniper berry (*Juniperus communis*: Cupressaceae) and Frankincense (*Boswellia carterii*: Burseraceae) respectively. *p*-Cymene has been demonstrated to have skin sensitising effects, so essential oils rich in *p*-cymene are therefore avoided in topical applications (Bowles, 2003).

With regard to sesquiterpene hydrocarbons, β-caryophyllene (**4**) is known from Black Pepper and chamazulene (**5**) from German Chamomile (*Chamomilla recutita*: Asteraceae) (Bowles, 2003). Azulene sesquiterpenes, such as chamazulene or guaiazulene from *Callitris intratropica* (Cupressaceae), are responsible for the blue

colour of their respective essential oils, if present at sufficient concentrations (Bowles, 2003; Sadgrove & Jones, 2014).


(**1**) α-pinene

(**2**) myrcene

(**3**) ρ-cymene

(**4**) β-caryophyllene

(**5**) chamazulene
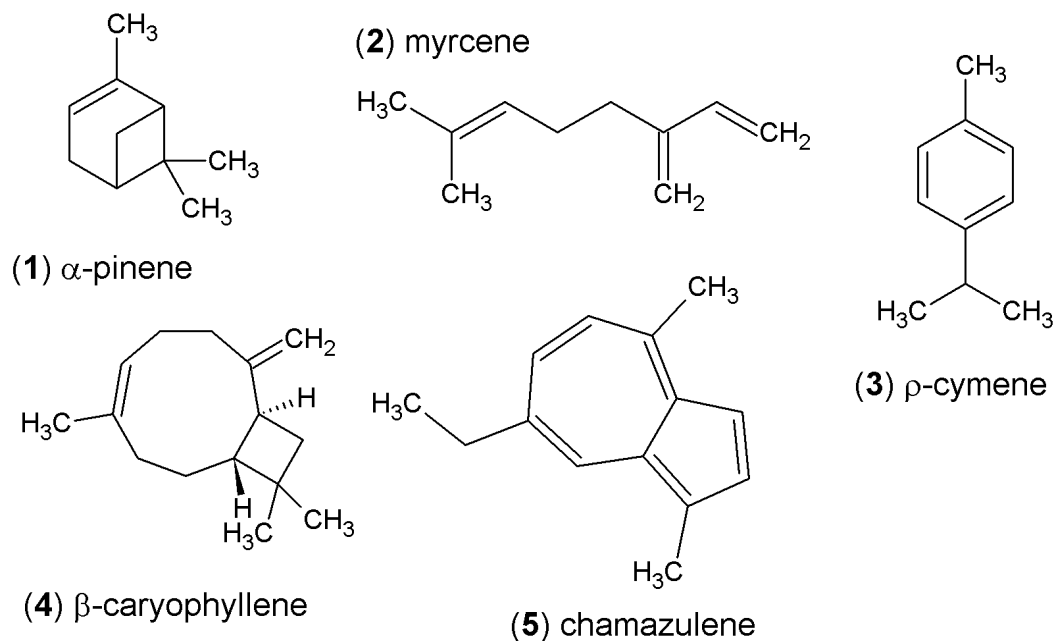
Monoterpenols such as linalool (**6**) from Lavender (*Lavendula angustifolium*: Lamiaceae), menthol (**7**) from Peppermint (*Mentha piperita*: Lamiaceae) or α-terpineol (**8**) from Tea Tree (*Melaleuca alternifolia*: Myrtaceae) are known for slight analgaesic effects if applied topically (Bowles, 2003). Furthermore, linalool has been associated with possible sedative effects as well (Elisabetsky *et al.*, 1995).

Well known sesquiterpenols are α-bisabolol (**9**), again from German Chamomile, α-eudesmol (**10**) from Cedarwood (*Juniperus virginiana*: Cupressaceae) or α-santalol (**11**) from Indian Sandalwood (*Santalum album*: Santalaceae). The sesquiterpenol α-bisabolol and the sesquiterpene chamazulene, have been associated with anti-inflammatory activity, particularly α-bisabolol (Bowles, 2003). *In vitro* blockage of neuronal Ca^{2+} channels by α-eudesmol has been linked to potential psychoactive effects (Horak *et al.*, 2009). This may be significant with regard to anecdotal accounts of Cedarwood essential oil associated with enhanced memory and creativity (Asakura *et al.*, 1999). Psychoactive and physiological effects consistent with sedation were observed when Indian Sandalwood (*S. album*) was transdermally absorbed, with activity attributed to α-santalol (Hongratanaworakit *et al.*, 2004). Sandalwood oil has also been associated with potential inhibition of the *Herpes simplex* virus (Bowles, 2003).

Other well known examples from the chemical groups described by Franchomme and Penoel (Table 1) include the phenol carvacrol (**12**) from Oregano (*Origanum vulgaris*: Lamiaceae), which has been potentially implicated in liver damage, along with a host of other phenols and more specifically, phenylpropanoids, such as the

aforementioned carvacrol and the potentially hepatotoxic safrole (**13**) and methyl eugenol (**14**), known to be available in high yields from various Australian *Zieria* (Rutaceae) species (Sadgrove & Jones, 2013a) and an unusual and rare chemotype of *Eremophila longifolia* (Scrophulariaceae) (Della & Jefferies, 1961; Sadgrove *et al.*, 2011).
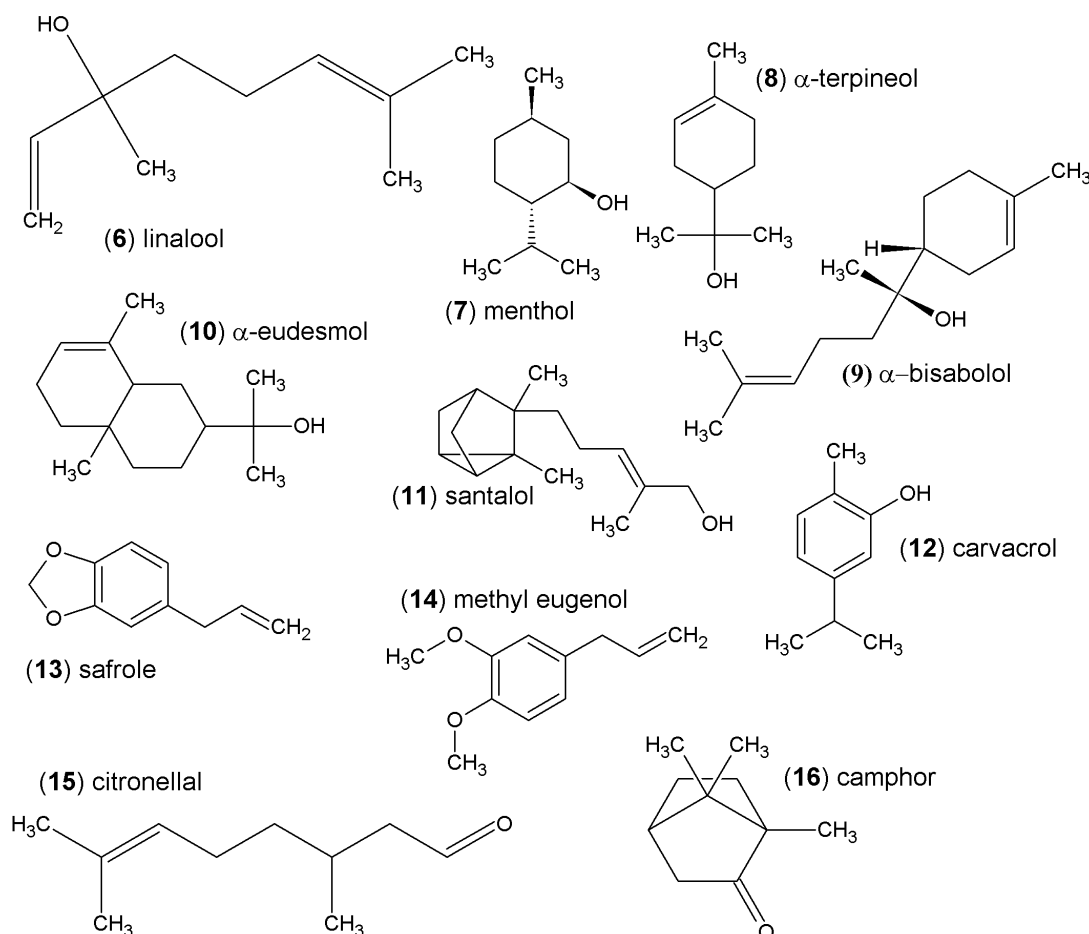

An example of a well-known component from the aldehyde class is citronellal (**15**) from *Eucalyptus citriodora* (Myrtaceae), which is used as an insect repellent with mosquitocidal activities (Maia & Moore, 2011). Camphor (**16**) is the best known example of a ketone, which is the major component in essential oils from the Spanish chemotype (CT1) of Rosemary. Although the use of camphor is treated with suspicion, after studies demonstrated potential convulsant and liver/central nervous system damage, the camphor and α-pinene rich chemotype of rosemary continues to be used as a liniment for muscle aches and pains (Bowles, 2003).

Because acids are more soluble in water, they do not often become part of an essential oil. An example of this would be the boswellic acids from various Frankincense species (*Boswellia* spp.). Small amounts of boswellic acids do appear in the essential oils but the majority are dissolved into the hydrosol. Thus, Frankincense

oils produced using supercritical CO_2 extraction have much higher concentrations (Bowles, 2003).

Acids and alcohols are usually precursors to esters and when esters form into closed rings they become lactones (Sell, 2010). Typically when alcohols are esterified by acetic acid, or another larger mass molecule, they are named according to the parent alcohol, thus, linalool (**6**) becomes linalyl acetate (**17**), borneol (**18**) becomes bornyl acetate (**19**) and fenchol (**20**) becomes fenchyl acetate (**21**). Linalyl acetate is another of the major components of Lavender oil and is additionally a significant component in the essential oil from Clary Sage (*Salvia sclarea*: Lamiaceae).

(17) linalyl acetate

(19) bornyl acetate

(18) borneol   **(20)** fenchol
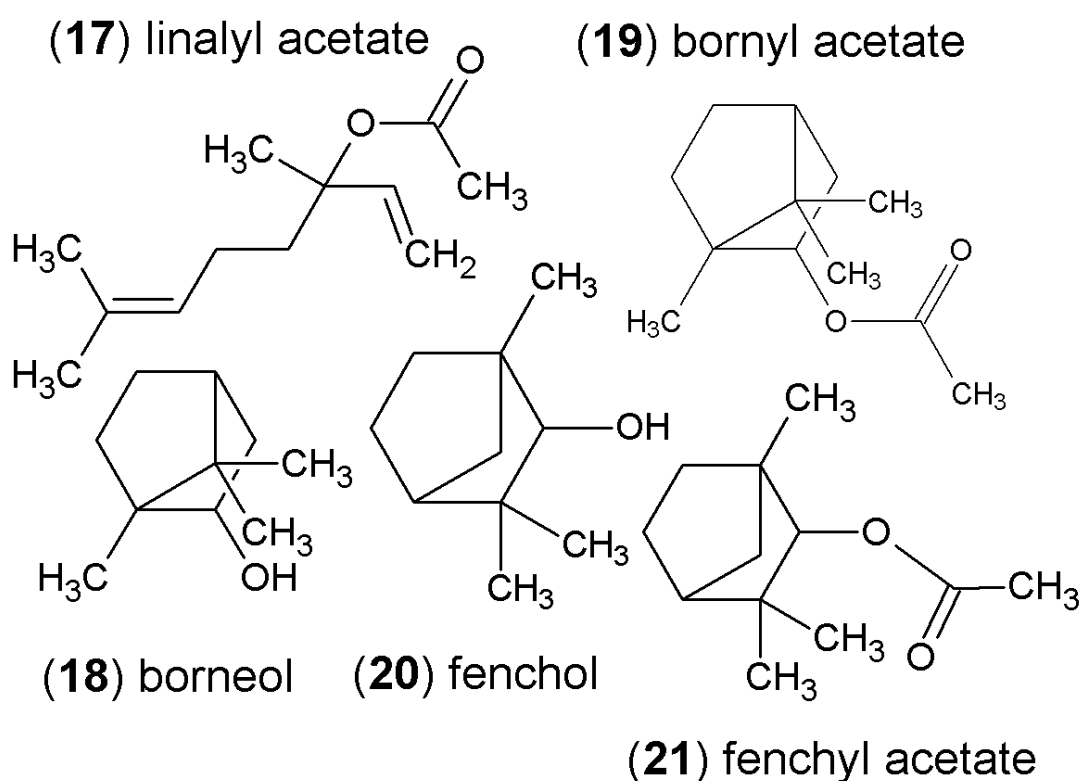
(21) fenchyl acetate

Linalyl acetate was associated with the previous mentioned analgaesia, along with linalool, in Lavender oil (Bowles, 2003). An oil rich in fenchyl- and bornyl acetate is that from *Eremophila bignoniiflora* (Scrophulariaceae), and these components are probably responsible for the demonstrated moderate to high activity against the yeast *Candida albicans* and the bacteria *Staphylococcus epidermidis* (Sadgrove *et al.*, 2013). Additionally, *E. bignoniiflora* was used in traditional medicinal applications by Australian Aboriginal people to treat headaches using volatile gases, and extracts from leaves as a laxative. Schnaubelt (1995) lists ester rich essential oils as having antispasmodic activity and are also effective in the treatment of central nervous system and stress related ailments. Thus, ester-rich essential oils from *E. bignoniiflora* may have been significantly involved in traditional medicinal uses.

Another well known ester, making up approximately 98-99% of the whole essential oil of Wintergreen (*Gualtheria procumbens*: Ericaceae), is methyl salicylate (**22**), which is thought to have analgaesic, anti-inflammatory and counter-irritant effects comparable to aspirin. Methyl salicylate is often used as a positive control in various pharmacological assays for analgaesia and anti-inflammatory activity (Semnani-Morteza *et al.*, 2004; Wang *et al.*, 2008).

With regard to ethers in essential oils, they typically occur as phenyl ethers, such as the phenylpropanoids eugenol (**23**), known from Clove bud oil (*Syzygium aromaticum*: Myrtaceae) in concentrations as high as 75%, and methyl chavicol (**24**) from Comoro Island Basil oil (*Ocimum basilicum*: Lamiaceae) at approximately 85% of the whole. Ethers are commonly associated with psychotropic effects, which can lead to death if taken in high dosages. The best known examples of these are the phenylpropanoids, myristicin (**25**) and elemicin (**26**), highly concentrated in essential oil produced from Nutmeg seed (*Myristica fragrans*: Myristaceae) (Kalbhen, 1971; Bowles, 2003; Beyer *et al.*, 2006).

(22) methyl salicylate   **(23**) eugenol
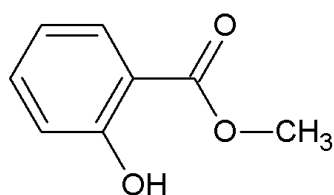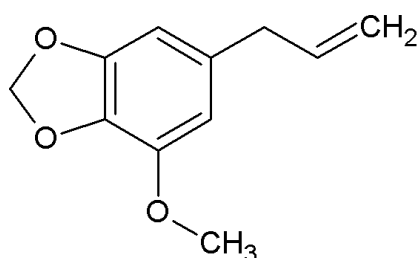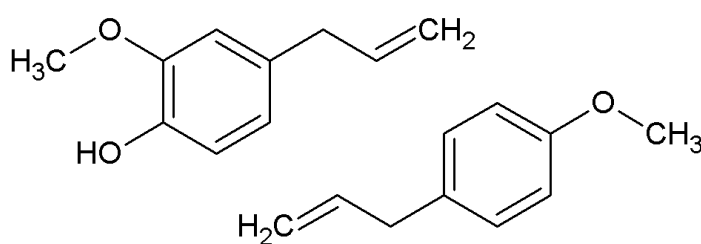
(24) methyl chavicol
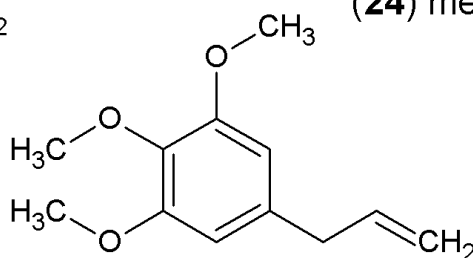
(25) myristicin   **(26**) elemicin

When ethers occur in closed cyclic structures, they are called oxides. Perhaps the best known of these is 1,8-cineole, also known as eucalyptol. In the Australian flora, Eucalyptus species are not the only ones exhibiting high yields of this compound, as 1,8-cineole also occurs in high concentrations in the essential oil of many other endemic genera, including *Prostanthera* spp. (Lamiaceae) along with a host of other sesquiterpenols. Species such as *P. ovalifolia*, *P. rotundifolia*, *P. caerulea*, *P. lasianthos*, *P. cineolifera* and *P. incisa* have high concentrations of 1,8-cineole in their essential oils (Baker & Smith, 1912; Southwell & Tucker, 1993; Dellar *et al.*, 1994;

Gersbach, 2002; Pala-Paul *et al.*, 2006). As 1,8-cineole (**27**) produces expectorant effects, it is not surprising that a large number of plants, rich in this compound, were used ethnomedicinally for decongestion by sufferers of coughs and colds.

(**27**) 1,8-cineole   (**28**) coumarine   (**29**) bergaptene
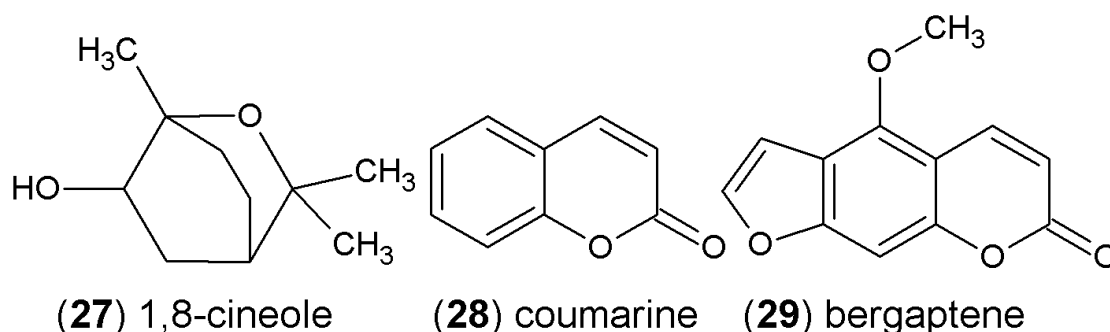
Lactones are constituents of many essential oils (Baser & Demirci, 2007). Lactones are produced by an intramolecular esterification reaction, where an aliphatic alcohol joins with an acid and closes into the respective cyclic ester (Bowles, 2003). The lactones are named after, and derived from, lactic acid ($C_3H_6O_3$). They usually occur in five- or six-membered heterocyclic rings in saturated or unsaturated forms, bonded to a carbonyl group. Lactones occurring in five-member rings are referred to as γ-lactones; those occurring in six-member rings they are referred to as δ-lactones (Baser & Demirci, 2007), and those occurring in four-member ring as β-lactones (Bruice, 2004).

Constituent γ-lactones, some with a peach-like flavour, are found in Fenugreek, coffee and Sake; representatives of δ-lactones are found in cheese, fruits and dairy products, typically with a creamy-coconut or peach-like odour. Lactones with larger carbon rings are found in essential oils from Ambrette seed or Angelica root. Angelica also contains pthalides, which are a lactone of 2-hydroxymethyl benzoic acid. Phthalides are restricted to the Apiaceae family, typically in Celery, Lovage and Angelica (Baser & Demirci, 2007).

Lactones also have demonstrated possible expectorant effects, but it is not yet clear if topical applications should be contraindicated, as some studies have highlighted the potential for skin-sensitisation to occur. Despite this, lactones have also demonstrated high *in vitro* activity consistent with anti-inflammatory effects, meaning lactone rich essential oils may be suitably used for topical applications to treat inflammation (Bowles, 2003). In this context then, given the widespread chronic nature of gastric inflammatory disease, it may be worth investigating the potential for lactones to treat inflammation of the bowel or alimentary canal.

When an aromatic lactone is adjacent to a benzenoid moiety it becomes a coumarin. In its simplest structural form it is simply called coumarin (**28**), which is the principal odour compound responsible for the aroma of freshly cut hay (Lassak & McCarthy,

2011). Perhaps the best known coumarin is the furanocoumarin bergaptene (**29**), found in Bergamot oil (*Citrus bergamia*: Rutaceae) and also in Australian species, such as *Philotheca trachyphylla* (Rutaceae) (previously *Eriostemon*) (Lassak & Pinhey, 1969). Bergaptene has a UV-sensitising effect, linking to melanin in the skin if applied topically in the sun (Bowles, 2003). This has the effect of intensifying the effects of the sun's rays. Interestingly, this bergaptene rich oil is most likely a consequence of the method of extraction, being mechanical processing. Bergamot essential oils produced by hydrodistillation, as opposed to those expressed oils, are not likely to have significant quantities of bergaptene.

Coumarins are also potentially associated with anticoagulant activity, but this has not yet been fully investigated. It is well known that the double coumarin 'dicoumarol' (**30**) is related to the occurrence of internal bleeding when herbivores consume large amounts of Yellow Sweet Clover (*Melilotus officinalis*: Fabaceae). If other coumarins could be associated with anticoagulant activity, this effect may be employed in the treatment of cardiovascular disease (Bowles, 2003).

A wide selection of both furano- and pyranocoumarins are known to produce bioactive effects *in vitro* and should form the basis for further pharmacological investigations in Australian plants used medicinally by Aboriginal people. The furanocoumarin geiparvarin (**31**) and methoxycoumarin dehydrogeijerin (**32**) are potentially responsible for differences in sheep palatability of leaves from *Geijera parviflora* (Rutaceae) (Lahey & MacLeod, 1967). Novel, as well as known coumarins were identified in *Phylotheca trachyphylla* (as *Eriostemon* in that study) (Lassak & Pinhey, 1969). A host of others are known from Australian plants.

(**30**) dicoumarol

(**32**) geiparvarin

(**32**) dehydrogeijerin

(**33**) anethole
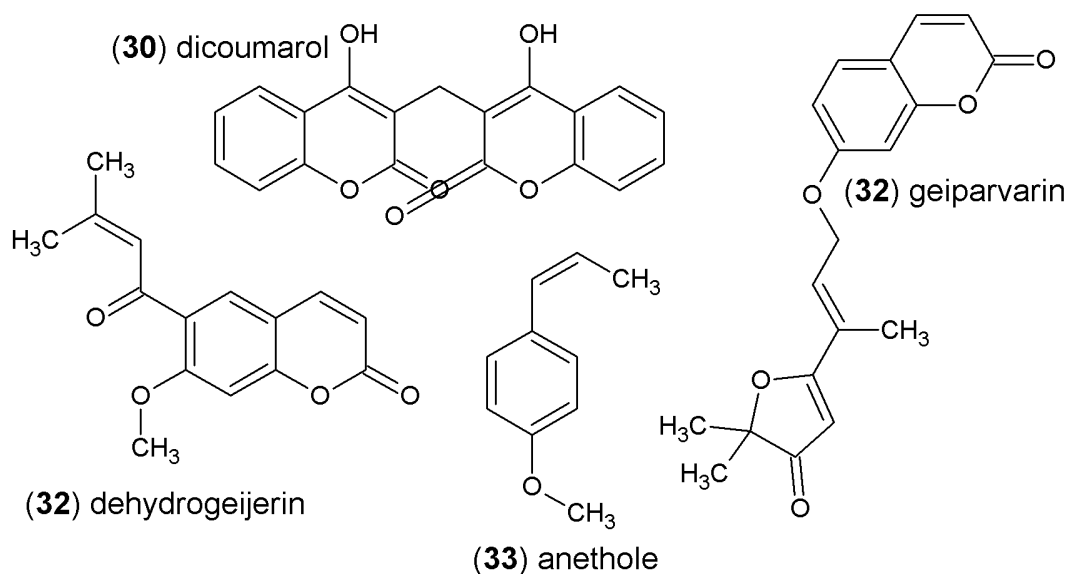
Because *G. parviflora* was used in various medicinal, ceremonial and recreational activities by Aboriginal Australian people, the involvement of coumarins in these

types of activities should be investigated. For example, the desmethyl congener of geiparvarin has already been demonstrated to have *in vitro* effects consistent with psychoactive sedation (Carotti *et al.*, 2002). This effect could be related to psychoactivity achieved in traditional smoking activities (Lassak & McCarthy, 2011).

With respect to using coumarins medicinally, as with other compounds, the chiral configuration strongly influences subjective and pharmacological effects (Tanaka *et al.*, 2004), which obviously makes synthesis more expensive. A corollary of this is the toxic *cis*-anethole (**33**) enantiomer, which is not produced in nature, but is rather a consequence of synthetically producing the medicinal compound, *trans*-anethole, which is sourced in enantiopure forms from Aniseed oil (*Pimpinella anisum*: Apiaceae) or Fennel seed oil (*Foeniculum vulgare*: Apiaceae) (Lassak & McCarthy, 2011). The effect of chirality on the pharmacokinetics and pharmacodynamics of drugs is now fully appreciated (Hutt, 2007) and researchers are beginning to seriously investigate the effects in natural product and synthetic medicine.

1.1.6 Essential oils and flavours as glycosides and glucosinolates

Aside from the phenylpropanoids mentioned earlier, or other shikimates, which are biosynthesised via the *shikimic acid pathway*, the above mentioned chemical classes can be either of terpenoid or non-terpenoid origin. Non-terpenoid essential oils may also form from degraded phospholipids and fatty acids. However terpenoid essential oil components are by far the most common.

Non-terpenoid components occur in a large number of forms, such as acids, esters, oxides et cetera, but are more commonly found as small chain hydrocarbons formed into alcohols and aldehydes, from saturated or unsaturated parent chains, such as alkanes or alkenes/alkynes respectively (Evans & Becerra, 2006; Baser & Demirci, 2007). Apart from simple hydrocarbons, non-terpenoid essential oils also may contain nitrogen or sulphur, or both, as with isothiocyanates (Baser & Demirci, 2007).

Isothiocyanates generally involve a sulphur, carbon and nitrogen chain, double bonded between atoms (Figure 1). The nitrogen acts as the bridge to the remainder of the molecule. Isothiocyanates are formed from hydrolysis of a glucosinolate precursor. Although a variety of essential oil components are freely converted between a glycoside and free form, the biosynthetic pathway of isothiocyanates is dependent upon the formation of this glucosinolate. Thus, isothiocyanates are always the aglycone of a hydrolysed glucosinolate (Baser & Demirci, 2007).
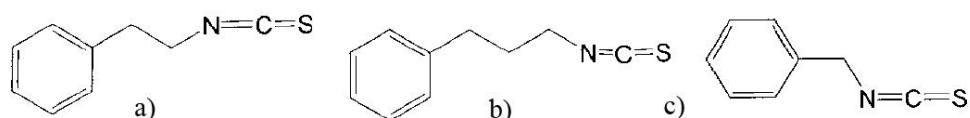
Figure 1 - Isothiocyanates contain both sulphur and nitrogen; a) Phenylethyl, b) 3-methylpropyl and c) Benzyl isothiocyanate (Baser & Demirci, 2007).

Hydrolysis of glycosinolate bonds typically occur when plant cells are broken, perhaps during animal, insect or human grazing, and the enzyme myrosinase, capable of cleaving the bond, is brought into contact with the glycoside. The subsequent formation of isothiocyanates gives flavour to various food items, such as cruciferous vegetables, mustard, broccoli, cauliflower, kale, turnips, collards, brussel sprouts, cabbage, radish, turnip and watercress (Baser & Demirci, 2007, pp. 70-71).

In addition to isothiocyanates, terpenoid compounds are also stored in plant tissues bonded to a sugar moiety through glycosidic linkages. This typically happens to alcohols such as α-terpineol (Skoula & Harborne, 2002). In these cases volatile oils become fixed (non-volatile), preventing them from being produced during steam distillation (Stahl-Biskup, 1987; Skoula & Harborne, 2002). When essential oil components are formed into glycosidic bonds, the respective component becomes hydrophilic, making it transportable throughout the plant tissues (Stahl-Biskup, 1987). Additionally, the formation of glycosides reduces the damaging power of essential oil components to plant tissues, serving the advantage of safe storage and minimal damage to the plant cells during circulation (Stahl-Biskup *et al.*, 1993).

In plant tissues, hydrolysis of glycosidic bonds typically occurs during wilting of plant leaves after harvest. Thus, with regard to a selection of Australian essential oil yielding species, it is common practice to allow the harvested leaves to dry for a short period before hydrodistillation (Bottcher *et al.*, 1999). It is recommended to retain leaf attachments to twigs during postharvest drying, as oil is sometimes transported from the twig to the leaf, which is known to occur during harvest of *Melaleuca alternifolia* (Myrtaceae) crops in the production of Tea Tree Oil (TTO) (Whish & Williams, 1996). Although it is common for Australian species to have this effect during post-harvest drying, other species may produce a net loss of essential oils during this wilting period, resulting from evaporation during this drying period, with no metabolic processes replenishing this lost oil.

1.2.0 Australian colonial and Aboriginal ethnopharmacology and utility of aromatic, medicinal or useful plants

1.2.1 Gums, resins and kinos

A consideration of colonial Australia's pharmacopoeia, in works such as those accumulated by Maiden (1889), Cribb and Cribb (1981b), Barr (1988), Low (1990), Lassak and McCarthy (2011), Williams (2011) and earlier sources from Everist or Penfold, demonstrates a recurring list of products including the oils (essential, expressed or animal oils), gums, resins, kinos, tannins and dyes. These useful and medicinal plants may have been involved in Australian Aboriginal usage and adopted by early colonialists, or may have been simply used by colonialists since they resemble, in some degree, European plants already familiar to them.

Gums, resins and kinos are apparently all various forms of a resinous exudate from the plant, but differ in their chemical character (Cribb & Cribb, 1981a). Although they are collectively referred to under the one name as gums, the distinction is occasionally made that gums are soluble in water, resins are not and kinos are either soluble or not, but distinguished on the basis of having a high tannin content. Frequently the exudate is a mixture of these, perhaps also with an oil, either fixed or volatile. Thus, resins may be referred to as oleo-resins, or oleo-gum-resins.

This system was not highly regarded as it was too ambiguous and subjective to allocate plant exudates into categories that were initially defined for less common, special cases, such as the Gum Arabic of African *Acacia* species, which is entirely composed of readily soluble simple and oxidised sugars (Lassak & McCarthy, 2011). With regard to kinos, apparently the original kino was produced by drying the juice of Indian and African species of *Pterocarpus*. These were widely distributed in Europe and Great Britain. When the Australian *Myrtaceae* kinos reached Europe, under the name of 'Botany Bay Kino' as one of the first exports from the fledgling colony, the reception was not great, as there was already a glut of inferior grade kinos available. Lassak and McCarthy (2011) however, point out that Australian kinos, produced mainly from the well known Ironbark (*Eucalyptus siderophloia*), were more effective than the oriental types, as the astringent in the former only mildly adhered to the mucous membranes and was not tolerated by a weak stomach. Interestingly, an earlier study made a connection between the distributions of oesophageal cancer and higher consumption of tannin rich drinks (Mortan, 1972), so consumption of tannin rich kinos is apparently not without a risk.

Australian colonial pharmacopoeia, in its time, provided the basis for an industry of considerable importance and promise. Early Australian settlers were more often than not isolated in the bush, separated from the more conventional health care system in larger urban areas by poor roads, insufficient transport and limited communication. In urban communities the foraging for plant-based medicines was largely replaced by commercial type (patent) medicines nearly a hundred years ago. This includes importantly the advent of antibiotics in the 1940s. However, it is not surprising that foraging for medicinal plants persisted for decades in more regional

areas, probably continuing during the childhood of the baby boomers (1970's), as the post war depression placed financial constraint on these regional families. Often such remedies focused on health complaints that were not severe enough to warrant a threat to one's life, such as diarrhoea, skin infections or sores, asthma or coughs and colds (Cribb & Cribb, 1981b).

Another type of plant exudate is latex, which is a milky white fluid containing resins, sugars and oils, but distinguished from the collective gums by the occurrence of proteins and caoutchouc, the latter being the primary source of rubber (Cribb & Cribb, 1981a). Although it had been thought that Australian latex yielding species could be utilised for a large scale commercial rubber industry, the advent of synthetics subsequently nullified this as a commercial proposition. Indeed, synthetic materials eventually came to replace the use of gums for adhesives, or cosmetics, pharmaceuticals, paints, matches or foodstuffs. Prior to the advent of synthetic materials, resins were also involved in waterproofing, as well as adhesives, paints, linoleum, torches, incense and embalming (Cribb & Cribb, 1981a). It is of more than simple historical interest to note that the early colonial uses of these naturally occurring materials overlapped extensively with traditional uses by Aboriginal Australian peoples.

The history of early natural product industry developments have been largely forgotten since the advent of synthetics and large-scale entrepreneurial industries, as well as advanced contemporary medical techniques. Having said this, in some quarters there appears to be resurgence in interest, due to the challenges involved in enantioselective synthesis and the changing views on dependence on fossil fuels (Lassak & McCarthy, 2011).

An early leading advocate for the potential of Australia's natural product industry was Joseph Henry Maiden (1859-1925), who enthusiastically communicated business prospects to various members of the public and fuelled initiatives to encourage small business ventures. Maiden was considered the foremost expert in this area and therefore became the focus of a large number of entrepreneurs who approached him with industry initiatives.

Interestingly, Maiden himself had received a letter with regard to the use of an infusion from the leaves of *Geijera parviflora* (Rutaceae) for analgaesia, taken either orally or topically, by a man from Dubbo, in regional New South Wales (Lassak & McCarthy, 2011). One may have thought that such a claim would warrant serious research, but it is only recently that this has begun (Sadgrove & Jones, 2014). As previously mentioned, further investigations on the coumarins from these species are required.

Maiden received a significant number of letters describing the botanical observations of species that were not well known at the time, but are well known for commercial production today, such as *Melaleuca quinquenervia* (Myrtaceae), a species responsible for two of the Tea Tree Oils (CT Niaouli and CT Nerolina), the nectar of which has apparent unusual effects on the behaviour of bees (Lassak & McCarthy, 2011). Maiden was also informed of a species claimed to have potent insecticidal activity (*Alstonia constricta*: Apocynaceae). Apparently foraging bees are killed or sedated as a consequence of the occurrence of alstonine in the pollen or the leaves.

Interestingly, in the light of our current results presented in Chapter 8, Maiden himself was particularly interested in the utilisation of the resinous exudate from injured or cut trunks of *Callitris* species (Cupressaceae), as an export oriented commercial product. At the time he had hoped that this could complement a timber industry that inevitably produced large amounts of this exudate a year or so after logging. Maiden encouraged small, family driven business as a means to make the industry viable, as the collecting efforts of the children would prove economically advantageous (Low, 1990; Williams, 2011). When the industry started to grow, the commercial name 'Sandarac' was used. At the time another product, using the same name, was produced from the African species *Tetraclinis articulata* (Cupressaceae) (Dieterich, 1920).

'Sandarac' was used as an enteric coating for pills, as it was insoluble in the stomach but digested once in the small intestine. The Australian 'Sandarac' was also used in dentistry and other applications similar to the better known Sandarac of African provenance. Although the two 'Sandarac' types were very similar, the African variety was and still is considered superior (Dieterich, 1920), so the Australian industry did not achieve the growth that may have been expected of it.

Maiden was best known for his involvement in the rise of Australia's *Eucalyptus* essential oil industry. Prior to Maiden's involvement, the pioneering Yorkshire pharmacist, Joseph Bosisto, after migrating to Australia in 1848, had studied the essential oil yielding flora, with particular emphasis on *Eucalyptus* spp. A German pharmacist turned botanist, known at that time as Dr Müller but later as Baron Sir Ferdinand von Müller, greatly assisted Bosisto to establish Australia's first commercially produced eucalyptus oil industry, after Müller himself encouraged Bosito to undertake the initiative (Lassak & McCarthy, 2011).

1.2.2 Uses of the Australian essential oils

The first eucalyptus oil to enter British pharmacopoeia, under the name *Oleum Eucalypti*, was the cineol rich form known widely today (Maiden, 1889). The most common eucalyptus (Myrtaceae) species used to produce the well known 1,8-

cineole rich essential oil are, among others, the 'blue mallee' (*E. polybractea*), the 'broad leaf peppermint' (*E. dives* var C) and *E. leucoxylon, E. sideroxylon, E. oleosa, E. radiata* var australiana et cetera (Lassak & McCarthy, 2011). Currently much of the global production of Eucalyptus oil from Eucalypts is carried out in Portugal and Spain, which have established *E. globulus* as the favoured cultivar; however, in terms of gross production China leads the way with Chinese Eucalyptus oil, a by-product of camphor production from *Cinnamomum camphora* (Lauraceae).

Another form of eucalyptus oil, rich in the ketone piperitone, is produced in Australia from commercial plantations of another chemotype of *E. dives*. In terms of volume, the major supplier of this oil is based in Swaziland in South Africa. This piperitone rich oil can also be produced from *E. piperita* (Myrtaceae), which was first distilled by First fleet Surgeon Denis Considen in 1788. The basis of Considen's attraction to this species was its odorous resemblance to *Mentha piperita* (Lamiaceae), hence the botanical name (Cribb & Cribb, 1981a). Although the subjective comparison is correct, *M. piperita* essential oils are dominated by menthol, menthone and pulegone (Behnam *et al.*, 2006), but contain no piperitone. This account reflects the natural tendency of early Australian colonialists to focus on species that resembled in some manner those already described in British or European pharmacopoeia. This could be seen as an impediment in terms of accessing the rich existing tradition of Aboriginal Australian medicines. Having said this, there are many examples of colonial medicines, taken from the Australian environment, that were not in fact used by Aboriginal people. In many such cases the Aboriginal people were aware of medicines that more effectively targeted the respective ailments than those chosen by early colonial settlers.

The piperitone rich oil produced by Considen from *E. piperita* apparently constitutes the first recorded distillation of an essential oil from an Australian *Eucalyptus* species. The resultant product was one of the first useful exports from the colony to Britain. Although, for over 100 years it was wrongly believed that the credit was owed to 'Surgeon-General to the Colony', John White, it was later clarified by Maiden that this was wrong, when he examined a letter addressed to Sir Joseph Banks from Considen, who had posted him a sample of the oil for use in medicinal applications (Lassak & McCarthy, 2011).

With regard to the cineol-rich oils from *Eucalyptus* species, apart from medicinal applications consistent with decongestion in coughs and colds, there are traditional reports of using the Tasmanian Blue Gum (*E. globulus*) in applications consistent with mosquitoe repellence. Accordingly, it was given the colloquial name 'fever tree' or 'fever prevention tree' as the leaves were hung in and around homes to prevent the occurrence of malaria and other mosquitoe borne diseases. Interestingly, the absence of malaria in New Caledonia at the time was attributed to the high

occurrence of the cineol rich chemotype of *Melaleuca quinquenervia* (Myrtaceae) (Lassak & McCarthy, 2011). Furthermore, *Prostanthera cineolifera* (Lamiaceae), named for it's higher yield of 1,8-cineol, was also used as an insect repellent by early colonialists (Baker & Smith, 1912). As an aside, Maiden (1889) reported that eucalyptus oil may be useful for treating malarial symptoms, albeit less effective than quinine but nevertheless, capable of providing relief. However, it was the insect repellent activity of 1,8-cineole that formed the basis for its use in and around homes.

Some of Australia's best known essential oils were listed by Maiden as early as 1889. These included species such as *Eucalyptus globulus*, *E. citriodora*, *Backhousia citriodora* (Myrtaceae) and *M. alternifolia* (as *M. linarifolia*: Myrtaceae). It is apparent from the text that Maiden (1889) favoured a number of species, which for a range of reasons have not achieved significant commercial value. For example, a potentially hepatotoxic essential oil from *Z. smithii* (Rytaceae) is listed for its flavour enhancing activity, although it has more recently been demonstrated to have potentially carcinogenic phenylpropanoids, safrole and methyl eugenol, in its essential oil (Sadgrove & Jones, 2013a). *Eucalyptus* species provided the greater part of Maiden's focus, but *Melaleuca* species were also given due attention.

Although the well known *Melaleuca alternifolia* (as *M. linarifolia*) is only given brief mention by Maiden (1889), *M. leucadendra* (as *M. leucadendron*) is probably the best described by him. Because of the similarity of this essential oil to that of the Malay Cajeput (*M. cajuputi*: Myrtaceae), the tree has been given the vernacular name 'Cajeput Tree'. Maiden's description of the preferred method for preparing leaves for hydrodistillation somewhat resembles the modern post-harvest leaf wilting technique in contemporary use for commercial production of TTO from *M. alternifolia*. Prior to hydrodistillation this method involved collection of the leaves, storing in a sack and wilting for approximately a day, before maceration of the leaves and soaking in water for fermentation, taking yet another day (Maiden, 1889).

Maiden recommended this method for essential oil extraction from any of the *Melaleuca* species. The latter part of this method, involving the fermentation of macerated leaves in water, is not commonly in use today, but it may be worth investigating the possibility that it can improve essential oil yield by facilitating the hydrolysis of glycosidically bonded essential oils. Leaf fermentation preparation may still be in common use for production of essential oils from *M. cajuputi* in Asia and India.

By the 1980's, only two Australian essential oils had achieved significant international market success. These were the cineol-rich *Eucalyptus* and *M. alternifolia* essential oils. With respect to addressing the international market place, Cribb and Cribb (1981a) hypothesise that the limiting factors include the availability

of commercial scale plantations and the lack of anecdotal reference describing traditional use modalities of the oil. The adoption of essential oils, addressing specific uses, into the international market place, would be fuelled tremendously from such anecdotal accounts. Contemporary pharmacological investigations, informed by traditional medicinal uses by Australian Aboriginal people, also facilitate the emergence of this market niche. To a large extent, the research that follows, in this thesis, is an attempt to do exactly that. Having said this, it is primarily the availability of viable plantations that is the limiting factor. At the moment moves to involve Aboriginal communities in plantation and harvest of suitable cultivars will address both these factors as well as providing a source of much needed employment.

An object lesson supporting the above hypothesis is provided by the history of the Australian Sandalwood (*Santalum spicatum*: Santalaceae) industry. The sesquiterpenol dominated essential oil is known for medicinal activity, as demonstrated firstly by Aboriginal Australian people, who made use of concoctions for coughs and colds, or as a liniment (from the nuts) for muscle stiffness (Lassak & McCarthy, 2011). Smoke from the Eastern Australian species of Sandalwood (*Santalum lanceolatum*: Santalaceae) was used to drive away mosquitoes in New South Wales (Cribb & Cribb, 1981b) or in aromatherapy applications for babies in the Northern Territory (Barr, 1988). Subsequent pharmacological testing has revealed good antimicrobial activities against such microbial species as *Candida albicans* or *Staphylococcus aureus* (Jirovetz *et al.*, 2006).

Because the distillation of *S. spicatum* required destruction of the heartwood of the tree, the procurement of essential oils had a negative impact on wild populations. Due to the tree's growth habit as a parasite on other trees, regrowth was very slow, so sustainability of the industry was threatened by the disappearance of wild populations (Lassak & McCarthy, 2011). Thus, the limiting factor was primarily the lack of viable plantations. Recently the industry recovered with the formation of initiatives such as the Australian Sandalwood Network or WA Sandalwood Plantations, so the product is once again available for consumers.

Another scenario which demonstrates how the availability of plantations is a limiting factor in establishing a commercial niche is the recent emergence of the *Callitris intratropica* (Cupressaceae) essential oil industry. At one time, *C. intratropica* was botanically classified as *C. columellaris*, together with *C. glaucophylla* (Thompson & Johnson, 1986). Because these species were known under the one name, many ethnobotanic records describing traditional Australian Aboriginal medicinal uses of *C. columellaris* included those for *C. intratropica*. Medicinal uses included topical applications using hydrophilic or animal fat extracts, as well as smoke fumigation treatments for various ailments (Lassak & McCarthy, 2011). Barr (1988) provides

clearer details of traditional medicinal use, specifically of *C. intratropica*, which apart from topical applications for effects consistent with antimicrobial activity, also involved the use of a concoction of the inner bark, applied topically for relief from abdominal pains and cramps, perhaps achieved via transdermal absorption of the relevant medicinal principles.

Early 19th century colonial settlers were also aware of medicinal uses of *Callitris* species and the needles were steamed and inhaled for chills and pains. Maiden declared that "there is nothing more delightful in the approach, on a winter evening, to a township where Cypress pine is used as a fuel. Its delicious perfume is borne on the air for miles, and is often the first intimation that the weary traveller experiences that he is approaching a human habitation, and that his long journey is drawing to a close" (Low, 1990).

After it was observed that houses built using *Callitris* timbers, had resisted termite infestation over several decades, an attempt was made to develop a timber industry for international export (Maiden, 1889). The formation of a plantation of *C. endlicheri* (Cupressaceae) was the plan. Much to the disappointment of Maiden, this proved not to be economically viable, due to the high costs involved in transporting timber from the proposed plantations in New South Wales, to the Northern Territory, where ships would transport further into south-east Asia and beyond.

It wasn't until the 1960's, long after Maiden had passed on, that a timber plantation of *C. intratropica* had been established in the Northern Territory. After the disastrous occurrence of Cyclone Tracey in Darwin during 1974, it was observed that structures built with *Callitris* timbers were not as resilient. The timbers were therefore not considered strong enough to be used in infrastructure and the plantations were abandoned. In 1995 the blue essential oil, from the timber of *C. intratropica*, was first discovered and an essential oil industry was quickly established, supported by pharmacological studies demonstrating antibacterial and possible anti-inflammatory activities and supplied by the existing plantations (Oprava *et al.*, 2010).

With regard to the pioneering efforts of earlier Australians to examine essential oil yielding flora of Australia, another of the names frequently highlighted in the literature is Arthur de Ramon Penfold (1890-1980), a phytochemist with a special interest in the Australian essential oils (McKern, 1980, 1981). Penfold achieved an international reputation for his work in chemistry when he started to characterise unusual essential oil components, unique to the Australian flora. Penfold was also the one to elucidate the structure of piperitone and demonstrated how menthol and thymol could be synthesised from it (McKern, 1980, 1981). Penfold substantially contributed to Ernest Guenther's six-volume work, *The Essential Oils* (Guenther, 1948a).

In 1915 Penfold became a research chemist and assistant work manager to the eucalyptus oil distillers, Gillard Gordon Ltd (McKern, 1980, 1981). *Eucalyptus* species have been apart of European pharmacopoeia for well over 100 years. In relative terms, *Melaleuca alternifolia* essential oil has only recently acquired an international niche. It was Penfold who demonstrated significant antibacterial activities of *M. alternifolia* essential oil in a series of papers published in the 1920s and 30s (Carson *et al.*, 2006).

Prior to this, antimicrobial activities of *M. alternifolia* were familiar to the Bundjalung people from north-eastern New South Wales, who didn't use essential oils *per se*, in medicinal applications, but rather inhaled the vapours from crushed leaves for coughs and colds (Carson *et al.*, 2006). Additionally, a topical compress was used for skin infections and so forth, or a concoction to achieve a similar effect, or as a gargle for sore throats. Interestingly, according to the oral history of the Aboriginal people, lakes that received large amounts of fallen leaf matter from riparian *M. alternifolia*, developed medicinal properties (Carson *et al.*, 2006).

Today the essential oil from *M. alternifolia* is officially known as Tea Tree Oil (TTO); however, a large number of *Melaleucas* and *Leptospermum* species are also called Tea Trees, which can confuse the nomenclature. The description Tea Tree in fact arises from the tannins which can cause a brownish colour in lakes and water courses; hence the name Tea Tree Lake on the north coast of New South Wales.

After the antimicrobial properties of TTO were promulgated by Penfold, its first significant documented use was in the mid-1920s when it was applied as an antiseptic in surgery and dentistry. Following this, during World War II, it was used as a surface disinfectant in munitions factories, to curb infections to the workers following skin injuries. Additionally, the WWII soldiers were also issued TTO in their first aid kids. Following the advent of antibiotics, TTO was eventually forgotten and by the 1960s the oil became a rare commodity. In 1976 Eric White, convinced of a resurgence of interest in TTO in modern society, established a plantation near Coraki in northern New South Wales, after a crown lease was granted on a Thursday. The company therefore became known as the Thursday Plantation. Today TTO is used in a selection of soaps, shampoos and disinfectant products. The oil is sourced from commercial scale plantations in New South Wales, Queensland and Western Australia (Carson *et al.*, 2006).

Another essential oil worth mentioning here, because of its long history in Australia, is from the Western Australian species, *Boronia megastigma* (Rutaceae) (Cribb & Cribb, 1981a). Although it is better known commercially for its fragrant flowers, an essential oil industry was trialled in the early 1900s and declined, as it was wild harvested at that time and faced similar problems to the industry centred around *S. spicatum*. In the recent 20 years plantations have been established in Tasmania,

which have had varying success, but essential oils from *B. megastigma*, rich in β-ionone and dodecyl acetate, as well as their absolutes produced from the flowers for food flavouring, are now available under the name 'Brown Boronia' (Plummer *et al.*, 1999).

1.2.3 Alkaloids from Duboisia (Solonaceae) *species*

Müller named two of the *Duboisia* species, *D. hopwoodii* (1865: revised in 1882) and *D. leichhardtii* (1867), which was incidentally before he was made aware of the possible psychotropic effects (Foley, 2006). The better known *D. myoporoides* was named by Robert Brown as early as 1805, when he first introduced the genus *Duboisia* to the world of botany. Later, *D. myoporoides* was given the vernacular name 'corkwood' as it was among a selection of prospective timbers on display in Paris (1855 World Exhibition) described as having cork-like bark, under the incorrect name of *Santalum obtusifolium*. When *D. myoporoides* was first named it was placed in the Solonaceae, but later moved to Scrophulariaceae. Müller eventually reinstated its place in Solonaceae (Foley, 2006).

Of the three species, *D. hopwoodii* and *D. myoporoides* appear to be the best known for their use by Australian Aboriginal people to achieve psychotropic effects (Latz, 2004; Foley, 2006; Lassak & McCarthy, 2011). Ambiguous earlier reports describe the use of *D. hopwoodii* as an hallucinogenic substance (Lassak & McCarthy, 2011), in the pursuit of shamanistic effects. Colonial Australians were not able to experience the alleged shamanistic effects of 'pituri', made from *D. hopwoodii* (Robinson, 1980). Perhaps the 'pituri' in alleged shamanistic preparations used material from the other *Duboisia* species.

The psychotropic effects of *D. hopwoodii* were first brought to light when John King, the sole survivor of the infamous 'Burke and Wills' expedition, received 'pituri' as a parting gift from Aboriginal people of the Central Queensland Coopers Creek region, after his time of refuge with them had come to an end and the return with his European rescue party, to more familiar urban surrounds, had begun (Foley, 2006). According to ethnobotanical accounts, 'pituri' was used to increase stamina and decrease hunger during trialling ordeals, and then rolled up into a 'bolus' and stored behind the ear during less challenging activities (Latz, 2004). It took several decades before the source material of this 'pituri' was identified by Müller as *D. hopwoodii*, only a few years after John King's demise (Foley, 2006).

Although Müller had botanically named *D. hopwoodii* several years earlier from specimens growing in Central Australia, at the time he was not aware of the cholinergic effects because Central Australian specimens were chemotypically different from Queensland and New South Wales varieties. The higher abundance of nicotine and nornicotine in Central Australian specimens meant that it was fatally

poisonous, so it was utilised by Aboriginal people as an animal poison for food procurement purposes (Sadgrove, 2009). However, Central Australian Aboriginal people also appreciated the cholinergic effects of 'pituri', which was made available to them via extensive trade routes, stretching into New South Wales (Robinson, 1980). At a later time, it was observed that the Queensland 'pituri' was more frequently produced from *Nicotiana* species than *D. hopwoodii* (Foley, 2006).

Although no commercial developments have derived from *D. hopwoodii* or *Nicotiana* species, plantations in Queensland of a cultivar produced by crossing *D. myoporoides* and *D. leichhardtii* now provide the world's bulk supply of scopolamine (Foley, 2006). The psychotropic activity of *D. myoporoides* was familiar to Australian Aboriginal people, who made a hole in the trunk, filled it with a liquid and returned the following morning to consume the stupefying drink (Cribb & Cribb, 1981b). During the developmental stages of scopolamine research, medical naturalist Joseph Bancroft, also involved in the first investigations of physiological effects derived from usage of *D. hopwoodii*, started to observe the physiological effects of the previous described liquid extract from *D. myoporoides*, after it was applied to animals. In a letter from Müller, Bancroft was also encouraged to observe the physiological effects, in a controlled environment, after an Aboriginal man consumed the drink in the usual, traditional manner. However, Bancroft continued to focus on physiological effects in animal experiments before trialling it on some of his patients, which demonstrated advantages in ophthalmological procedures (Cribb & Cribb, 1981b).

Alkaloids from *D. myoporoides* were involved in a number of experimental medicinal applications. By 1893 both scopolamine and hyoscyamine had been identified as the active constituents and were in common use. About that time also, Maiden had described the method of commercial preparation and also the market niche, which he believed was primarily in Germany. The drug didn't achieve much more attention until after WWII, when over 7000 ounces were produced in Australia to treat sea sickness (Cribb & Cribb, 1981b). Again, sadly, Maiden passed on before seeing his industry grow to these proportions.

After the 1940s the scopolamine/hyoscyamine industry came close to closing down due to a lack of government interest. However, as Maiden had presciently suggested, German interest in the industry would lead to its expansion. This came to pass when, in the 1950s, the firm Boehringer Ingelheim made significant investments. From that time until now, the industry has maintained a steady level of production (Foley, 2006).

Another Australian plant reportedly producing psychotropic effects consistent with sedation is *Geijera parviflora* (Rutaceae), which was apparently smoked by Aboriginal people, unlike the *Duboisia* or *Nicotiana* (Solonaceae) species. As previously mentioned the contribution of coumarins in these types of activities should be

investigated. Studies should focus on the possible involvement of geiparvarin, as its desmethyl congener has been demonstrated to have *in vitro* effects consistent with psychoactive sedation (Carotti *et al.*, 2002).

Another possibility would be to investigate if alkaloids are produced in the smoke. A qualitative pharmacology test demonstrated the potential occurrence of alkaloids in these smoke extracts in Chapter 9 of this thesis. The origin of these alkaloids could be from cleavage of the ether bond between the coumarin and alkaloid moieties of parvifloranines A and B (Shou *et al.*, 2013), producing rearranged, potentially volatile alkaloids, in the smoke. A similar type of mechanism occurring in *Eremophila longifolia* smoking ceremonies leads to the production of genifuranal, Chapter 4.

1.3.0 Current trends in ethnomedicinal and chemical research on Australian aromatic plants

1.3.1 Today's essential oil industry

Due to a recent surge in interest in healthy living, complementary therapies and the non-synthetic health product sector, coupled with concerns raised about the growing resistance of pathogens to conventional antibiotics, the market for essential oils and suitably formulated creams and lotions has initiated surprising new developments. Although a significant number of antibiotic compounds have been isolated from Australian plants, the greater focus has been on essential oils.

Essential oils today are either sourced from plantations or wild harvested from populations that have grown to apparent unnatural densities because of a change in fire regime. A good example of this would be *Eremophila mitchellii* (Scrophulariaceae). In the early 20th century, when the *S. spicatum* (Santalaceae) populations started to decline from over harvesting, the fragrant eremophilane rich essential oils from heartwood of *E. mitchellii* were temporarily used as an alternative, but the subjective and chemical differences between the two essential oils prevented this change from taking effect.

Although some vernacular names include 'Buddah Wood', 'False Sandalwood' and 'Native Sandalwood', the other name 'Bastard Sandalwood' is perhaps the most cognisant of previous attempts to use it as a *S. spicatum* alternative. The *E. mitchellii* essential oil industry today owes its viability to the overgrowth of populations in the South Australian Flinders Ranges. Although the timber and essential oils are known for anti-termite activity (Beattie *et al.*, 2011), the essential oil is marketed as an aromatherapy complement to meditation.

Another plant known for termite resistant timbers is the Tasmanian native *Kunzea ambigua* (Myrtaceae). In a similar way to the discovery of termite resistance in *E. mitchellii*, Tasmanian farmers observed that fence posts produced from *K. ambigua*

remained intact when others did not. Most famously, in 1993 John Hood produced an essential oil from the species when he noted that his north boundary fence, constructed from *K. ambigua* wood, remained intact after 35 years. Interestingly, the vernacular name 'Tick Bush' derives from observations by early colonialists of the preference that wild animals had for sleeping under the bush, eventually demonstrated to reflect protective benefits from tick infestation.

The essential oils produced from *K. ambigua* leaves show a high degree of variation from predominantly monoterpenoid to predominantly sesquiterpenoid compositions, characterised by components such as α-pinene, 1,8-cineol, spathulenol, bicyclogermacrene, globulol, ledol and viridiflorol (Thomas *et al.*, 2010). Some of these oils are unusual because of the higher abundance of sesquiterpenes. In terms of the biological activity, the oil is best known anecdotally for its anti-inflammatory activity, which has led to its involvement in topical applications for the treatment of insect bites, itching and irritation.

Like *E. mitchellii*, commercial quantities of *Kunzea* oils, known as 'Ducane Kunzea', are also produced from wild harvest. However, unlike the previous mentioned *S. spicatum* and *E. mitchellii*, essential oils are produced from the leaves, not the heartwood. In ecological terms, wild populations have been grazed by wild animals for millennia, so leaf harvesting is not a new occurrence and is therefore sustainable. Thus, commercial growth of the Kunzea industry is not expected to be restricted by a decline in species density or a threat to the density of wild populations, but rather to the rejuvenation rate of the leaves.

Apart from the previously mentioned *Eucalyptus* spp., as well as *E. mitchellii*, *S. spicatum* and *M. alternifolia*, other examples of commercial scale essential oil plantations in full production today include; Anise Myrtle (*Syzygium anisata*), Fragonia (*Agonis fragrans*: Myrtaceae), Lemon Myrtle (*Backhousia citriodora*), Lemon Tea Tree (*Leptospermum petersonii*: Myrtaceae), Bracelet Honey Myrtle (*Melaleuca armillaris*) (Amri *et al.*, 2012), Nerolina (*Melaleuca quinquenervia* CT Nerolina), Niaoulina (*M. quinquenervia* CT Niaouli) and Rosalina or Lavender Tea Tree (*M. ericifolia*). This latter essential oil, Rosalina, is produced from both wild harvest and commercial plantations.

1.3.2 The research focus of this thesis

As previously mentioned, possibly the most important factor, with regard to the establishment of viable industries focused on select essential oils and natural products, is the establishment of commercial plantations. Following this, the next steps are to perform chemical character studies and pharmacological activities, to complement ethnobotanical records of traditional use by Australian Aboriginal people. Chemogeographic studies demonstrate the variation of naturally occurring

chemotypes, and in concert with respective pharmacological activities, they aid in the identification and promotion of significant cultivar chemotypes. Botanical and chemotaxonomic investigations are also significant with regard to identifying these cultivar chemotypes.

With regard to recent research focused on ethnopharmacological investigations of Australian plants, a significant number of novel chemical structures have been elucidated since the 1960s. A large number of these structures were sourced from *Eremophila* and *Myoporum* (Scrophylariaceae) species (Hegarty *et al.*, 1970; Blackburne *et al.*, 1972; Blackburne & Sutherland, 1972; Hamilton *et al.*, 1973; Blackburne *et al.*, 1974; Allen *et al.*, 1978; Dimitriadis & Massy-Westropp, 1979, 1980; Grant *et al.*, 1985; Ghisalberti, 1995), including multiple studies examining serrulatanes (Abell *et al.*, 1985; Forster *et al.*, 1986; Tippett & Massy-Westropp, 1993; Ndi *et al.*, 2007; Smith *et al.*, 2007). Various sesquiterpenes (Dastlik *et al.*, 1989) and other diterpenes (Ghisalberti *et al.*, 1990), have also been demonstrated in a selection of *Eremophila* species.

Ethnopharmacological studies of the *Eremophila* genus have also demonstrated some significant research findings. Ghisalberti (1994a) and Richmond (1993) provide comprehensive reviews of the ethnopharmacology of the *Eremophila* genus, and Ghisalberti (1994b) also reviewed Myoporaceae, which at the time included *Eremophila* spp. With regard to the serrulatanes in *Eremophila* species, some have been linked to high antimicrobial activities (Smith *et al.*, 2007). The antibacterial activities of various extracts, such as *E. duttonii* (Shah *et al.*, 2004), or other *Eremophila* species (Pennacchio *et al.*, 2005), or various other medicinal plants (Palombo & Semple, 2001), have received much attention.

Extracts from *Eremophila* species have therefore also been seriously considered in the development of antibacterial coatings to combat infections associated with surgical implants (Vasilev *et al.*, 2009). Cardioactive compounds were identified in *E. longifolia* (Pennacchio *et al.*, 1996; Pennacchio *et al.*, 1997). With regard to essential oils, *E. longifolia* was known for a long time by an essential oil hydrodistilled from a rare chemotype occurring in north-west Western Australia (Della & Jefferies, 1961), which yielded 5.5% w/w wet leaves, of an essential oil comprised almost entirely of the potentially hepatotoxic phenylpropanoids safrole and methyl eugenol. This served to put a dampener on medicinal research of essential oils from *E. longifolia*.

Several years later Smith *et al.*, (2010) identified three other essential oil chemotypes of *E. longifolia*, occurring in New South Wales, one with a particularly high yielding monoterpene ketone dominated essential oil that shows considerable promise that given the high oil yield and localised abundance shows considerable promise on a commercial level (isomenthone/menthone; CT.A). The other two chemotypes comprised CT.B, made up predominantly of karahanaenone, and CT.C,

made up predominantly of monoterpenes, such as α-pinene, limonene, α-terpinolene and significant amounts of borneol.

The isomenthone/menthone rich oil (CT.A) is hydrodistilled to produce a yield ranging from 3-8% w/w of fresh leaves. In conjunction with reports of another chemotype in the Northern Territory, identified by Barr (1988) with a monoterpenoid character predominantly made up of α-pinene and limonene, it is surprising that the initiatives to implement a commercial crop of *E. longifolia* for essential oil production, are to an extent still compromised by claims that the species in general yields the potentially harmful safrole/methyl eugenol essential oil. Of course as previously mentioned plants yielding this oil have a relatively restricted geographic range (Murchison area, Western Australia). Clearly, misconceptions regarding the oil of *E. longifolia* should be brought up to date.

In Part 1 of this thesis (Chapters 2-5) we are concerned with further defining essential oil chemotypes of *E. longifolia*, investigating factors related to essential oil character, including cell nuclei ploidy, and antimicrobial activities of extracts and essential oils. Additionally, some investigations, informed by ethnopharmacological considerations, were performed to further clarify the role of essential oils and other volatiles in traditional medicinal uses by Australian Aboriginal people.

Essential oils of *E. bignoniiflora* are characterised in Part 2 of this thesis (Chapter 6) and investigated for antimicrobial activities. Investigations, informed by ethnopharmacological investigations, of the previously mentioned *Callitris* spp., *endlicheri* and *glaucophylla* was also undertaken (Chapter 7). This investigation included an examination of the chemical character and antimicrobial activity of suitable extracts. Extracts included essential oils, solvent extracts and smoke extracts produced during laboratory simulation of smoke fumigation practices.

The chemical character of essential oils, from the chemogeographically varied *Geijera parviflora*, were examined and their respective antimicrobial activities were measured and reported in Chapters 9 and 10, with a focus on identifying candidate cultivar chemotypes for possible use in a natural product industry. Previously a number of essential oil chemotypes were demonstrated (Brophy *et al.*, 2005), along with coumarin chemotypes (Lahey & MacLeod, 1967; Shou *et al.*, 2013) that may or may not correlate with essential oil defined chemotypes. Although four essential oil chemotypes were demonstrated previously, being 1) the geijerene/pregeijerene, 2) the linalool, 3) the xanthoxylin and 4) the α-pinene/camphene types, until now no antimicrobial activities were ever examined to complement these findings.

In Chapter 8, essential oils were extracted from *Pittosporum angustifolium* (Pittosporaceae) and *P. undulatum* and chemically characterised. In addition, antimicrobial activities for the essential oils and other solvent extracts were

demonstrated for the first time. With regard to ethnomedicinal uses, *P. angustifolium* was involved in applications requiring the use of both solvent extracts (lipophilic and hydrophilic) and volatiles, to treat ailments such as eczema or psoriasis (Lassak & McCarthy, 2011) or coughs, colds and breast milk supply (Latz, 2004) respectively. Interestingly, we have been recently approached by Kamillaroi members of the Aboriginal community in relation to their intentions to produce a commercial plantation of *P. angustifolium* (known to them as Gumby Gumby), for the development of a range of medicinal products, targeting psoriasis and eczema. The suggested anticancer effects of this plant, also present as a topic worthy of further discussion and research.

Part 3 of this thesis is dedicated exclusively to a consideration of essential oils and their antimicrobial activities in the further chemotaxonomy of plants with no known ethnopharmacological significance. Chemotaxonomy of *Zieria* (Rutaceae) species has already been comprehensively investigated by various authors (Lassak & Southwell, 1974; Southwell, 1981; Flynn & Southwell, 1987b, 1987a; Southwell & Armstrong, 1987) and antimicrobial activity of essential oils has also been adequately demonstrated (Griffin *et al.*, 1998). Previous chemotaxonomic investigations have demonstrated a deal of success in using this method to complement traditional morphology in redefining taxonomic boundaries.

Accordingly, in Chapter 11 we examined antimicrobial activities of a broader range of extracts from a selection of *Zieria* species, using a broader range of bacteria and pathogenic fungi. In addition, we put the chemotaxonomic approach to the test by clarifying whether the essential oil character of *Z. floydii* complements the morphological observations made by A. G. Floyd, after he first discovered the species, when he described it as an apparent morphological cross of *Z. granulata* and *Z. furfuraceae*.

In Chapters 12 and 13 we address chemotaxonomic questions of considerable importance for the *Phebalium* (Rutaceae) species. Morphological observations had demonstrated *P. squamulosum* subsp. *verrucosum* was more similar to the *P. glandulosum* complex, so essential oil compositions were examined to see if phytochemical data supported this. With regard to the remainder of the *P. squamulosum* complex, a number of subspecies have been placed into this complex by Paul G. Wilson (1970), but this decision was not welcomed by other botanists who believed such subspecies should themselves be raised to species rank. The work presented in this thesis, also supports the proposition that *P. squamulsoum* subsp. *ozothamnoides* is made up of two morphologically different varieties.

The essential oil composition of *P. squamulosum* subspecies was investigated and then clustered using principal component analysis (PCA), to determine if the demonstrated phytochemical differences would support morphology, with regard to

species revision. Antimicrobial activities of the essential oils were also produced, as a starting point for defining bioactive roles, and this will be subject to future studies.

In chapter 14 a preliminary study was conducted which demonstrated the occurrence of various essential oil characters within established species that show considerable morphological variation. It is therefore proposed that essential oil variants within the species *Prostanthera lasianthos*, *P. ovalifolia* and *P. rotundifolia* (Lamiaceae) are reflective of actual chemotypes, which will be demonstrated in subsequent, more elaborate chemotaxonomic studies. Preliminary data describing the essential oil character of two other species, *P. caerulea* and *P. cineolifera*, were also included in this study. Additionally, antimicrobial activity was investigated and the results discussed with reference to potential and previous medicinal use.

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