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Review

A Contemporary Introduction to Essential Oils: Chemistry, Bioactivity and Prospects for Australian Agriculture

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Abstract: This review is a comprehensive introduction to pertinent aspects of the extraction methodology, chemistry, analysis and pharmacology of essential oils, whilst providing a background of general organic chemistry concepts to readers from non-chemistry oriented backgrounds. Furthermore, it describes the historical aspects of essential oil research whilst exploring contentious issues of terminology. This follows with an examination of essential oil producing plants in the Australian context with particular attention to Aboriginal custom use, historical successes and contemporary commercial prospects. Due to the harsh dry environment of the Australian landmass, particularly to the cyclical climatic variation attendant upon repeated glaciation/post-glaciation cycles, the arid regions have evolved a rich assortment of unique endemic essential oil yielding plants. Though some of these aromatic plants (particularly myrtaceous species) have given birth to commercially valuable industries, much remains to be discovered. Given the market potential, it is likely that recent discoveries in our laboratory and elsewhere will lead to new product development. This review concludes with an emphasis on the use of chemotaxonomy in selection of commercially viable cultivar chemotypes from the Australian continent. Finally, drawing largely from our own results we propose a list of Australian endemic species with novel commercial potential.

Keywords: essential oil; organic chemistry; pharmacology; Australian; cultivation; chemotype; cultivar; history; Aboriginal

1. Introduction

1.1. Terminology of Essential Oils and Methodologies of Production

Essential oils are a mixture of volatile lipophilic (fat loving, *i.e.*, soluble in fat) constituents, most commonly sourced from leaf, twig, wood pulp or bark tissue of higher plants, but also widely found in bryophytes, such as the liverworts [1]. Although essential oils are only slightly soluble in water, the aqueous solubility of individual essential oil components varies with respect to polarity (magnetic activity). Generally, components with more polar functional 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. A variation of this is steam distillation, which places the source tissue (leaves, stem or bark) in the path of steam and not in the boiling water itself, as in hydrodistillation.

During hydrodistillation, mixed gases (steam and oil vapour) are produced and expand into a condenser where they are cooled to below 30 °C and condensed into two separated (non-mixing) 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 they are separated. Problems occur when hydrodistillation is performed at higher temperatures, because the subsequent temperature of the hydrosol is not sufficiently lowered before entering the separation funnel. The consequence is fractionation of the essential oil, with a greater representation of components with higher boiling points. In addition, there may also be a failure to condense any essential oil at all; or if condensed oils are observed they may be subject to re-evaporation if the hydrosol temperature is too high. Thus, it is generally a priority to regulate the boiling temperature in order to optimise the hydrodistillation to maximise essential oil yield.

Most authorities contend that if a process other than hydro- or steam distillation is used, such as solvent extraction or mechanical pressing, to collect liquids containing volatile compounds, the product should not be regarded as an essential oil and may instead be called an “absolute” or an “expressed oil”, respectively. In the preparation of an “absolute” a hexane extract is first taken of the raw material and evaporated to produce a “concrete”. The concrete is dissolved into ethanol and cooled to −20 °C. At this lower temperature waxes, sterols and other lipids solidify and are removed, which concentrates the volatile compounds in the product; the absolute.

A host of other names can be used to describe aromatic preparations, such as “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 [2,3].

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 to introduce further confusion in terminology. Essential oils can now be extracted using modern microwave-assisted hydrodistillation [4,5] or microwave-assisted distillation techniques that require no additional water, other than cytosolic and vascular fluids already present in the source tissue [6]. These methods result in differences, both qualitative and quantitative, in the composition and yield of the subsequent essential oil [4,6]. Strictly speaking the latter technique, requiring no additional water, is not hydrodistilled but merely distilled.

In this regard, the International Organisation for Standardization (ISO) 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" [2,3].

With regard to the classification of expressed oil from citrus peel, such as orange or bergamot oil, these are commonly referred to as essential oils [7,8]. 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) and other larger compounds that are not volatile, such as the coumarin bergaptene (**31**) (see all chemical structure diagrams in Figure A1) in the case of bergamot oil (*Citrus bergamia* Risso.) [8,9].

Further implications of definition of essential oils appear when considering the traditional 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 [10] or in the lungs through inhalation [11]. In this context, medicinal effects may sometimes be produced via a molecular interaction between multiple compounds that could be of both lipophilic and hydrophilic character. Such potentially synergistic interactions will not occur using only the pure essential oil as produced in hydrodistillation [10]. However, more often than not there is a single active compound involved that produces the greater part of the medicinal effect [12], which can be of either lipophilic or hydrophilic in character.

In this regard, slightly larger intact or modified compounds are evaporated, in significant quantities, when higher temperatures are involved, such as in the Australian Aboriginal smoke fumigation practices [12], or indeed in microwave assisted distillation [4,6]. These slightly larger compounds can be found in simulated 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 establish 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 (plus non-volatiles) 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 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* [13], as is commonly accepted [7,14].

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 (**17**), were described by the ancient Romans and Greeks in the first century [13]. 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 400 BC (Terracotta distillation apparatus dated to approx. 400 BC, now stored in the Taxila Museum, Pakistan) until the ninth century, this method was primarily used to produce distilled waters where fractionated essential oils, such as camphor (**17**), were often produced as a by-product [13]. Using such primitive hydrodistillation, distilled waters or "hydrosols" could be achieved without any difficulty, but intact essential oils could not be captured without modern methods enabling the cooling of steam to the required lower temperatures. Thus, only essential oil components with higher boiling points, such as camphor (**17**), could be retained.

Distillation technology was improved in the ninth century by earlier Arabic scientists [15,16], but again it is not clear if they used this technology to deliberately produce essential oils or if the primary 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 [13].

Essential oil components are usually no larger than 300 Daltons (amu) in size [17], except in unusual cases involving larger diterpenoids such as incensole acetate from *Boswellia* spp. [18–20] and these require longer periods of hydrodistillation (perhaps higher temperatures) with cohobation before they are measured in the whole essential oil. 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 [12]. In such cases these constituents have greater polarity than other essential oil components, making them more soluble in water. Minimisation of distillation waters and recycling of the hydrosol can significantly enhance the yield of oil obtained from a hydrodistillation. Cohobation is one method used to reduce the loss of essential oil via solubility into the hydrosol, where the hydrosol is manually returned to the still throughout the duration of the hydrodistillation.

Another method used to reduce the loss of essential oil to dissolution in the hydrosol, employs the Clevenger-type apparatus (Figure 1), which returns the hydrosol to the still in real-time, during the course of the distillation. This also reduces the overall volume of water, initially required for the distillation. Unfortunately one of the challenges in using the Clevenger-type apparatus is in maintaining the hydrosol at a lower temperature, as higher temperatures can lead to a reduction in oil yield or an emulsion of the oil in the hydrosol. A disadvantage of the Clevenger apparatus is that essential oils must float (be less dense than water), otherwise they will be lost back into the still with the hydrosol.

As depicted in Figure 1, the positioning of the condenser (D) is directly above the Clevenger-type apparatus (C). Here we have tilted the condenser so that the condensates run along the sides of the glass and meet the liquid at a reduced velocity. In the traditional Clevenger-type spatial configuration, the condenser is positioned vertical to the liquid surface, but in our experience the fall of liquid from the condenser disrupts the phase separation of the essential oil and the hydrosol. In addition, the positioning of the condenser so that condensed liquids return via the passage of steam, is why there is difficulty in reducing the temperature of the hydrosol. To combat this we have adjusted the heating mantle (B) to a lower temperature, using a power regulator (A). However the best measure would involve reinventing the Clevenger-apparatus to include a water jacket around the essential oil and hydrosol phases to maintain a lower temperature and therefore prevent re-evaporation.

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 [21]. For example, some phenylpropanoids, such as safrole (**14**) and methyl eugenol (**15**), 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 [22], which is a mixture of safrole (**14**) and methyl eugenol (**15**), comprising approximately 97% of the entire essential oil. In this particular case, a hydrodistillation of this species using the Clevenger-type apparatus would fail to capture the essential oil because it would return to the still with the hydrosol.

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 [17]. 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 a less obvious ecological role, such as in fire tolerance, attracting pollinators and/or herbivores for seed dispersal, drought tolerance or plant-to-plant biosemiosis (pheromones).

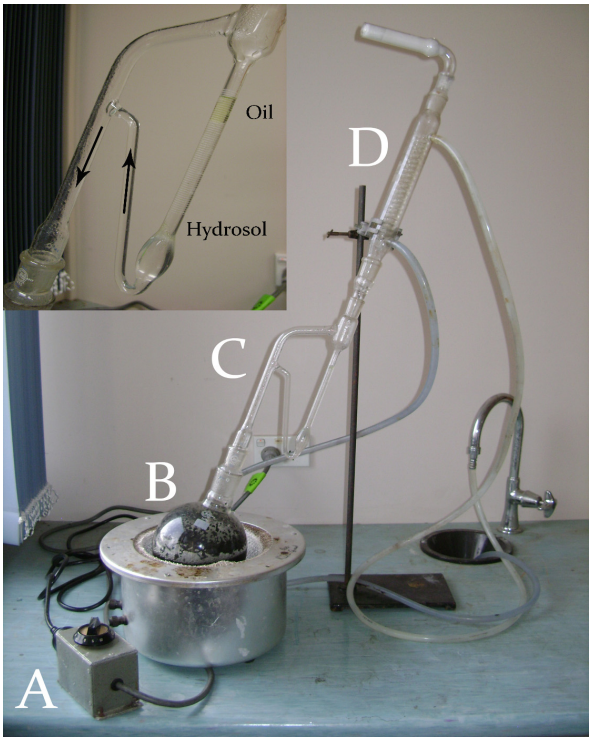


Figure 1. Hydrodistillation using the Clevenger-type apparatus. (**A**) Power regulator; (**B**) Heating mantle with round bottom flask containing water and aromatic leaves; (**C**) Clevenger-type apparatus which returns the hydrosol to the still and maintains the essential oil phase, but only for essential oils that are less dense than water and therefore float; (**D**) The condenser.

Although essential oils may contribute significantly toward the evolutionary survival of the respective organism, the term "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. However, the term "essential oil" actually 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 [13]. 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. 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 [17].

A variety of other names are given to the essential oil. These include essence, fragrant oil, volatile oil, etheric oil, aetheroleum or aromatic oil [21]. 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.

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 [21]; 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 [23]. 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 describe essential oil yielding varieties, most essential oil compounds are aliphatic in the strict chemical sense.

1.2. Chemistry, Chirality and Stereochemistry of Essential Oils

Essential oil conferences attract attendees from a multitude of professions with a diversity of expertise. Some of the participants will be expert chemists whilst others will have no previous exposure to chemistry at all. Generally it is expected that our readers will have at least a basic level of chemistry, but for readers with no previous exposure, we have included an image and explanation in the Appendix to help in the understanding of molecular diagrams, in Figure A2; lessons A–E. Furthermore, information related to understanding chirality and stereochemistry has also been included in the Appendix.

In the chemistry of essential oils, chirality and stereochemistry of components are of considerable importance. This is because the spatial orientation of connective parts of a molecule can significantly influence the chemical behaviour and pharmacological activity of the compound. In this regard, molecules with the same molecular formula and the same bonds between atoms, but different spatial arrangements of these atoms, are called stereoisomers. The two main types of stereoisomers that are relevant to the discussion of essential oils are diastereomers and enantiomers.

Generally a pair of stereoisomers are called diastereomers, which are distinguished as separate entities in routine chemical analysis, such as in gas chromatography (GC) or nuclear magnetic resonance spectroscopy (NMR). However, some stereoisomers are exact mirror images of each other that cannot be superimposed, like a left and right hand (Figure 2). Each of these are called enantiomers of a chiral molecule. An example of a chiral compound is carvone (**1**) (Figure 2). Because carvone (**1**) is a chiral molecule, differences between enantiomers cannot be observed using routine GC or NMR [24].

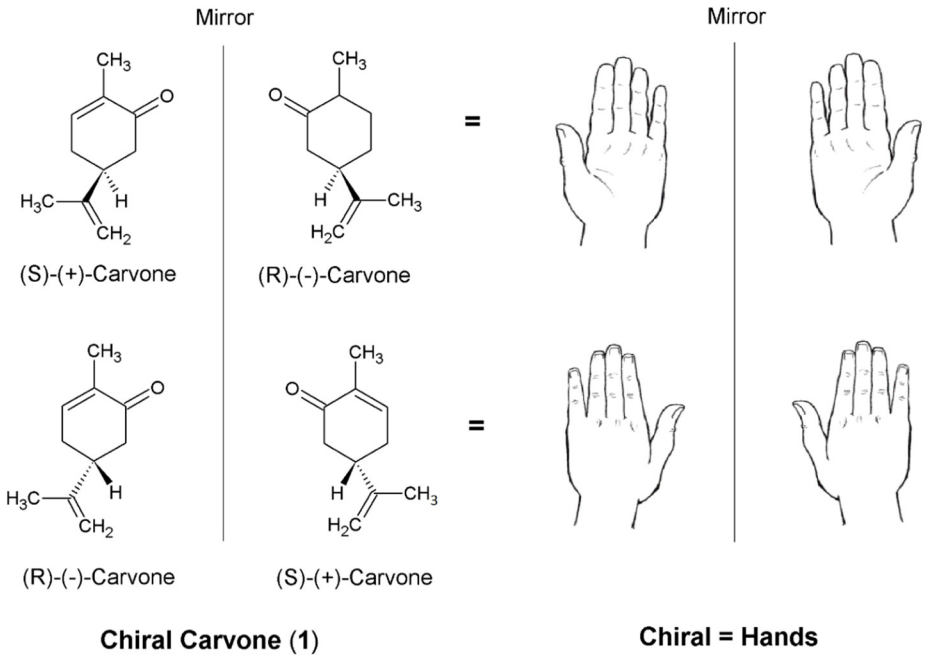


Figure 2. The two enantiomers of a carvone (**1**).

The term "chiral" first joined the language of chemistry after it was coined by Lord Kelvin in 1893 [25]. It derives from the Greek word for hand, the most familiar chiral object in nature. Before the technology was available to elucidate the absolute stereochemistry of chiral compounds they were identified on the basis of being able to rotate plane polarised light. Compounds that are able to rotate plane polarised light are known as "optically active" and are assigned a "specific rotation" measurement that is generally unique for each chiral compound. However, each enantiomer in a chiral compound will rotate equally in opposite directions, one to the right and the other to the left. Thus, one will have a negative specific rotation, which is to the left, and the other will have an equal positive value, which is to the right. Using terminology deriving from Latin, rotation to the right is *dextrorotatory* (*dextro*- derived from *dexter* for "right") and rotation to the left is *laevorotatory* (*laevo*- derived from *laevus* for left). Thus, in the older language of chemistry the prefix to specify enantiomers was either D- or L-. Examples of where this language has survived include D-alpha-pinene (**2**) and D-limonene (**3**) (Figure A2), which are sometimes shown as D-(+)-alpha-pinene and D-(+)-limonene, respectively. However, the use of D- and L- has more recently been dropped and replaced by the symbols for positive (+) and negative (−) [21,24].

An example of an achiral (not chiral) molecule is ρ-cymene (**4**). This molecule is achiral because it is superimposable on its mirror image but it does not have a chiral centre (bear in mind that an aromatic ring has delocalised electrons, so the placing of double bonds is arbitrary). A clearer picture of what a chiral centre looks like is elucidated in Figure 3.

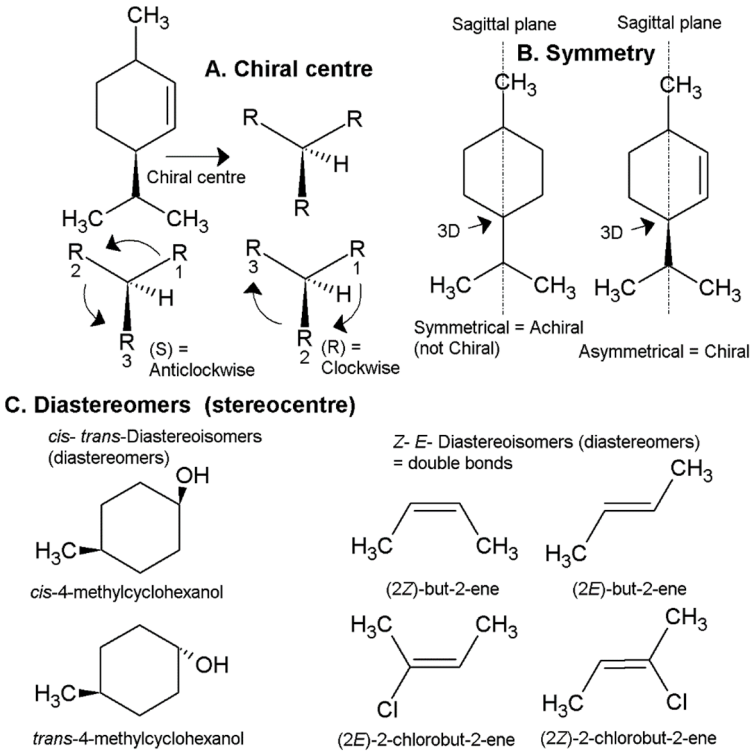


Figure 3. The stereochemistry of a molecule where the rotation of priority bonds around a chiral centre are defined as either *S* or *R* (**A**), where symmetry can influence whether something is chiral or achiral (**B**) and where two chiral centres (or stereocenter for double bonds) can result in either *cis*- or *trans*- isomerism (**C**), which can be more accurately denoted with *Z*- and *E*- if isomerism is over a double bond.

The specific rotation of a chiral compound is measured using a polarimeter. Although this technology is quite old, its use in chemistry has continued to this day. However, because chemists are now aware of the absolute stereochemistry of each enantiomer (the exact 3D configuration of bonds), the convention for describing an enantiomer is complemented with either *S* or *R* descriptors (Figure 3A). This helps chemists to communicate the 3D spatial arrangement of atoms or groups around the bond without resorting to drawing a diagram. Such descriptors are generally only used in the common name of an essential oil component where only one chiral centre is present, otherwise each chiral centre should be assigned an *S* or *R* configuration, *i.e.*, (1*R*,5*R*)-(+)-alpha-Pinene (**2**). However, where the configurations are identical, it is conventional to use the descriptor just once, *i.e.*, (*R*)-(+)-alpha-Pinene (**2**) [24].

A common misconception is to believe that *S* or *R* indicate the direction of rotation of plane polarised light, but this is wrong. A chiral centre that is in the *S* configuration can be either the positive (+) or the negative (−) enantiomer, but will always be opposite to the *R* configuration. Thus, when *S* = (+) then *R* = (−) and *vice versa* [24].

A chiral centre is defined as either *S* or *R* by the rotation of priority groups around the main carbon in the bond (Figure 3A); it is purely a constitutional concept. Priority groups are defined by the Cahn-Ingold-Prelog priority rules (CIP), which prioritises groups based on higher atomic number (higher numbers = higher priority). The CIP rules are explained in detail in any modern organic chemistry textbook [24].

In a chiral centre, the rotation of priority bonds in an anticlockwise direction (to the left from 12 O'clock) is called *S*, which derives from the Latin word *sinister* (Figure 3A) meaning wrong or to the left. In a clockwise direction it is called *R* for *rectus*, which means straight or correct, but in this context it could mean to the right or correct. Because influences from the left side were regarded as evil in ancient societies, the latin word *sinister* for left, survives today in the English language to mean evil or threatening, no longer associated with the left. However, it is clear that the modern chemical use of these words is reflective of the ancient concept that left and right had moral connotations [24].

In essential oil chemistry it is also common for differences at a chiral centre to result in an entirely different molecule, not just a different enantiomer. Such compounds are asymmetrical but not chiral (no mirror image), so they are diastereomers as mentioned previously. Diastereomers can result from a variation in one of two or more chiral centres, or alternatively from the stereochemistry of substituent groups about a double bond. For example, a compound with two chiral centres can lead to epimers where the two compounds differ at just one chiral centre (globulol (**52**) and ledol (**53**) are epimers). This has the capacity to substantially alter the chemical and pharmacological properties of the compound (Figure 3C). Such changes will be more pronounced between diastereomers than between enantiomers. However, if groups at both chiral centres are changed, the resulting compound is its enantiomer [24].

A compound with a double bond can occur as either a *cis*- and *trans*- isomer, which makes it one of two diastereomers. This convention can also be used on single bonds but only in an alicyclic molecule (non-aromatic ring structure) where rotation about the single bond cannot occur. These isomers are defined by substituent groups, which are the non-hydrogen attachment at the chiral centre or stereocenter (sterecenter is a more general expression that includes stereochemistry on a double bond as well as chiral centres). Where the substituent group at one stereocenter is on the same side of the molecule as the group on the other stereocenter, it is called *cis*- and on opposite sides called *trans*- (Figure 3C). Specifically where the stereocenters are on opposite sides of a double bond, *Z*- and *E*- notation can be used to replace *cis*- and *trans*-, respectively (*Z*- is from the German word *zusammen*, meaning together; *E*- is from the German word *entgegen*, meaning opposite) [24].

However, *E*- and *Z*- notation uses the CIP priority rules mentioned earlier. *Cis*- and *trans*- isomerism does not, but alternatively prioritises for non-hydrogen groups that are in a different position compared to its known diastereomer. Furthermore, *cis*- and *trans*- substituent groups are not always obvious, making this convention rather arbitrary. Such ambiguity is more common in complex molecules or where more than two different types of elements are present at the stereocenter. That is why *cis*- and *trans*- isomerism is regarded as relative stereochemistry (relative to its known diastereomer), whereas *Z*- and *E*- notation is regarded as absolute. Thus, in a similar way to the *R* and *S* descriptors, *E*- and *Z*- notation in alkenes (molecules with double bonds) does not always translate to the *cis*- and *trans*- isomerism of the same alkene. However, this inconsistency should only occur if more than two different types of elements are present at the stereocenter, such as in the case of 2-chlorobut-2-ene depicted in Figure 3C. Therefore such ambiguity is avoided by using only *E*- and *Z*- notation on alkenes, where the convention of using *cis*- and *trans*- has not already been established [24].

As previously mentioned, generally diastereomers can be detected using basic gas chromatography or NMR to the level of its relative (*cis*- or *trans*-) or absolute (*Z*- or *E*-) stereochemistry if it has previously been reported, but the absolute stereochemistry of known chiral molecules (*S* or *R* configuration of chiral centres) cannot be realised without further more comprehensive investigation.

Therefore, in using such non-specialist instrumentation, it is not possible to differentiate between enantiomers in an essential oil [24].

The importance of chirality arises in essential oil chemistry when samples are corrupted by profit oriented manufacturers who adulterate natural products with cheaper synthetics. Consequently measures need to be routinely undertaken to prove or disprove claims of authenticity made by sellers and manufacturers in the marketplace. It is also common practice for producers of essential oils to optimise profit by adulterating natural essential oils with more common natural but cheaper essential oils. Among other things, the consequence of any kind of adulteration is that the composition of enantiomers is not reflective of the naturally occurring essential oil. Cheap manufacturers are dependent on the fact that no specialist methods will be employed to investigate the composition of their essential oil products, but times are changing [21,26].

Essential oils that are biosynthesised by plants are composed of a variety of components that may include chiral compounds. Generally if these chiral compounds are synthesised in a laboratory a 50:50 mixture of the enantiomers will be produced, but only if the chiral centre is a part of the reaction or if the precursor is racemic, meaning an equal proportion (50:50) of enantiomers. Such a mixture is very difficult to separate using common inexpensive chromatography, in contrast to the separation of diastereomers, which are also produced in synthetic reactions and are more easily separated using basic chromatography. Because it is expensive and difficult to separate enantiomers, the occurrence of racemic mixtures in natural products is therefore a reliable indication of cost cutting and adulteration [21].

The specific rotation of a racemate is zero because the rotation from each of the enantiomers sums to zero. Although such a mixture is called a racemate, this should not to be mistaken for a mixture with unequal proportions of enantiomers, which can occur in natural products, often at a ratio of 40:60 [27]. Such a mixture is referred to as enantioenriched or can be defined as having an enantiomeric excess (ee) which is a figure that shows the amount of unpaired enantiomer as a mass percentage of the whole (g/g), or how much one enantiomer outweighs the other. Thus, enantiopure samples (just one enantiomer) have an ee of 100%, whereas a racemate has an ee of 0%. Furthermore, a mixture with 75% of the *R* or *S* enantiomer will have an ee of 50% (75 − (100 − 75) = 50). Another way to look at this is to imagine that enantiomeric excess describes the part of the mixture that is not racemic (not a one to one mixture of enantiomers) [21,24].

As high stereospecificity is achieved in enzyme-catalysed reactions (biological reactions), such chiral compounds from nature are generally enantiopure, but not always. Regardless, studies focused on elucidating the enantiomeric excess of essential oils provide guidelines as to the expected enantiotypes from the selected species under investigation. This rising field of enantiotaxonomy has already identified single or multiple enantiotypes within the one species, which may serve as fingerprints where authentication is an issue [21].

The consequence of adulterated essential oils is that they won't display the same pharmacological or aesthetic qualities of the natural essential oils. This was made implicit in earlier studies where double blind olfactometry studies demonstrated that enantiomers were differentiated by the character of the odour as perceived by a specialist odour panel. In one such study, the two enantiomers of carvone (**1**) were synthesised from enantiopure samples of Limonene (**3**), where the chiral centres were not altered in the reaction, so high enantiomeric purity of the carvones was achieved. Using a panel of 21 reliable odor specialists, the odor of (+)-carvone was characterised as caraway-like and (−)-carvone as

spearmint-like [28]. This is in agreement with the predominating enantiomers found in caraway (*Carum carvi* L.: Apiaceae) and spearmint (*Mentha spicata* L.: Lamiaceae), respectively [21].

1.3. Chemical Analysis and Standardisation/Legislation of Essential Oils

The most common technique employed in the chemical characterisation of essential oils is gas chromatography coupled with mass spectrometry (GC-MS). The relatively small size of essential oil components means that they are all volatile and can therefore be separated according to boiling points. This process takes place in a long thin column (*i.e.*, 30 m) that has the appearance of coiled wire. This column is prepacked with a porous stationary phase that is either polar (slightly charged), such as a wax column (polyethylene glycol; *i.e.*, DB-wax, Carbowax 20M, PEG-20M) or apolar, such as polymethylsiloxane (*i.e.*, HP-1MS or 5% diphenyl- *i.e.*, HP-5MS). The apolar HP-5MS column is the most commonly used [29].

The essential oil is diluted into a solvent and injected into a heated injection chamber (*i.e.*, 300 °C) so all components of the essential oil are evaporated and delivered by a benign (non-reactive) gas (*i.e.*, nitrogen or helium) to the start of the column, which is usually at a lower temperature (*i.e.*, 60 °C), so essential oil components precipitate onto the column [29].

In gas chromatography, separation is performed by heating the column in an oven, most commonly employing a programmed temperature ramp, from a lower to a higher temperature; however, occasionally isothermal (constant temperature) programs are used. When a temperature program is used, the separation of components occurs when the temperature is raised to each of the component's individual boiling points. At this point the component vaporises and is carried by the non-reactive gas to the detector [29]. The most common detector used in gas chromatography is a mass spectrometer, but flame ionisation detection (FID) is often used where the accuracy of quantification is a concern.

In using FID the identity of the compound cannot be known. However, if the identity of the compound and its retention time (how long it takes to come out of the column) is already known from a previous experiment, then GC-FID can be used to calculate an accurate relative abundance of each component in the essential oil. Generally prior to GC-FID, GC-MS is used to identify components in the essential oil. Although GC-FID gives more accurate quantification data, it is more common to use the less accurate quantification method, which is calculated from the GC-MS chromatogram (Figure 4).

In a GC-MS chromatogram the retention time of components generally reflects their sizes and the presence of functional groups (Figure 4). In Figure 4 the elution of components starts with monoterpenes (10 carbon compounds), which is then followed by oxygenated monoterpenes and finishes with sesquiterpenes (15 carbon compounds). Although Figure 4 shows the mass spectrum of a few selected components, such information is always depicted in a separate window.

In mass spectrometry the separated component (say, Limonene, **3**) is fragmented by electron impact ionisation, which produces a spectrum of ions that are separated according to mass, with heavier components exerting a greater inertial resistance to a magnetic field than lighter components. In the process of magnetic deflection of their paths, ions are diverted onto a detector. The result is a spectrum showing ions of different sizes with different relative abundances. This spectrum can be generally considered a fingerprint of each component in the essential oil. Because of the reproducibility of this

experiment, each mass spectrum can be compared across a spectral library. Using other pieces of information, such as retention time, a fairly reliable match can be made, with minor exceptions [29].

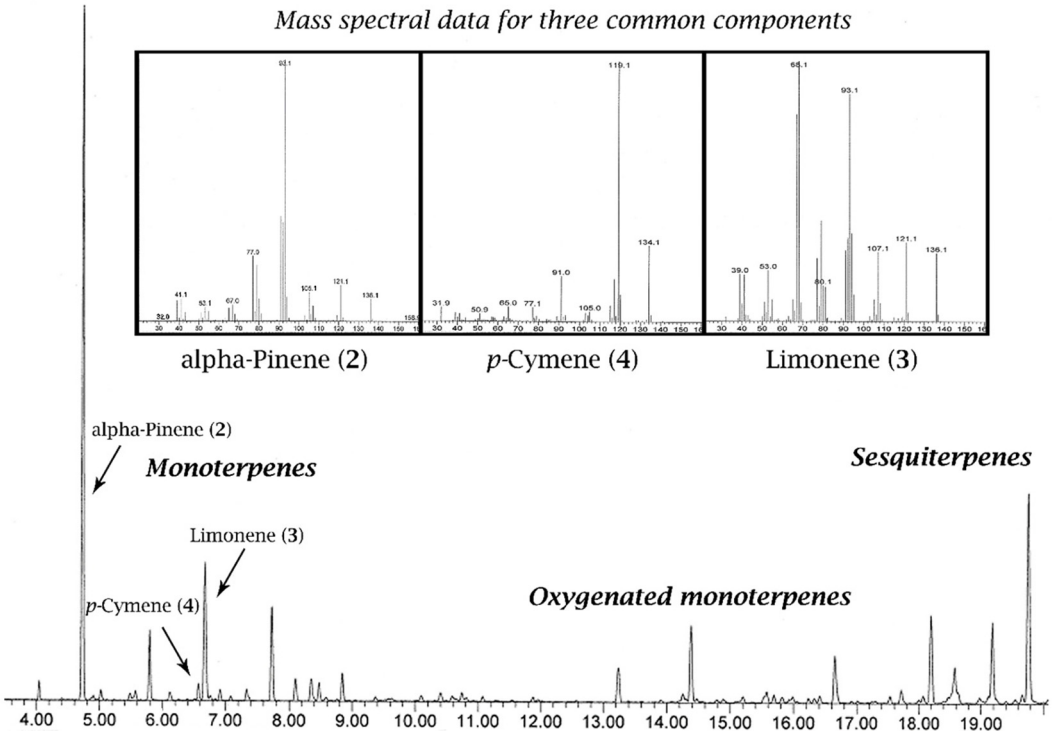


Figure 4. A gas chromatogram with mass spectral data superimposed for three common essential oil components. On the chromatogram *x-axis* is retention time (RT) in minutes and on the *y-axis* is abundance in arbitrary units.

Another method worthy of mention here is solid phase microextraction (SPME) or to put it more simply, head-space analysis or absorption [30]. This method delivers volatiles to the GC-MS but by-passes the solvent extraction or distillation process. This technique employs an adsorptive solid phase that is positioned in the headspace of the aromatic preparation. Following this either the solid phase can be injected directly into the GC-MS or the volatiles can be desorbed into a solvent before injection. A number of adsorbents are used in SPME and these are listed by Reineccius [30].

After GC-MS analysis is performed, where discrepancies in identification are involved, essential oil components can be purified using flash chromatography before analysis using nuclear magnetic resonance spectroscope (NMR). Flash chromatography is achieved by packing silica gel (polar phase) into a vertical column with a solvent mixture. The essential oil is added to the top of the column. Because each component has a different binding affinity to the silica gel, the components can be subsequently washed out individually by the same solvent under pressure. The solvent mixture is customised relative to the polarity of the target compound to optimise separation [29].

The NMR of the purified component will provide a unique spectrum of ^{13}C or ^{1}H shifts that can be matched to a published value if it is a known compound. If not, more comprehensive structural elucidation can be performed using 2D-NMR experiments. In addition, by using 2D-NMR experiments new structures can be discovered. The theory behind NMR is explained in detail in any modern organic chemistry textbook [24].

The spectral data generated by NMR, as well as mass spectral data from GC-MS, will not help in differentiating between enantiomers. In addition, enantiomers cannot be separated by either a polar or apolar stationary phase in chromatography, unless a different binding affinity is employed. To achieve this, an enantiopure additive is included in the stationary phase, with the most common being enantiopure aryl or alkyl derivatives of a cyclodextrin (α-, β- or γ-cyclodextrins). Such a stationary phase is called a "chiral column", which can be fitted to a GC-MS for chromatographic separation of enantiomers. This methodology, called enantioselective chiral gas chromatography (enantio-cGC) [31] is the preferred choice in authenticating essential oils because it requires no prior separations. The whole essential oil can be injected and the chiral compound will separate into two peaks. Without access to a chiral column a more primitive method involving flash chromatography and a polarimeter can be used, but this proves to be very time consuming and resource wasteful.

Knowledge of the chemical composition of an essential oil, as well as the enantiomeric excess of chiral components, comprises the most significant part of the standardisation process. If both of these chemical aspects conform to the published standard, other physical parameters of the essential oil should fall into line. Thus, with the correct number of components at relative abundances within the defined range and the correct enantiomeric composition, the optical rotation and refractive index of the whole essential oil, its colour, density and appearance should conform to the defined standard [3].

However, these other parameters may be elevated to become more significant authenticating tools when essential oils are adulterated with carrier oils or diluted into alcohol. Additionally, this can also be of relevance if the essential oil is produced using a method other than hydro- or steam distillation. This is pertinent in the production of absolutes or expressed oils, such as the previous mentioned example of Bergamot (*Citrus bergamia* Risso and Poit). In this case components other than those identified in GC-MS will be present; components that are not volatile or gaseous. Thus, all of the authentication parameters are necessary for standardisation purposes, not just the chemical character as deduced from gas chromatography.

Another factor worthy of consideration when preparing or examining published standards of essential oils is the occurrence of chemotypes. It is extremely common for a single essential oil yielding species to produce varieties of essential oils, called chemotypes. Chemotypes often occur where a geographical or geological difference influences diversification of biosynthetic pathways. Chemotypes may result from diverging evolutionary pathways, or from environmental cues, such as soil type or altitude. Where chemotypes occur in a species, published standards are generally specific, *i.e.*, the standard of Tea Tree Oil (*Melaleuca alternifolia* Cheel) as specified by the International Standards Organisation (ISO: Geneva Switzerland) clarifies the chemotype being described: "Oil of Melaleuca, terpinene-4-ol type (Tea Tree Oil)' (ISO 4730:2004) [3]. The standard described by ISO is identical to that described by Standards Australia (AS 2782-2009) [32] (Figure 5).

Requirements:		
Appearance	Clear, mobile liquid	
Colour	Colourless to pale yellow	
Odour	Characteristic	
Relative density (20° C)	Min: 0.885	Max: 0.906
Refractive index (20° C)	Min: 1.475	Max: 1.482
Optical rotation (20° C)	between + 5° and + 15°	
Miscibility in ethanol (20° C)	not necessary to use more than 2 volumes of ethanol, 85% (volume fraction) to obtain a clear solution with 1 volume of essential oil	
Flashpoint (closed cup)	mean value	mean value
Min volume of test sample	50 ml	

Chromatographic profile:		
Component	**Min %**	**Max %**
α-Pinene	1	6
Sabinene	trace	3.5
α-Terpinene	5	13
Limonene	0.5	1.5
p-Cymene	0.5	8
1,8-Cineole	trace	15
γ-Terpinene	10	28
Terpinolene	1.5	5
Terpinen-4-ol	30	48
α-Terpineol	1.5	8
Aromadendrene	trace	3
Ledene (syn. viridiflorene)	trace	3
δ-Cadinene	trace	3
Globulol	trace	1
Viridiflorol	trace	1

Figure 5. The Australian standard of chemical components in Tea Tree Oil (AS 2782: 2009), the terpinen-4-ol chemotype, Standards Australia [32].

The ISO reference defines Tea Tree Oil as:

> "Essential oil obtained by steam distillation of the foliage and terminal branchlets of *Melaleuca alternifolia* (Maiden et Betche) Cheel, *Melaleuca linariifolia* Smith, and *Melaleuca dissitiflora* F. Mueller, as well as other species of *Melaleuca* provided that the oil obtained conforms to the requirements given in this International Standard".

With regard to the occurrence of chemotypes also requiring published standards, as well as information related to enantiotaxonomy, the standardisation bodies world-wide have much catching up to do. However, those standardisation bodies regarded as most reliable in terms of the description of chemical components of essential oils, are the previous mentioned ISO and the "Association Française de Normalisation" (AFNOR: France) [33]. Other standardization bodies include the British [34], International, European and United States Pharmacopoeia. Although Australia does not have a pharmacopoeia *per se*, Australian standards are kept by Standards Australia [32].

Briefly, all standards are kept under a reference number, which includes a file number and the year it was last revised. Published standards must also conform to standards of measurement and presentation, which are also defined by ISO, however Australia's equivalent references are listed alongside the ISO references in Table 1. To view any of these standards they must first be purchased from the organisation. Although standards are not enforced by law, manufacturers of essential oils can provide guarantee of reproducibility and reliability if their product conforms to a widely accepted standard.

Table 1. References equivalent between the International Standards Organisation (ISO) and Australian Standards (AS) in relation to essential oils and preparation of standards for publication.

Reference to International Standard (ISO)		Australian Standard (AS)	
212	Essential oils—Sampling	4550	Essential oils—Sampling
11024	Essential oils—General guidance on chromatographic profiles	5025	Essential oils—General guidance on chromatographic profiles
11024-1	Part 1: Preparation of chromatographic profiles for presentation in standards	5025.1	Part 1: Preparation of chromatographic profiles for presentation in standards
11024-2	Part 2: Utilization of chromatographic profiles of samples of essential oils	5025.2	Part 2: Utilization of chromatographic profiles of samples of essential oils

Recent challenges in the authentication of Tea Tree Oil were met when it was revealed that enantiomeric excess of most of the chiral components of Tea Tree Oil were similar to Eucalyptus oil. Thus, manufacturers of Tea Tree Oil could fraudulently add components from Eucalyptus oil to bring levels into accordance with the ISO standard and enantio-cGC had limited capability to detect this. In this case enantio-cGC could be complemented with isotope ratio mass spectrometry. Because of varying kinetic and thermodynamic factors across species, during primary carbon dioxide fixation in photosynthesis, isotopic ratios of carbon vary between Melaleuca and Eucalyptus species [31]. Therefore, where isotopic ratios of carbon fail to meet known ratios for Melaleuca, the essential oil could be regarded as adulterated.

It is not only standardisation that governs essential oil manufacture and production. Standardisation merely ensures reproducibility and conformity, but where such standards are used in the formulation of products, legislation provides the framework. The primary objective of legislation is to govern the amount of biologically active material added to a product, at what concentration and the amount of information provided to the consumer (as ingredients), so that health and safety controls are in place. Although legislation may be regarded with distaste by many, with conflicting views and indecision across the board, such legislation does impact upon essential oil usage and therefore should be given due attention.

Generally the safety of an essential oil is difficult to predict from merely examining its chemical composition. This is because naturally occurring combinations rarely demonstrate the same biological activity as the individual separated components. However, the first indicator of an essential oil's biological activity utilises a constituent based approach [35]. Using the constituent based approach, the most common cited examples of potentially dangerous compounds are related to hepatotoxicity, phototoxicity and skin sensitisation. By far the vast majority of these contain aromatic moieties, such as a phenyl, phenol or methoxy phenol group. Phototoxicity is quite common in essential oils derived from *Citrus* species, which is attributed to the UV sensitising components belonging to the coumarin or furanocoumarin groups (*i.e.*, bergaptene (**31**)) [36]. Within the context of hepatotoxicity, phenylpropanoid derived compounds such as the methoxy phenols safrole (**14**) and methyl eugenol (**15**) are considered dangerous and carcinogenic [37]. Indeed, within the Australian context the occurrence of a rare chemotype of *Eremophila longifolia* F. Muell which yields an essential oil made up entirely of these two

components, cast doubt over the safe use of this species for a long time, before other safe chemotypes were described [38].

Phenols such as carvacrol (**13**) and thymol are considered dangerous if consumed in higher quantities or at lower dosages but over long time periods, leading to cases of hepatotoxicity. Oils high in ketones, such as the abortifacient pulegone (**40**), or aldehydes should also be used cautiously, but of course there are many exceptions to the rule, such as the essential oil from *Mentha piperita* L, which contains high quantities of menthone (**39**) [37].

The main message from texts describing essential oil safety is that although most of them may be considered safe if used in an informed way, by merely being "natural" it does not mean that they can be utilised without consideration of dose, "… no substance is safe independent of consideration of dose" [35]. The first comprehensive text to examine essential oil safety was compiled in 1995 by Tisserand and Balac [36] and in it are described several cases of hospitalisation of adults or children who have consumed "safe" essential oils at unsafe dosages. This book also includes a multitude of anecdotes, such as the case of an 11 year old child carrying a bottle of *Cinnamomum zeylanicum* Blume essential oil in his pocket. When the bottle broke, the boy wore the same pants for another 48 h and sustained a severe burn around the pocket area. In another case, camphor (**17**) applied to a child's nostrils resulted in instant collapse, thereby demonstrating the effects of location of application [36].

A concept of considerable importance in essential oil usage is the occurrence of sensitisation, where allergic reactions occur in particular locations. A list of these allergens has been compiled in a recent study, where the frequency of sensitisation earns it a grouping classification. In order of descending priority, Group 1 allergens are more serious than Group 2 or 3. According to the first amendment of the "Detergent Regulation and Allergen Labelling", if any of these allergens are present in a detergent or cosmetic product it is mandatory for them to be declared, irrespective of the way they are added (such as part of an essential oil). Under the flavouring directive, various maximum and minimum levels are specified for biologically active substances [35].

Whilst the European Union controls legislation of therapeutic goods in Europe, in the Australian context it is the Therapeutic Goods Administration (TGA) that governs such approval [39]. When the therapeutic goods act of 1989 was enacted, a number of household natural products were "grandfathered" onto the "Australian Register of Therapeutic Goods" (ARTG), such as Eucalyptus or Tea Tree Oil. However, Australian natural products that had not become a household name, such as other Aboriginal medicines, could not be included on the ARTG without 75 years of documented use, with clear guidelines as to the exact method of preparation and safe use. Because the Aboriginal *materia medica* of Australia has primarily been transmitted orally, or documented in books with minimal details, the process of getting ARTG approval for such natural products is a herculean task. Thus, comprehensive documentation of such use should start in an altruistic sense, for the benefit of future generations.

1.4. Biosynthesis and Subjective Classification of Essential Oils

Essential oils are classed as secondary metabolites derived by various biosynthetic processes, starting with phosphoenolpyruvate, a glycolysis product of the glucose produced in photosynthesis. Secondary metabolites can be defined as components that are present in some species but not others, which is in stark contrast to the four primary metabolites that constitute the basic building blocks of life and are

therefore found in all life forms, consisting of proteins, carbohydrates, nucleic acids and lipids. Of the secondary metabolites the most significant with regard to essential oils are terpenoids and shikimates, although polyketides also occur in essential oils and also rarely alkaloids [17].

Phosphoenolpyruvate, is the precursor to both shikimic acid and acetyl coenzyme-A. Therefore, phosphoenolpyruvate marks a major crossroad in the synthesis of secondary metabolites and some primary metabolites, such as lipids. At this crossroad, those metabolites along the acetyl coenzyme-A path include lipids, polyketides and terpenes, whilst those along the shikimic acid path include coumarins, flavonoids (colour compounds) and lignin. Of particular interest, lignin is a complex monomer of aromatic alcohols called monolignols, an integral part of the secondary cell wall of plants. The lipids also include free fatty acids that appear in fixed oils and sometimes in essential oils. Furthermore, some essential oils are decomposition products of lipids [17].

Where the path also splits at acetyl coenzyme-A, on one side mevalonic acid is produced, made from three molecules of acetyl coenzyme-A. This serves as the precursor to the isoprenes, which are the building block of terpenes. On the other side there is the carboxylation of acetyl coenzyme-A to give malonyl coenzyme-A. This combines with acetic acid then decarboxylates to give a β-ketoester. If this process is repeated a molecule will form with a carbonyl group on every alternating carbon, hence the name polyketide. Alternatively the ketone function can be reduced to an alcohol, which is then eliminated with the corresponding carbon hydrogenated and therefore giving a higher homologue, giving the start of a fatty acid (lipid). Since this lipid pathway corresponds to polyketide synthesis, there lies an explanation for why fatty acids are mostly even numbered [17].

The biosynthetic origin of shikimates (or the phenylpropanoids) is from the *shikimic acid* pathway. The biosynthesis of shikimic acid itself starts with the previously mentioned phosphoenolpyruvate and erythrose-4 phosphate, which is a precursor to carbohydrates. This means that the biosynthesis of shikimates diverges from the carbohydrate pathway [17].

As previously mentioned, terpenoid essential oils are biosynthesised via the *mevalonate pathway* involving the derivatisation and polymerisation of 5-membered isoprene alkenes from isoprenyldiphosphate (IPP) and dimethylallyldiphosphate (DMAPP). Isoprene units therefore combine to build terpenes, involving repeated carbon chains in multiples of five. 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 [21].

The term "terpene" was coined by Kekulé in 1880, because terpenes were first discovered in turpentine oil, as the main constituents [19]. 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 (including oxygenated terpenes), in modern terminology "terpene" only describes monoterpene hydrocarbons (two isoprenes with only carbon and hydrogen) [17].

With regard to the conventions for qualitatively or subjectively describing the 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 [40]. 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.

Essential oils can also be described and to an extent classified according to their aroma. Oils dominated by monoterpene components could be described as "top note" because the aroma is sharp and perceived immediately upon application. Well-known top notes are citrus (*Citrus bergamia* Risso) and ginger (*Zingiber officinale* Roscoe). Such top note (or head note) oils contain small components that evaporate quickly. However, although not apparent at first, the middle notes (heart notes) and base notes strongly affect the aesthetics of the odour and mask the sharpness of the head notes. In addition, they are perceived immediately after the top notes dissipate. Well-known heart note oils are lavender (*Lavandula angustifolia* Mill.) and rose (*Rosa damascena* Mill.), which are described as having a more mellow or rounded smell. These oils are composed of components that are slightly larger than simple monoterpenes, such as monoterpenols and esters. Oils that are dominated by sesquiterpenes are generally regarded as producing base note odour. Base notes are typically regarded as rich, earthy and deep, with the most common example being musk (*Angelica archangelica* L.). Generally perfume designers seek to combine notes to achieve an optimisation and orchestration of ingredients that please the senses [41].

In returning to the concept of biosynthesis of essential oils, microbial endophytes can also play a significant role in their synthesis. Although not a great deal is yet known about exactly how involved endophytes can be in this process, it is clear that at the very least such endophytes can give the final chemical alteration before the essential oil becomes the end product. Such microbial reactions are referred to as biotransformation, which is a process that can be utilised *in vitro* to create less common components from precursor compounds that are available in abundance. It is already known that endophytes are responsible for the biosynthesis of an array of natural products, some of which provide defence against herbivores or other competing plants, such as the cytotoxic quinoline alkaloid camptothecin, biosynthesised *de novo* (from the start) by the endophytic fungus *Fusarium solani* and accumulated in the tissues of *Camptotheca acuminata* Decaisne [42].

In the case of bacterial endophytes it is becoming increasingly evident that they influence plant physiology, nutrient uptake and plant growth vigour via the biosynthesis of phytohormones, such as ethylene, indol-3-acetic acid and acetoin, 2,3-butanediol [43]. Such bacterial endophytes from *Lavandula angustifolia* Mill were isolated and demonstrated to secrete metabolites that were inhibitory to human pathogens. Due to the similarity of inhibition demonstrated between the endophytes and the essential oil it was hypothesised that the endophytes may have been involved in the synthesis of the essential oil. However, the metabolites from the endophytes were not chemically characterised in that study [43]. In a ground breaking study of the fungal endophytes of *Mentha piperita* L. it was demonstrated that the fungal organism itself was biosynthesising all of the essential oil components *de novo* in the rhizosphere of the plant, although the composition of components was regulated by a plant interaction [44].

In the Lamiaceae the essential oil secretory structures, called glandular trichomes, are well studied and regarded as the site of biosynthesis, accumulation and secretion of the essential oils [45]. Very little research has been dedicated to identifying possible endophytic bacterial communities that may be involved in this biosynthetic path and in what capacity.

Currently there are approximately 100 or so molecules in the flavour industry derived from enzymatic or microbial processes [46]. This is because ingredients derived from transformation of natural raw materials by microbial or enzymatic processes can be labelled as "natural" under European and United States legislation. Of course such flavouring compounds could be sourced more expensively from plants or crops. In the classical flavour industry, the predominant source of ingredients was from plants, but as synthetic chemistry evolved botanical sources became out-dated, particularly in larger industries where cost cutting takes precedence. This phase eventually faded when "natural" products started to attract significantly higher market prices of up to two orders of magnitude. At that time research on microbial biotransformations started to expand until the industry was dominated by flavours of microbial origin. The rise of biotransformation and *de novo* biosynthetic products of microbial origin in the industrial flavours realm owes its success to the biological or "natural" origin of products, which yields such flavours at a substantially lower cost than classical methods of production [46]. A comprehensive coverage of microbial transformations are provided by Noma and Asakawa [47] for biotransformation of monoterpenes and Asakawa and Noma [48] for sesquiterpenes.

Although such microbial processes are dominating the market where individual isolated flavour compounds are in demand, whole essential oils are still predominantly sourced from classical agriculture or wild harvest. However, in an attempt to lower the cost and challenges of traditional agriculture and reduce dependence on wild harvesting, researchers are undertaking to develop essential oil industries where large-scale plant tissue cultures are involved in an attempt to create suitable alternatives to essential oil production [49]. At such a large scale this technology is still in its infancy. Unknown variables such as the role of endophytes and their survival in these culture environments, needs to be taken into consideration. Additionally, if the essential oils biosynthesised by the respective "plant" are shown to be synthesised *de novo* from the bacterial endophytes, such plant culturing would be purely a waste of resources, unless synthetic precursors are made by the plant itself.

1.5. Essential Oils in Agriculture

When it comes to the discussion of essential oils in agriculture, the major focus of course is of growing essential oil yielding species to maximise yield whilst still producing an essential oil that is in accordance with published standards. There is minimal discussion on how such oils could be employed more generally in agriculture itself. This is perhaps due to the perceived costs involved in applications involving essential oils as pesticides or herbicides. However, one might argue that the decline in popular demand for synthetic flavours in human foods could mean that such products will find a use in agriculture as a substitute for much less popular pesticides and herbicides. Where agriculture aligns with organic methods of production, such compounds could be sourced from bacterial or fungal cultures.

The vast majority of essential oil research today is confined to the pharmacological or drug discovery laboratory. Perhaps this is inspired by the financial stimulus from large pharmaceutical firms that seek to buy out patents to biologically active substances, with the promise of huge financial gain to the patent holder. Alternatively it may be a consequence of a gravitation toward popular science, which seeks to celebrate highly specialised research and purely academic discoveries. However, areas of more practical significance have subsequently been neglected, as explained by Murray;

> "Academic researchers tend to be more concerned about maintaining the rigour of science, judged by their peers in journals and conference proceedings, rather than research that contributes directly to the exploitation of essential oils and development of the industry" [50].

The advantage of using volatile "natural" herbicides in the field is that they don't persist and become part of the post-harvest product and in some cases the product may be marketed as "organic" and therefore attract a premium price. Although this may be an advantage, efforts need to be taken to prolong the residence time during the growth phase, by using surfactants or encapsulation technology. The possibility of using essential oils as herbicides has already been demonstrated, particularly where seed germination has been inhibited, but again there is minimal movement toward employing such technology in broad acre farms due to costs. Another advantage of the use of volatiles as a complement to herbicides is that they may facilitate pollination by acting as specific desirable insect attractants, while repelling others [51].

Essential oils may also serve agriculture by acting as antimicrobial compounds. Recently EU legislation has prohibited the use of antibiotics in the rearing of animals for slaughter. This is partly because of the growing resistance of pathogens [51] but primarily concerns the quality of end-of-use meat products. In this regard, natural products provide the best alternative, particularly essential oils as they serve other advantages. In conjunction with advantages gained in using essential oils as antimicrobials, they also act as appetite stimulants and as stimulants of saliva production, gastric and pancreatic juices [52].

The antimicrobial activity of essential oils can also be exploited to combat fungal or bacterial spoilage of shelf foods or as a treatment of fruits and vegetables at the harvest stages of production. A challenge in using this methodology is that the concentrations required to achieve such inhibition may add other flavours and fragrances unfamiliar to the product [51]. However, recent innovative techniques have utilised low pressures and warm air flow as a means to significantly enhance antimicrobial activity of essential oils applied to fruit and vegetable produce [53].

In returning to the optimization of agricultural production of essential oils, much attention has shifted toward genetic modification to enhance yield. Traditionally optimisation of yield focused on plant ontogeny, which means selection at a particular growth stage or for particular plant organ, cell or tissue structures. A suggested biotechnological innovation involves modification of plants to optimise for phenotypic characters that support essential oil production, such as trichomes or epidermal hairs *et cetera* [54]. Generally where genetic engineering of the plant aims to upregulate the biosynthetic pathway the limiting factor is a precursor compound, making things difficult. Whilst some have attempted to recreate the entire terpenoid biosynthetic pathway in recombinant microbes, thereby overcoming precursor supply by adding them manually after biosynthesis in another microbe [55], the next challenge lies in using a microbe that has resistance to the target compound. In practice, a microbe sourced from the plant itself is the answer [56].

In light of the greater interest and easier manipulation of microbes as biosynthetic factories, in particular the ease by which genes can be cloned from plants into microbes, there is not a great deal of interest in modifying plant organisms themselves to enhance essential oil production. Whilst this might seem threatening at first to the agricultural industry, the feasibility of employing a single microbe to biosynthesise a complex mixture of components identical to an essential oil is still out of reach.

Contrarily, using a set of microbes to individually biosynthesise the ingredients and therefore create the mixture, will probably not be met with approval by the vast majority who enjoy having essential oils of plant origin.

2. Pharmacology of Well-Known Essential Oil Components of World-Wide Origin

2.1. Bioactivity Testing

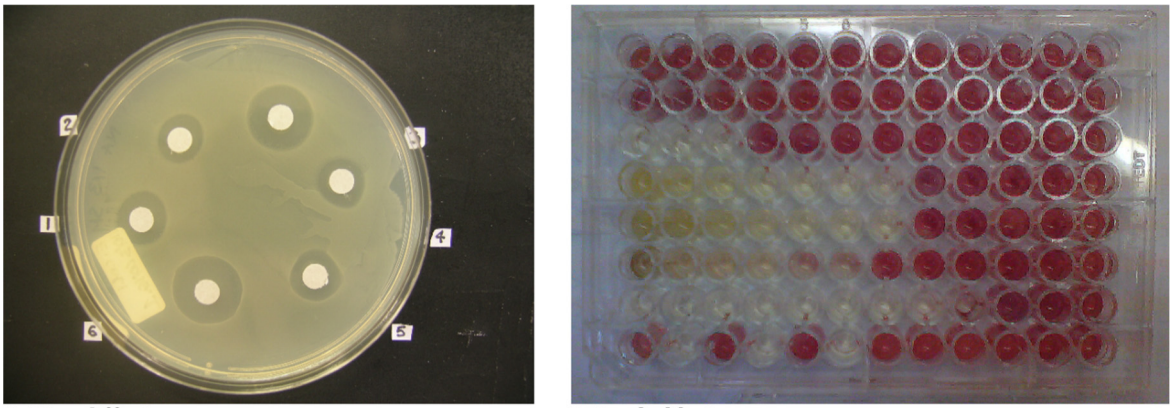
Essential oils may be characterized by either high or low biological activities, but this subjective description is of relative importance, exclusively within the context of essential oils. An inhibitory concentration of a "highly" antimicrobial essential oil may not necessarily be as low as an over the counter antibiotic. Therefore, the use of such terminology to describe the biological activities of essential oils must always be complemented with data values to show context.

The standardization of methods for biological testing is still evolving, which means that outcomes described in the literature may not necessarily be easily reproducible. In this regard, the more commonly described bioactivities in the literature are related to antimicrobial, antiviral, antinociceptive (analgaesic), anticancer, anti-inflammatory (antiphlogistic), digestive, semiochemical and free radical scavenging activity. The methodology behind these tests will be briefly described here [57,58].

Although there is a range of biological activities demonstrated in the literature, by far the most commonly cited activity for an essential oil is its antimicrobial activity. This is perhaps because antimicrobial susceptibility testing is a simple, inexpensive and straightforward technique. Alternatively, the growing popularity of essential oils as antimicrobial substances could be related to concerns about the growing resistance of pathogenic organisms against mainstream antibiotics.

In a similar way to the generalizations about essential oil toxicity to humans, a constituent-based approach can provide a simple guide in predicting the antimicrobial activity of an essential oil, but the actual activity cannot be known until a sample is tested and even then the results can be surprising. This simple generalization gives highest priority (highest activity) to essential oil components with high lipophilic character on the hydrocarbon skeleton, but high hydrophilic character on its functional group, with a ranking as follows: phenols > aldehydes > ketones > alcohols > esters > hydrocarbons [58].

To test antimicrobial activity two methods are primarily used, which are the disc diffusion and broth dilution assays. A variation of the disc diffusion assay is the well diffusion assay, but they both convey the same type of result, which is a more qualitative inhibition assay. A disc diffusion is performed on an agar plate, whilst a broth dilution is performed in a nutrient broth. Briefly, in microbiology agar plates are utilized in general as a medium for the growth of bacterial or fungal organisms (Figure 6A). The agar itself is a gelatinous substance discovered as early as 1650–60 AD in Japan, derived from the cell walls of algae. Its primary purpose in an agar plate is to act as a solidifying agent in a nutrient-enriched medium, to create a flat moist nutrient-rich surface for easy manipulation of growing microbial or fungal colonies. In a disc diffusion assay this surface is completely covered in a thin layer of microbes and paper discs are placed onto the surface. Each paper disc is inoculated with the treatment, in this case being a volume of the essential oil. If the essential oil is inhibitory to the chosen microbe, there will be a zone of clearance around the disc, representing the diffusion of the antimicrobial substance across and into the agar (Figure 6A) [59].



A. Disc diffusion **B.** Broth dilution

Figure 6. Examples of common methods employed for antimicrobial testing. Subjective antimicrobial activity is demonstrated by a disc diffusion assay (**A**) and mean inhibitory concentrations (MIC) are calculated from a broth dilution assay performed in a 96-well microtitre plate, with colorimetric detection of organism growth, with red indicating organism growth (**B**).

The disc diffusion method is not regarded as an accurate method for the determination of a representative inhibitory concentration. This technique is merely employed as a primary screening tool before a more rigorous method is employed, which will be the broth dilution assay. The broth dilution assay is almost always performed in a 96-well microtitre plate (Figure 6B), where the antimicrobial substance is mixed with a nutrient rich broth and serially diluted to progressively lower concentrations (*i.e.*, 5%, 2.5%, 1.25% *et cetera*), usually moving from left to right. In essential oil assays it is common practice to add something to help with the formation of an emulsion, due to the usual phase separation of oil and water. In some cases a small amount of agar is used to make the broth "sloppy" [60], but more often a detergent is used, such as Tween 20 [61].

Although the bacterial growth can be visualized by turbidity in the broth, it is common practice to add a metabolisable salt to the medium (*i.e.*, p-iodonitrotetrazolium) before recording the results. The salt is converted to a coloured compound by live bacteria and the results can be read according to the appearance of colour (Figure 6B). The concentration at which no colour is observed is reported as the mean inhibitory concentration (MIC). At this concentration it is not clear if the organism was inhibited or destroyed. However, if the broth from this well is spread onto an agar plate and no organisms grow on the agar, then this concentration can also be reported as the mean bactericidal concentration (MBC). The lower the MIC or MBC, the higher the activity of the oil [61].

A problem often faced in broth dilution experiments using essential oils is that they are continuously evaporating. This has the effect of lowering the concentrations being measured and leads to fractionation of the oils due to some components having a higher vapor pressure than others. Perhaps more problematic is the diffusion of components from one well to another, which has the capacity to create the appearance of synergistic or antagonistic interactions, which again reduces the reproducibility of the results. Whilst using parafilm to lock all volatiles into the 96-well plate has the advantage of slowing the evaporation of essential oils during the experiment [62], the disadvantage is that synergistic or antagonistic interactions can be enhanced. Thus, a more reliable method uses a sterile plastic sticky sheet to cover all wells individually [63].

In both cases, often authors try to report their data with an average and standard error but due to variability and lack of reproducibility, this is misleading. This is because the starting concentration will influence the atmospheric concentration of essential oil vapours, which has the capacity to influence the observed MIC. Furthermore, MIC data is ordinal, which requires a different type of statistical analysis. Generally if a particular MIC value can be repeated at least 3 out of 4 times it is regarded as significant and can be reported.

Whilst the use of a sticky sterile plastic sheet to cover all wells on the 96-well microtitre plate could be considered the most reliable in terms of the reproducibility of results, there still is the challenge of translating results achieved in experimentation (viz, *in vitro*) to actual effects in an application on an animal or human being (viz, *in vivo*). This problem of *in vitro versus in vivo* is encountered by all biological assays, not just antimicrobial tests.

Aside from antimicrobial assays, other biological tests generally use animal models or cell or tissue culture assays where known pathways toward pathogenesis are inhibited. For example, in the inflammatory model a number of known inflammatory pathways can be induced in an assay (or kit) and the test substance (essential oil) can be introduced to attempt to inhibit transcription or activity of the relevant factors. Some well-known inflammatory pathways include the NO (nitric oxide), TNF-α or PGE2 just to name a few [57]. An interesting development for testing the TNF-α pathway is by transfecting the RAW264.7 murine monocytic macrophage cell line with pDNA that encodes for reporter proteins (proteins that can indicate the outcome of the experiment) [64]. In one particular experiment, activation of NF-κB (that leads to TNF-α production) can be visualized via the simultaneous transcription of the same phosphorescent luciferase protein that is better known from a species of firefly (*Photinus pyralis*). The cell "glows" when triggered by the inflammatory signal compound lipopolysaccharide. If the cell does not glow then inhibition of the TNF-α pathway may have occurred [65].

In some tests for antiviral activity mammalian cells are transfected with the virus and cell survival detected by any of a number of colourimetric or flow-cytometric methods [66]. In the most basic test for anticancer activity immortalized cancer cells are grown in a culture and examined for survival or suppression under conditions of the respective treatment [67]. For antioxidant or free radical scavenging activity, a coloured free radical can be reduced by the treatment and made invisible to the naked eye, with the results quantified by a spectrophotometer on the visible spectrum [68]. A variation of this is that the free radical becomes coloured when reduced, also measured with a spectrophotometer [69]. For digestive activity related to blockage of Ca^{2+} gated ionic channels, the caecum segment of an animal intestine can be placed into a buffer and attached to an assembly that measures spasmolytic activity related to contraction of the caecum. An excess of a particular ion in the buffer demonstrates a blockage of the channel [70].

In semiochemical activity a number of insect species are shown to be repelled by a particular treatment or essential oil. For example, the Australian essential oil from *Eremophila mitchellii* Benth proved to be a strong repellent against termites [56]. A common animal model used to demonstrate the topical analgaesic properties of an essential oil is the "hot plate test" where the response of a mouse or rat after placing its paw on a hot plate can be delayed by treatment with a particular essential oil [71].

Analgaesia is also commonly related to activity on the central nervous system but in this regard it is called antinociceptive activity. Antinociceptive activity can be more broadly associated with psychological effects where other diseased states include depression, anxiety or hysteria. Antinociceptive activity is not

regarded as a biological property *per se* [57] but is nevertheless of importance in biological activity of essential oils. Methods commonly employed to measure such effects extensively use animal models where pain or intestinal writhing from intraperitoneal injections is induced. The treatment aims to reduce these observed effects [57].

A comprehensive coverage of the results of such biological activities in the context of essential oils is provided by Buchbauer [57] and Koroch *et al.* [58]. In this regard, due to the overwhelmingly high amount of data that is now available related to biological activities, there is currently a paradigm shift taking place in the biological research of essential oils. Increasing concern for the translation of *in vitro* results to *in vivo* use has prompted a number of experiments aimed at modifying the application of essential oils to reproduce *in vitro* results in the human or animal model.

Of particular consequence to essential oils are the problems of evaporation, solubility and absorption. There are now several studies that seek to enhance encapsulation of essential oils, which may include encapsulation of essential oils into various substrates, such as chitosan-coated liposomes, to slow evaporation and increase the antimicrobial activity [72]. Another approach involves the entrapment of essential oils into dissolved cyclodextrins, which can be used as a feed additive to disguise taste, as well as to slow evaporation of essential oils and therefore increase the shelf life of topical ointments and creams [73]. Penetration experiments where essential oils act as the carrier to another antimicrobial drug have also been undertaken with demonstrated success, which may be related to the interaction of the oils with liquid crystals of skin lipids [57]. It could be argued that such innovative techniques will comprise a large part of the future of biological research on essential oils.

2.2. Pharmacological Character of Internationally Recognized Essential Oils

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 [2]. This took place when the well-known French authors Pierre Franchomme and Daniel Penoel published *L'Aromathérapie éxactement* [2], 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 according to structural functional, which are listed in Table 2 [2]. Some of these pharmacological groups do not occur as common components in essential oils, and these have been italicised to aid the reader. A pictorial representation of common groups is provided in Figure 7.

Table 2. Essential oil pharmacological types defined by functional group [2].

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*	-

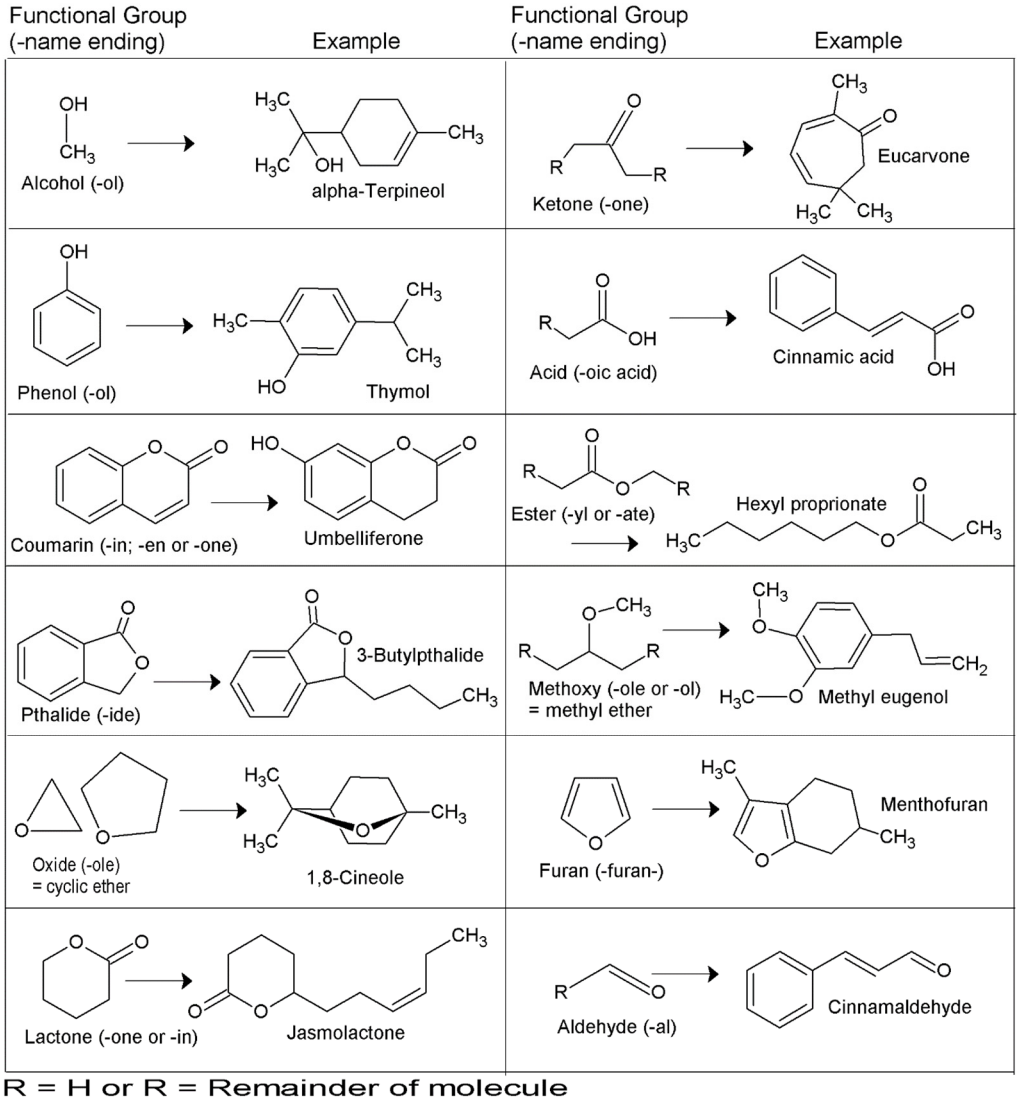


Figure 7. Examples of common functional groups.

Pharmacologically significant essential oil functional groups are of both terpenoid and non-terpenoid origin unless specified. With regard to commercially significant essential oils, monoterpene hydrocarbons, such as α-pinene (**2**), limonene (**3**) or ρ-cymene (**4**) are major components in grapefruit (*Citrus paradise* Macfad*: Rutaceae*), pine (*Pinus pinaster* Aiton: *Pinaceae*), juniper berry (*Juniperus communis* L.: *Cupressaceae*) and frankincense (*Boswellia carteri* Birdw: *Burseraceae*), respectively.

The *S*-enantiomer of limonene (**3**) is best known from Citrus, whereas the *R*-enantiomer is known from Turpentine [26].

Such monoterpene dominated essential oils have pronounced antiviral effects and produce a drying effect on the skin [2]. The phenyl hydrocarbon ρ-cymene (**4**) has been demonstrated to have skin sensitising effects, so essential oils rich in ρ-cymene are therefore avoided in topical applications [40]. Phenols can also demonstrate such effects, together with hepatotoxicity if ingested in high concentrations or moderate concentrations over a long period of time [2].

With regard to sesquiterpene hydrocarbons, β-caryophyllene (**5**) is known from Black Pepper and chamazulene (**6**) from German chamomile (*Chamomilla recutita* (L.) Rauschert*:* Asteraceae) [40]. Azulene sesquiterpenes, such as chamazulene (**6**) or guaiazulene from *Callitris intratropica* R.T.Baker and H.G.Sm (*Cupressaceae*), are responsible for the blue colour of their respective essential oils, if present at sufficient concentrations [40,74].

Monoterpenols such as linalool (**7**) from Lavender (*Lavandula angustifolia* Mill: *Lamiaceae*), menthol (**8**) from peppermint (*Mentha piperita* L.: *Lamiaceae*) or α-terpineol (**9**) from Tea Tree (*Melaleuca alternifolia* Cheel: *Myrtaceae*) are known for slight analgaesic effects if applied topically [40]. Furthermore, linalool (**7**) has been associated with possible sedative effects as well [75]. Strictly within the context of essential oils, monoterpene alcohols are generally of high inhibitory character against bacterial pathogens [2]. In the context of adulteration of essential oils, the *R*-enantiomer of linalool (**7**) predominates in bergamot oil, so its adulteration is signalled by the presence of the *S*-enantiomer. The opposite is true for coriander oil, which has an excess of the *S*-enantiomer [26].

Well known sesquiterpenols are α-bisabolol (**10**), again from German chamomile, α-eudesmol (**11**) from cedarwood (*Juniperus virginiana* L.: *Cupressaceae*) or β-santalol (**12**) from Indian sandalwood (*Santalum album* L.: *Santalaceae*). The sesquiterpenol α-bisabolol and the sesquiterpene chamazulene (**6**), have been associated with anti-inflammatory activity, particularly α-bisabolol [40]. The (−)-enantiomer of α-bisabolol is a signature for chamomile oil (*Chamomilla recutita* (L.) Rauschert: *Asteraceae*) [26].

In vitro blockage of neuronal Ca^{2+} channels by α-eudesmol (**11**) has been linked to potential psychoactive effects [76]. This may be significant with regard to anecdotal accounts of Cedarwood essential oil associated with enhanced memory and creativity [77]. Psychoactive and physiological effects consistent with sedation were observed when Indian sandalwood (*S. album* L.) was transdermally absorbed, with activity attributed to α-santalol [78]. The (−)-enantiomer of β-santalol (**12**) is a signature compound for Sandalwood oil (*S. album* L.). Sandalwood oil has also been associated with potential inhibition of the *Herpes simplex* virus [40].

Other well-known examples from the chemical groups described by Franchomme and Penoel (Table 2) include the phenol carvacrol (**13**) from oregano (*Origanum vulgare* L.: *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 (**13**) and the potentially hepatotoxic safrole (**14**) and methyl eugenol (**15**), known to be available in high yields from various Australian *Zieria* (*Rutaceae*) species [79] and an unusual and rare chemotype of *Eremophila longifolia* F.Muell (*Scrophulariaceae*) [22,62], as mentioned previously.

An example of a well-known component from the aldehyde class is citronellal (**16**) from *Eucalyptus citriodora* Hook (Myrtaceae), which is used as an insect repellent with mosquitocidal activities [80].

The (−)-enantiomer of citronellal is sourced in enantiopure form from balm oil (*Melissa officinalis* L.), making it a useful for establishing authenticity [26].

Camphor (**17**) 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 (**17**) is treated with suspicion, after studies demonstrated potential convulsant activity and liver/central nervous system damage, the camphor (**17**) and α-pinene (**2**) rich chemotype of rosemary continues to be used as a liniment for muscle aches and pains [40]. Camphor (**17**) is the ketone of the alcohol borneol (**18**), which occurs abundantly in a specific essential oil chemotype of the Australian species *E. longifolia* that demonstrated moderate antimicrobial activity [62].

Because acids are more soluble in water, they do not often become part of an essential oil. An example of this is the boswellic acids group from various Frankincense species (*Boswellia* spp.). Small amounts of boswellic acids do appear in the essential oils but the majority are dissolved in the hydrosol. Thus, Frankincense oils produced using supercritical CO_2 extraction have much higher concentrations [40].

Acids and alcohols are usually precursors to esters and when esters form into closed rings they become lactones [17]. Typically when alcohols are esterified by acetic acid, or another larger mass molecule, the resulting esters are named according to the parent alcohol, thus, linalool (**7**) becomes linalyl acetate (**19**), borneol (**18**) becomes bornyl acetate (**20**) and fenchol (**21**) becomes fenchyl acetate (**22**). The ketone of fenchol is fenchone (**23**).

Linalyl acetate (**19**) is another of the major components of Lavender oil and is additionally a significant component in the essential oil from Clary Sage (*Salvia sclarea* L.: *Lamiaceae*). Linalyl acetate was associated with the previous mentioned analgaesia, along with linalool (**7**) in Lavender oil [40]. An oil rich in fenchyl- (**22**) and bornyl acetate (**20**) is that from the Australian species *Eremophila bignoniiflora* F.Muell (*Scrophulariaceae*), and these components are probably responsible for the demonstrated moderate to high activity against the yeast *Candida albicans* and the bacteria *Staphylococcus epidermidis* [81]. 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 [37] 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 (*Gaultheria procumbens* L: *Ericaceae*), is methyl salicylate (**24**), 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 [82,83].

In essential oils it is extremely rare for an ether to occur in any other form than as a methoxy group or closed into a ring structure (cyclic ether). With regard to methoxy groups (methyl ethers) in essential oils, they typically occur as phenyl ethers, such as the phenylpropanoids eugenol (**25**), known from clove bud oil (*Syzygium aromaticum* L: *Myrtaceae*) in concentrations as high as 75%, and methyl chavicol (estragole) (**26**) from Comoro Island basil oil (*Ocimum basilicum* L: *Lamiaceae*) at approximately 85% of the whole. In general such 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

(**27**) and elemicin (**28**), highly concentrated in essential oil produced from nutmeg seed (*Myristica fragrans* Houtt: *Myristaceae*) [40,84,85].

When ethers occur in closed cyclic structures, they are called oxides. Perhaps the best known of these is 1,8-cineole (**29**), 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 (**29**) 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* R.Br., *P. rotundifolia* R.Br., *P. caerulea* R.Br., *P. lasianthos* Labill., *P. cineolifera* R.T.Baker and H.G.Sm and *P. incisa* R.Br. have high concentrations of 1,8-cineole (**29**) in their essential oils [45,86–89]. As 1,8-cineole (**29**) 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.

Lactones are constituents of many essential oils [21]. Lactones are produced by an intramolecular esterification reaction, where an aliphatic alcohol joins with an acid and closes into the respective cyclic ester [40]. 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 [21], and those occurring in four-member ring as β-lactones [21].

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 phthalides, which are a lactone of 2-hydroxymethyl benzoic acid. Phthalides are restricted to the Apiaceae family, typically in celery, lovage and angelica [21].

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 [40]. 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 (**30**), which is the principal odour compound responsible for the aroma of freshly cut hay [90]. Perhaps the best known coumarin is the furanocoumarin bergaptene (**31**), found in bergamot oil (*Citrus bergamia* Risso: *Rutaceae*) and also in Australian species, such as *Philotheca trachyphylla* (F.Muell) Paul G. Wilson (*Rutaceae*) (previously *Eriostemon*) [91]. Bergaptene (**31**) has a UV-sensitising effect, linking to melanin in the skin if applied topically in the sun [40]. This has the effect of intensifying the effects of the sun's rays. Interestingly, this bergaptene (**31**) 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 (**31**). This is because these types of coumarins are not easy to evaporate at the relatively lower temperatures employed in hydrodistillation, so they are generally present in trace

quantities only, unless the oil is expressed or produced by a solvent extraction technique leading to an absolute.

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" (**32**) is related to the occurrence of internal bleeding when herbivores consume large amounts of Yellow Sweet Clover (*Melilotus officinalis* Lam: *Fabaceae*). If other coumarins could be associated with anticoagulant activity, this effect may be employed in the treatment of cardiovascular disease [40]. To the best of our knowledge dicoumarol (**32**) has not been observed in an essential oil. However, in one such study the biologically active coumarins isopsoralen (**33**), xanthyletine (**34**) and osthole (**35**) were discovered in trace quantities in the hydrosol and essential oil following hydrodistillation of leaves from *Geijera parviflora* Lindl. (*Rutaceae*) [38]. Such coumarins can be implicated in the traditional therapeutic uses of these plants where smoking modalities were used by the Australian Aboriginal peoples.

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 and the methoxycoumarin dehydrogeijerin (**36**) are potentially responsible for differences in sheep palatability of leaves from *G. parviflora* [92]. The chemically similar osthole (**35**) has already been demonstrated in essential oils from *G. parviflora* [38], which makes it plausible that dehydrogeijerin (**36**) may occur in essential oils as well. However, although it is not possible for geiparvarin to appear in a hydrodistilled essential oil, this coumarin would no doubt be abundantly present in a hexane derived extract or concrete.

Novel, as well as known, coumarins were identified in *P. trachyphylla* (as *Eriostemon* in that study) [91]. A host of others are known from Australian plants, but it is not yet known if any of these have been observed in a hydrodistilled essential oil, however they would certainly be abundantly present in aromatic preparations as absolutes.

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 [93]. Due to the relatively large size of this molecule it is not clear if this effect could be related to psychoactivity achieved in traditional smoking activities [90].

With respect to using coumarins medicinally, as with other compounds, the relative and absolute stereochemistry strongly influences subjective and pharmacological effects [94], which obviously make synthesis more expensive. A corollary of this is the toxic *cis*-anethole (**37**) diastereomer, which is not produced in nature, but is rather a consequence of synthetically producing the medicinal compound, *trans*-anethole (**37**), which is sourced in stereo-pure forms from aniseed oil (*Pimpinella anisum* L.: *Apiaceae*) or fennel seed oil (*Foeniculum vulgare* Mill: *Apiaceae*) [90]. The effect of stereochemistry and chirality on the pharmacokinetics and pharmacodynamics of drugs is now fully appreciated [95] and researchers are beginning to seriously investigate the effects in natural product and synthetic medicine.

3. More on Essential Oils in the Australian Context

3.1. Historical Uses of the Australian Essential Oils

The first eucalyptus oil to enter British pharmacopoeia, under the name *Oleum Eucalypti*, was the cineole rich form known widely today [96]. The most common eucalyptus (*Myrtaceae*) species used to produce the well-known 1,8-cineole (**29**) rich essential oil are, among others, the "blue mallee" (*E. polybractea* R.T.Baker), the "broad leaf peppermint" (*E. dives* Schauer var C) and *E. leucoxylon* F.Muell, *E. sideroxylon* A.Cunn, *E. oleosa* F.Muell, *E. radiata* Sieber var australiana *et cetera* [90]. Currently much of the global production of Eucalyptus oil from Eucalypts is carried out in Portugal and Spain, which have established *E. globulus* Labill. as the favoured cultivar; however, in terms of gross production China leads the way with Chinese Eucalyptus oil, a by-product of camphor (**17**) production from *Cinnamomum camphora* L. (*Lauraceae*).

Another form of eucalyptus oil, rich in the ketone piperitone (**38**), 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 (**38**) rich oil can also be produced from *E. piperita* Sm (*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* L. (*Lamiaceae*), hence the botanical name [97,98]. Although the subjective comparison is correct, *M. piperita* essential oils are dominated by menthol (**8**), menthone (**39**) and pulegone (**40**) [99], but contain no piperitone (**38**).

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 (**38**) 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 [90].

With regard to the cineole-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 cineole rich chemotype of *Melaleuca quinquenervia* (Myrtaceae) [90]. Furthermore, *Prostanthera cineolifera* (Lamiaceae), named for its higher yield of 1,8-cineole (**29**), was also used as an insect repellent by early colonialists [86].

As an aside, Maiden [96] 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 (**29**) that formed the basis for its use in and around homes.

Some of Australia's best0known 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 [96] 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 *Zieria smithii* (*Rutaceae*) is listed for its flavour enhancing activity, although it has more recently been demonstrated to have potentially carcinogenic phenylpropanoids, safrole (**14**) and methyl eugenol (**15**), in its essential oil [79]. *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 [96], *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 [96].

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 1980s, only two Australian essential oils had achieved significant international market success. These were the cineole-rich *Eucalyptus* and *M. alternifolia* essential oils. With respect to addressing the international market place, Cribb and Cribb [96] 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* R.Br: *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 [90]. Smoke from the Eastern Australian species of Sandalwood (*Santalum lanceolatum* R.Br: *Santalaceae*) was used to drive away mosquitoes in New South Wales [98] or in aromatherapy applications for babies

in the Northern Territory [100]. Subsequent pharmacological testing has revealed good antimicrobial activities against such microbial species as *Candida albicans* or *Staphylococcus aureus* [101].

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 [90]. 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* Benth. (*Cupressaceae*) essential oil industry. At one time, *C. intratropica* was botanically classified as *C. columellaris* F.Muell, together with *C. glaucophylla* Joy Thomps. & L.A.S.Johnson [102]. 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 [90]. Barr [100] 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, and 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" [103].

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 [96]. The formation of a plantation of *C. endlicheri* F.M.Bailey (*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 [104].

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 [105,106]. 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 (**38**) and demonstrated how menthol (**8**) and thymol could be synthesised from it [105,106]. Penfold substantially contributed to Ernest Guenther's six-volume work, *The Essential Oils* [107].

In 1915 Penfold became a research chemist and assistant work manager to the eucalyptus oil distillers, Gillard Gordon Ltd. [105,106]. *Eucalyptus* species have been a part of European pharmacopoeia for well over 100 years. In relative terms, *Melaleuca alternifolia* Cheel. 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 1930s [108].

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 [108]. 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 [108].

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 [56].

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 [108].

Another essential oil worth mentioning here, because of its long history in Australia, is from the Western Australian species, *Boronia megastigma* Bartl. (Rutaceae) [98]. 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 on *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" [109].

3.2. 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 [56].

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* Benth. (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 [56].

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 [110], the essential oil is marketed as an aromatherapy complement to meditation [56].

Another plant known for termite resistant timbers is the Tasmanian native *Kunzea ambigua* Sm. (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 [56].

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 (**2**), 1,8-cineole (**29**), spathulenol, bicyclogermacrene, globulol (**52**), ledol (**53**) and viridiflorol [111]. 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 [56].

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 [56].

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 anisatum* Craven and Biffin: Myrtaceae), fragonia (*Agonis fragrans* J.R.Wheeler and N.G.Marchant: *Myrtaceae*), lemon myrtle (*Backhousia citriodora* F.Muell: *Myrtaceae*), lemon tea tree (*Leptospermum petersonii* F.M.Bailey: *Myrtaceae*), bracelet honey myrtle (*Melaleuca armillaris* Sm. *Myrtaceae*) [112,113], nerolina (*Melaleuca quinquenervia* S.T.Blake CT Nerolina: *Myrtaceae*), niaoulina (*M. quinquenervia* S.T.Blake CT Niaouli: *Myrtaceae*) and rosalina or lavender tea tree (*Melaleuca ericifolia* Sm: *Myrtaceae*) [111]. This latter essential oil, rosalina, is produced from both wild harvest and commercial plantations [56].

3.3. Recent Innovation in Australian Essential Oils

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. As a necessary prelude, it is important 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 significant proportion of these novel structures were extracted from *Eremophila* and *Myoporum* (*Scrophylariaceae*) species. With regard to essential oils, for several decades all wild specimens of *Eremophila longifolia* were wrongly considered within the context of an essential oil hydrodistilled from a rare chemotype occurring in north-west Western Australia [22], which yielded 5.5% w/w wet leaves, of an essential oil comprised almost entirely of the potentially hepatotoxic phenylpropanoids safrole (**14**) and methyl eugenol (**15**). This served to put a dampener on medicinal research of the other essential oils from *E. longifolia*.

In conjunction with reports of another chemotype in the Northern Territory, identified by Barr [100], with a monoterpenoid character predominantly made up of α-pinene (**2**) and limonene (**3**), 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 (**14**)/methyl eugenol (**15**) 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.

Several years after Barr [100] characterised the limonene (**3**) chemotype of *E. longifolia*, Smith *et al.* [114] identified three other essential oil chemotypes, occurring in New South Wales. One of these chemotypes produces a particularly high yield of a monoterpene ketone dominated essential oil (isomenthone (**41**)/menthone (**39**); CT.A) that shows considerable promise on a commercial level, given the high oil yield and localised abundance (Table 3). This isomenthone (**41**)/menthone (**39**) rich oil (CT.A) is hydrodistilled to produce a yield ranging from 3% to 8% w/w of fresh leaves. The other

two chemotypes firstly included CT.B, made up predominantly of karahanaenone (**42**), and secondly CT.C, made up predominantly of monoterpenes, such as α-pinene (**2**), limonene (**3**), α-terpinolene and significant amounts of borneol (**18**) [114].

With regard to the identification and delineation of essential oil chemotypes of *Eremophila longifolia*, it is now clear that the first such chemotype identified in 1971 by Della and Jefferies, with an essential oil made up predominantly of the potentially hepatotoxic carcinogenic phenyopropanoids safrole (**14**) and methyl eugenol (**15**), is confined to a small geographic region in Australia's far west, in central-west Western Australia. This is important since although *E. longifolia* has a widespread distribution throughout the Australian landmass, perceptions still prevail that this single chemotype reflects the constituents of all individuals of the species. This is simply not true. Further clarification reveals that this chemotype is an unusual biotype with diploid cytology [115].

In all, a total of three diploid populations of *E. longifolia* were identified in Australia, the other two being geographically clustered in western New South Wales and producing terpenoid based essential oils via the *mevalonate pathway*. These ketone rich chemotypes, as is the case for the phenylpropanoid type, produce significantly high yields of essential oils, making them potentially suitable for commercial development. The first of these types is the isomenthone (**41**)/menthone (**39**) type (CT.A), described above.

The second is a recently discovered high yielding karahanaenone (**42**) type (CT.B), yielding at a range of 1%–5% for diploid specimens [115]. The previously known tetraploid karyotype yields at 0.3%–0.7% [113]. Both these high yielding diploid types are good candidates as cultivars for commercial plantations. Should such plantations be established and developed this would make a significant contribution to Australia's essential oil industry. Essential oils and or/extracts from the high yielding CT.A isomenthone (**41**)/menthone (**39**) type could be used to make ointments and lotions suitable for topical, antifungal, aromatherapeutic and cosmeceutical/aesthetic applications (Table 3). At present it is unclear how CT.B could be utilised, but karahanaenone (**42**) is already in demand as a feedstock in the flavour and fragrance industry and may also be useful as a chemical scaffold for further drug development.

In addition to the five essential oil chemotypes of *E. longifolia* described above, another four have also been discovered [115]. One of these new essential oils, with dominant components of bornyl- (**20**) and fenchyl-acetate (**22**), is similar in composition to the antimicrobial essential oil produced from *Eremophila bignoniiflora* [81]. Traditional ethnomedicinal use of *E. bignoniiflora* by Australian Aboriginal people involved applications consistent with antispasmodic activity and headache therapy. Because essential oils rich in esters are often associated with antispasmodic and nervous calming activity, the essential oils from *E. bignoniiflora* may have contributed to this effect. The same essential oil produced from the new chemotype of *E. longifolia*, in significantly higher yields, could be marketed for treatment of headaches, nervous tension or gastrointestinal disorders.

Interestingly, another of the newly characterised chemotypes of *E. longifolia* produces an essential oil comprised predominantly of fenchone (**23**) and camphor (2-bornanone) (**17**), which are analogues of the previous mentioned fenchyl- (**22**) and bornyl acetate (**20**), respectively, after removal of the acetate groups [115]. In the case of fenchone (**23**) and camphor (**17**), a ketone is in the place of the ester; however, in the case of the other known chemotype, dominated by fenchol (**21**) and borneol (**18**), an alcohol functional group is in the place of the ester. Clearly, the oils produced by these three chemotypes are of very similar biosynthetic provenance. The structural resemblances are depicted in the following image.

The essential oils dominated by the alcohols, fenchol (**21**) and borneol (**18**), demonstrated high antimicrobial activity against the yeast *C. albicans*, bacterial species, such as *Staphylococcus aureus*, *S. epidermidis*, and the human pathogenic fungal species *Trichophyton rubrum*, *T. mentagrophytes* and *T. interdigitalis* [62]. Similar activity was demonstrated by the fenchyl- (**22**) and bornyl acetate (**20**) oils against *C. albicans* and *S. epidermidis* [81]. The fenchone (**23**) rich essential oil is yet to be tested for antimicrobial activity.

Another of the new essential oil chemotypes of *E. longifolia* is rich in α-pinene (**2**), sabinene, limonene (**3**) and α-terpinolene [115]. At first this essential oil appeared to be consistent with an earlier type reported from an individual *E. longifolia* collected from Alice Springs, in the Northern Territory. However, the unusually high concentration of α-terpinolene in the former, makes this new essential oil unique. To date, the last of the new chemotypes identified by Sadgrove and Jones [115] is dominated by ρ-cymen-8-ol, along with a host of other unidentified compounds.

Currently then, at least nine chemotypes of *E. longifolia* have been characterised but preliminary results suggest that others wait to be confirmed. All essential oil chemotypes occurring outside the small region of the safrole (**14**)/methyl eugenol (**15**) diploid type, the isomenthone (**41**)/menthone (**39**) diploid type and the karahanaenone (**42**) diploid type show tetraploid cytology. The karahanaenone (**42**) and isomenthone (**41**)/menthone (**39**) types also exist as tetraploid forms but produce relatively low essential oil yields by comparison with the diploid varieties. Such tetraploid types appear as randomly emerging individuals in isolated patches throughout the range of *E. longifolia*, probably emerging as a result of sexual reproduction and assortment of recessive allelic traits related to biosynthesis [115].

Considered within the context of proposals to cultivate commercial scale crops of *E. longifolia* species, quality control of plantations of tetraploid chemotypes may involve the elimination of karahanaenone (**42**) and isomenthone (**41**)/menthone (**39**) chemotypes emerging in plantations from sexual reproduction. However, in any case, this is not expected to occur with any great frequency since this species has a preference for reproduction by root suckers.

With regard to the emergence of unintended chemotypes in populations of known chemotypes, one may consider the emergence of the safrole (**14**)/methyl eugenol (**15**) type a potential risk in a commercial scale plantation, particularly since safrole (**14**) and methyl eugenol (**15**) have been red flagged as potential hepatotoxic carcinogens. Our research indicates that the risk of this occurring is vanishingly small. Thus far the safrole (**14**)/methyl eugenol (**15**) type has not been demonstrated to occur in the tetraploid form. However, even if this did occur, the parent chemotype would produce essential oils via the *shikimic acid pathway*, because emergent chemotypes may not contradict the biosynthetic origins of the parent chemotype. However, if a tetraploid chemotype is discovered with both phenylpropanoid components, such as safrole (**14**) or methyl eugenol (**15**), and terpenoid components in the essential oil, then this genealogy would be diligently avoided during the development of a cultivar chemotype.

With regard to the role of volatiles in the medicinal efficacy of smoke or steam fumigation rituals, using *E. longifolia*, both partially pyrolysed essential oils and the more hydrophilic component "(−)-genifuranal" (**43**) may be involved [12]. Most of the essential oil components are present in the leaf tissue before heating, but are accompanied by other derived artefacts in the steamy smoke, produced when the leaves are placed on hot embers for use in medicinal applications consistent with antibacterial or antifungal applications, as well as lactagogue activity. The smoking procedure was also used to prepare surgical tools, no doubt for sterilization but conceptualised as a type of exorcism ritual. The

essential oils and artefacts were also accompanied by pyrolysed derivatives including radical essential oil fragments and lignin decomposition products such as phenolic or benzoid constituents; together producing significantly enhanced antimicrobial activity [10].

The heat derivative "genifuranal" (**43**) itself exhibited significant antimicrobial activity, with a mean inhibitory concentration as low as 100 μg/mL against some species [12]. In traditional Aboriginal medicinal fumigation rituals using *E. longifolia*, "genifuranal" (**43**) and partially pyrolysed essential oils are delivered in warm air to the patient. Although the transdermal absorption of components such as "genifuranal" (**43**) is expected to produce significant biological activity, the first application with warm air is itself expected to have enhanced activity, relative to cooler applications [10].

It is proposed that genifuranal (**43**) derives from the cleavage of geniposidic acid (**44**) [12] at the glycosidic bond, to produce glucose and a hemiacetal. The hemiacetal transforms into the product genifuranal, (**43**) a stable furan aldehyde. Geniposidic acid is one of two non-volatile cardioactive glycosides that occur in *E. longifolia*, the other being verbascoside. The occurrence of volatile heat derivatives from verbascoside has not yet been demonstrated. However, in light of the occurrence of genifuranal (**43**), with demonstrated biological activity, there is potential for the development of a therapeutic lotion or use as a chemical scaffold for further drug development.

3.4. Ethnopharmacology of Aromatic Medicinal Plants Used Traditionally by Aboriginal Australians

The medicinal potential of the essential oil of *E. bignoniiflora* has already been summarised above. In other studies a dichloromethane extract of the leaves of this plant demonstrated calcium channel blockage that may be consistent with a number of traditional medicinal uses. Because the calcium channel subtype was not clarified in this earlier study, the results have implications for both therapeutic activity related to headaches and spasmolysis of the intestine [81].

According to ethnobotanical accounts, therapeutic activity from the use of *E. bignoniiflora* is expected to vary from use of the leaves to the fruits. The leaves were reportedly used as a laxative and the fruits as a purgative. In the modern context a laxative often means something that restores elimination activity to the colon, however in the historic language used by colonial ethnobotanists it may have referred to merely correcting digestive complaints or treating/reversing diarrhoea. Activity as a spasmolytic in this context could therefore be related to the abundance of the two fragrant esters bornyl- (**20**) and fenchyl-acetate (**22**). Interestingly this reflects advice given to us by an elder of the Kamillaroi tribe, who reported seeing his grandfather forage for the most aromatic specimen when employed in therapeutic use [116]. However, the biological activity of the fruit as a purgative requires a more comprehensive investigation.

Although essential oils from the fruit of *Pittosporum undulatum* Vent. (Pittosporaceae) have already been partly characterised in an earlier study completed in 1905, a recent characterisations enhanced and extended this earlier study [117]. In this study Sadgrove and Jones [117] tentatively identified the optically inactive compound referred to in the earlier study from 1905, conducted over a hundred years ago. It was believed to be bicyclogermacrene.

Unlike *P. undulatum*, *Pittosporum angustifolium* Lodd. was involved in a significant number of traditional medicinal applications [117]. The most common of these to be recorded in the literature is related to the treatment of coughs and colds, for lactagogue activity or in the treatment of eczema.

More recently, a number of anecdotal reports have surfaced related to *ia* cancer inhibition, autoimmune conditions in the intestines and antimicrobial activity. Previous studies have supported potential anticancer activity [118–120], as well as possible antiviral activity (using the older name *P. phylliraeoides*), particularly the Ross River Fever virus [121].

Jones and Sadgrove [117] examined the chemical character of volatiles from *P. angustifolium*, demonstrating a degree of variation. Compounds with structural similarities to previously described chemosemiotic compounds identified in mother-infant communications, were also noted, including acetic acid decyl ester and 1-dodecanol. These compounds may be involved in the traditional application as a lactagogue, particularly because the modality of usage involved heating a compress of leaves to produce such volatiles, which were then used to fumigate the breasts of the nursing woman.

In another study examining the essential oils from *Geijera parviflora* and *G. salicifolia*, the chemical character was consistent with previous identified chemotypes; however some variation was noted and new potential chemotypes were identified [38]. One of these, from a specimen of *G. parviflora*, yielded oil comprised of a larger abundance of bicyclogermacrene and *trans*-caryophyllene (and unknown B), which may be the first known sesquiterpene dominated essential oil from *Geijera* species. As previously mentioned, following hydrodistillation performed on this specimen a dichloromethane partition of the hydrosol produced a residue that was rich in pyranocoumarin xanthyletine (**34**), furanocoumarin isopsoralen (**33**) and the methoxy coumarin osthole (**35**). This hydrosol partition was attempted using other chemotypes but they did not yield these same coumarins [38].

This comprehensive study of the essential oils from species of *Geijera* also presented antimicrobial and free radical scavenging activity of these essential oils. The most active of these oils was the green oil from *G. parviflora*, made up predominantly of green compounds pregeijerene (not to be confused with the methoxy coumarin) (**45**)/geijerene (**46**) and linalool (**7**). Previous studies on these components indicate that this green essential oil may have applications as an insect repellent (particularly mosquitoes) as well as a topical analgaesic agent. Another interesting chemotype from this species is that dominated by the acetophenone xanthoxylin (**47**). Although this compound is known to possess cytotoxic and fungicidal activity, it is not clear if this was ever utilised in the *materia medica* of Aboriginal Australians [38].

3.5. Phytochemical and Chemotaxonomic Investigations

Phytochemical investigations of *Zieria* species corroborated previously published data on representatives of this genus [79]. Sadgrove and Jones [79] expanded the available information on this genus by, for the first time, detailing the chemical character of essential oils from the two species, *Z. odorifera* J.A.Armstr. subsp. *williamsii* and *Z. floydii* J.A.Armstr. Considered within the context of the chemotaxonomic approach undertaken in earlier studies, the remarks of the discoverer of the species, A. G. Floyd, now seem somewhat prescient.

> "This is a quite oddity! This specimen does not match any known *Zieria* taxon. It appears to be allied to 3 closely related species; *Z. furfuracea*, *Z. granulata* and *Z. smithii*".

The former two species mentioned above, being *Z. furfuracea* R.Br. and *Z. granulate* C.Moore, produce an essential oil rich in car-3-en-2-one (**48**). The essential oil from *Z. floydii* was also dominated by this component.

Although essential oils from species of *Zieria* have been previously examined for antimicrobial activity, extracts and essential oils were tested against a broader range of organisms [79]. In that particular study the activity of essential oils with solvent extracts from the same species. High antimicrobial activity in both solvent extracts and essential oils was found. Therefore, a putative essential oil industry based on species of *Zieria* would provide a novel range of essential oils, attractive to the aromatherapy community, as well as providing purified compounds useful as scaffolds in pharmaceutical development.

In a further chemotaxanomic study addressing existing taxanomic concerns regarding the *Phebalium squamulosum* Vent. heterogenous species aggregate, some headway was made using the chemical character of essential oils to demonstrate specific differences between so-called subspecies [122]. The first species examined was *P. squamulosum* subsp. *verrucosum* Paul G.Wilson, which was regarded as having greater morphological alliance with the *Phebalium glandulosum* Hook. complex. Essential oils of *P. squamulosum* subsp. *verrucosum* were dominated by dihydrotagetone (**49**) at concentrations ranging from 95% to 98% [27]. An identical essential oil, with the same yield g/g wet weight of leaves, was produced in an earlier study from *P. glandulosum* subsp. *macrocalyx* R.L.Giles. In another study this was also demonstrated to be the case with *P. glandulosum* subsp. *glandulosum*. An almost identical essential oil was characterised from *P. glandulosum* subsp. *nitudum* Paul G.Wilson and *P. squamulsoum* subsp. *eglandulosum* Paul G.Wilson. Therefore, dihydrotagetone (**49**) dominated essential oils is a general characteristic of the *P. glandulosum* subspecies complex.

In a subsequent study other members of the *P. squamulosum* heterogenous species aggregate were phytochemically investigated [122]. It was demonstrated that all apparent subspecies currently assigned to this assemblage are characterised by separate individual essential oil chemotypes. Interestingly, several separate chemotypes were demonstrated from specimens currently assigned to *P. squamulosum* subsp. *squamulosum* Paul G.Wilson. In this regard, a notable chemical characteristic of oils from southern specimens (collected near Sydney and in the Hunter Valley) was the almost total predominance of a tricyclic sesquiterpene ketone; squamulosone (**50**). By contrast, northern specimens were characterised by essential oils rich in the heat derivative elemol (**51**); derived from the hedycaryol precursor [122].

A study as yet unpublished demonstrated significant potential for the use of essential oils from specimens in the genus *Prostanthera*. Essential oils from species of *Prostanthera* (in particular series racemosae) are almost always characterised by a major representation of 1,8-cineole (**29**). However the differentiating factor is the existence and relative abundances of tricyclic sesquiterpene alcohols, such as globulol (**52**), its epimer ledol (**53**), prostantherol (**54**) and maaliol (**55**), which are characterised by a cyclopropane moiety, attached to either a decahydro-napthalene or -azulene structure. Again, further significant differentiating components of some essential oils were the tricyclic sesquiterpenes, but with heterocycle substituents in place of the cyclopropane moiety. Examples include cis-dihydroagarofuran (**56**) or kessane (**57**), also on a decahydro-napthalene or -azulene structure, respectively [87].

As with other genera, *Prostanthera* essential oils were considered within the context of possible pharmacological activities. It was demonstrate that oils dominated by the sesquiterpene alcohols provided the greatest antimicrobial activity against a range of organisms, most pronounced against some Gram-positive species (results unpublished). Individual components found in significant amounts in the essential oils were related to this enhanced antimicrobial activity, particularly prostantherol (**54**). In a separate study of *P. centralis* a prostantherol-rich essential oil demonstrated significantly low

antimicrobial activity against Gram-positive bacterial organisms and the yeast *Candida albicans* [123]. In one specimen currently assigned to *P. prunelloides* maaliol (**55**) was found in significant amounts. This is of considerable potential pharmacological interest, given the importance of maaliol (**55**) in the antinociceptive activity of a widely used Indian medicinal plant species (*Valeriana wallichii*) [124]. This antinociceptive activity is therefore expected to also be produced by oils from maaliol (**55**) rich species of *Prostanthera*.

In a study conducted by Lassak [125], the occurrence of maaliol (**55**) was demonstrated in a specimen of "*P. ovalifolia*" R.Br., at approx. 2% of the whole essential oil. This tricyclic sesquiterpene alcohol was formerly only known to occur in one other *Prostanthera* species, *P. prunellioides* R.Br. [126] at approximately 60%. However, it may also be known from *P. ringens* Benth. (as *P. lepidota*) [127]. Maaliol (**55**) was first characterized from the oleoresin "maali", from *Canarium samonensa* (*C. vitiense* A.Gray: Burseraceae), a Samoan native plant [128]. Members of the *Canarium* genus have been used extensively in traditional medicinal applications by Polynesian people to the north and north-east of the Australian landmass. The Philippine oleoresin "elemi" from *Canarium luzonicum* Miq. is the best known of these traditional medicines, used in applications to treat bronchitis, catarrh, extreme coughing, aged, damaged or injured skin and generalised stress [56].

The other maaliol (**55**) rich traditional medicine mentioned previously, from the north Indian Himalayan plant *Valeriana wallichii* DC (Valerianaceae) (maaliol chemotype), was used in applications similar to "elemi" but with a greater focus on psychotropic activity, in the treatment of a broad range of psychological disorders including stress, epilepsy and "insanity" [124]; and also in the treatment of a range of skin disorders. The study by Sah *et al.* [124] demonstrated activity from the maaliol (**55**) rich essential oil consistent with sedation or analgaesia via inhibition of the opioidergic pathway; or consistent with a peripheral antinociceptive effect via inhibition of prostaglandin synthesis. Maaliol (**55**) may be involved in these activities. It would be interesting to know if Australian Aboriginal people were aware of similar effects following the use of maaliol (**55**) rich *Prostanthera* species. Possible anti-inflammatory and analgaesic activity has also been demonstrated using 1,8-cineole (**29**) [129], indicating that oils containing both maaliol (**55**) and 1,8-cineole (**29**) are good candidates for further pharmacological tests. Again, *Prostanthera* essential oils have great potential as novel additions to Australia's aromatherapy and/or natural product industry.

The potential for cultivation of novel essential oil yielding crops utilizing species endemic to the Australian landmass is implicit in the literature. Such endemic species yield appreciable amounts of secondary metabolites with known *in vitro* pharmacological activities, such as antimicrobial activity. Table 3 gives a brief summary of the species broached by this review, but a substantially greater number of species are yet to be fully describes in the literature.

Table 3. Possible commercial scale applications from essential oil yielding flora in Australia.

Species	Chemotype	Use
Geijera parviflora	geijerene (**46**)/pregeijerene (**45**) (and germacrene D)	Commercial plantation: Insect repellent, topical analgaesia (linalool content). "Australian Green Lavender".
Geijera parviflora	osthole (**35**), isopsoralen (**33**), xanthyletine (**34**)	Commercial plantations: therapeutic effects
Zieria floydii	car-3-en-2-one (**48**)	Commercial plantations: Chemical scaffold for further drug development and antimicrobial activities
Prostanthera prunelloides	maaliol (**55**)	Commercial plantations: Medicinal applications consistent with the Indian *Valeriana willichii*
Prostanthera rotundifolia, P. centralis	prostantherol (**54**)	Commercial plantations: Antimicrobial activities
Eremophila dalyana	NA	Essential oil requires characterisation—useful in topical applications to treat fungal or bacterial infections. Also an effective decongestant in coughs and colds.
Eremophila deserti	ngaione	Commercial plantation: antifungal treatment
Eremophila deserti	methoxymyodesert-3-ene	Commercial plantation: chemical scaffold
Eremophila longifolia	isomenthone (**41**)/menthone (**39**)	Commercial plantation: topical, gastrointestinal for antimicrobial activities, topical for muscle aches and pains, active in applications for treatment of thrush (*Candida*)
Eremophila longifolia	fenchyl- (**22**)/bornyl acetate (**20**)	Commerical plantations: possible activity in gastrointestinal disease, possible activity in aromatherapy for headache sufferers
Eremophila longifolia	Limonene (**3**)/sabinene/ α-terpinolene, (−)-genifuranal (**43**)	Commercial plantations: derive (−)-genifuranal for therapeutic effects (*i.e.*, treatment of MRSA)
Callitris glaucophylla	NA	(**1**) Bioactive γ-lactones; ferruginol, pisiferal, pisiferol. (**2**) Occurrence of slightly hydrophilic antibiotic highly active against *S. aureus* (MRSA) and *B. subtilis*—requires purification and structure elucidation. Medicinal applications consistent with the Japanese species *Chamaecyparis pisifera*

4. Conclusions: Suggested Areas for Further Research

The demonstration of multiple chemotypes in *E. longifolia* emphasises the chemical variability expressed by this species, which may be an intrinsic general character of this genus. Thus, it is quite probable that other species from *Eremophila* may demonstrate similar geographical chemical variability. The observed correlation of diploidy with higher abundance of secondary metabolites may have more general implications. Therefore it would be worthwhile examining other species for both chemogeography and karyotype. Perhaps this search should start in *E. deserti*, as this has already been shown to possess an abundance of essential oil chemotypes each with high yields of essential oil. Another species, *E. glabra*, produces no essential oil at all; however NSW specimens are either hexaploid or tetraploid, but a diploid biotype can be found in far western WA. It may be worthwhile

seeing if this diploid specimen yields any amount of essential oil. This may be a fruitful area of investigation for all *Eremophila* and its allied genus, *Myoporum*.

With regard to further investigation of species of *Eremophila* for derivatives produced in smoke fumigation rituals, no other species was as frequently used for this purpose as was *E. longifolia*, implying a lower likelihood that volatile therapeutic compounds could be found in other species of *Eremophila*. Perhaps a few exceptions would be *Eremophila freelingii* F.Muell and *E. neglecta* J.M.Black.

Derivatives or larger molecular mass compounds produced/evaporated during smoke fumigation methodology may alternatively be produced from hydrodistillation. However, due to the less destructive nature of conventional hydrodistillation as compared to smoke fumigation methodology, such derivatives or larger molecules could possibly be distilled in higher abundance at shorter time-intervals. This is particularly true if higher temperatures and pressures are employed, not without the risk of decomposition or fragmentation of volatiles. In this regard a modified pressure cooker, with a 5–15 psi pressure release valve positioned for horizontal airflow into an adjacent condenser, could be used to achieve this end. It has already been used successfully at 15 psi to derive genifuranal (**43**) from *E. longifolia* over a very short time span, but with the consequence of complete decomposition of the essential oil and most of the genifuranal (**43**). This effect may be reduced if adjusted to an optimal lower pressure.

An interesting and unexpected consequence of the current review is the "resurrection" of chemotaxonomy, which was utilised in Australia by botanists in the 70's and 80's before molecular fingerprinting became possible and quickly grew in popularity. In this regard, the question still begs an answer "how do you define a species"? Chemotaxonomy is challenged by the divide between "new species" and "new chemotype of the one species". To complicate the matter further, in some cases it has been demonstrated that a correlation could be made between genetics (karyotype) and chemotype such as with *E. longifolia* [68]. This is in stark contrast to the classical view that chemotypes result from differences in soil climate. In the former "genetics" view, seedlings from one chemotype could be transplanted into different soil types and different climates without any serious variation to the chemical character of its essential oil. In the latter more classical view, most certainly there would be a difference.

The view that chemotype derives from soil type is borrowed from Europe and Great Britain, where cultivar selection over thousands of years has caused a kind of genetic uniformity across many species used in cultivation. However, because this cultivar selection was not a practice employed by Australian Aboriginal people it is more likely that unique soil types and various climates favour certain biotypes—meaning the plant itself is different and better suited to that environment.

Over long stretches of time, geographically isolated chemotypes may diverge into new species, but again, the challenge lies in deciding exactly what amount of divergence warrants delimitation of a new species. Because of the inherent ambiguity in answering such a question, the best resolution for now is that consistent morphological differences should stand alone in defining a new species, but chemotaxonomy and phylogenetics may be utilised to demonstrate that such morphological variability is not merely a consequence of naturally occurring variability within the one species.

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Author Contributions

This manuscript was compiled by Nicholas Sadgrove with extensive English editing, comments and suggestions provided by Graham Jones. This work summarises research with emphasis on collaborations between the two authors.

Appendix

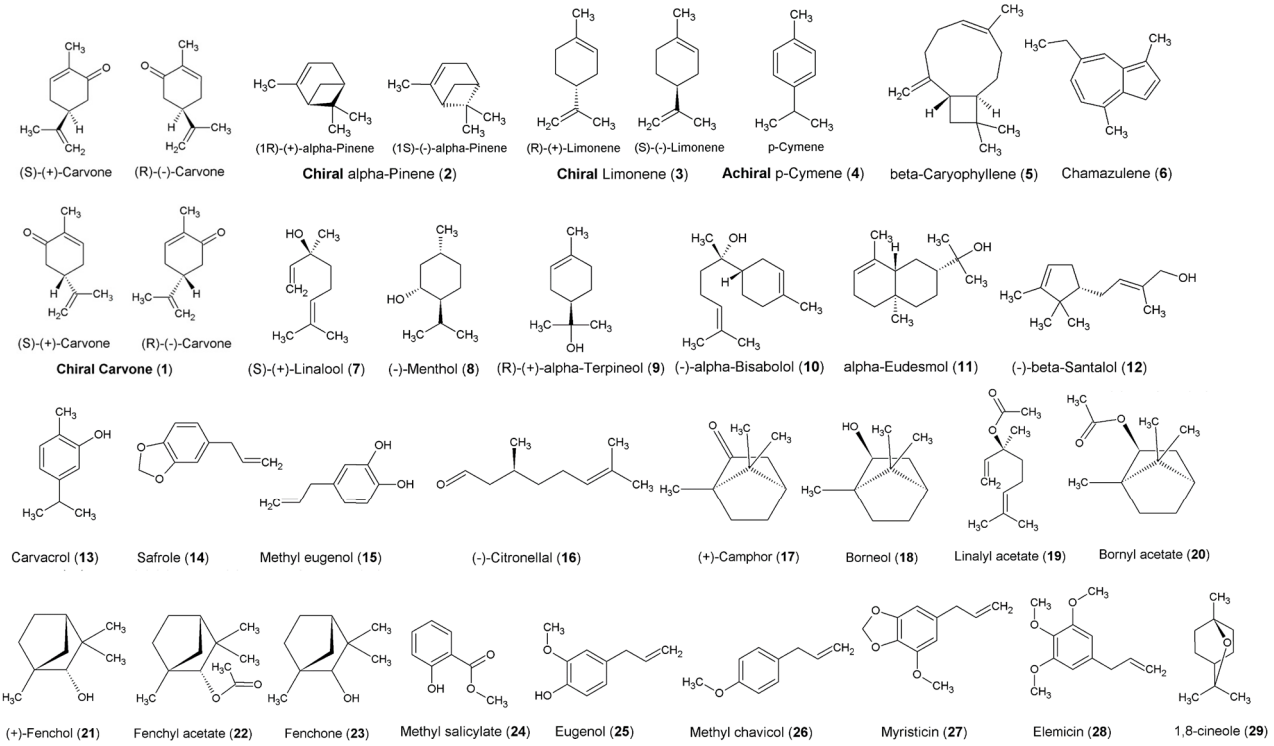

Figure A1. *Cont.*

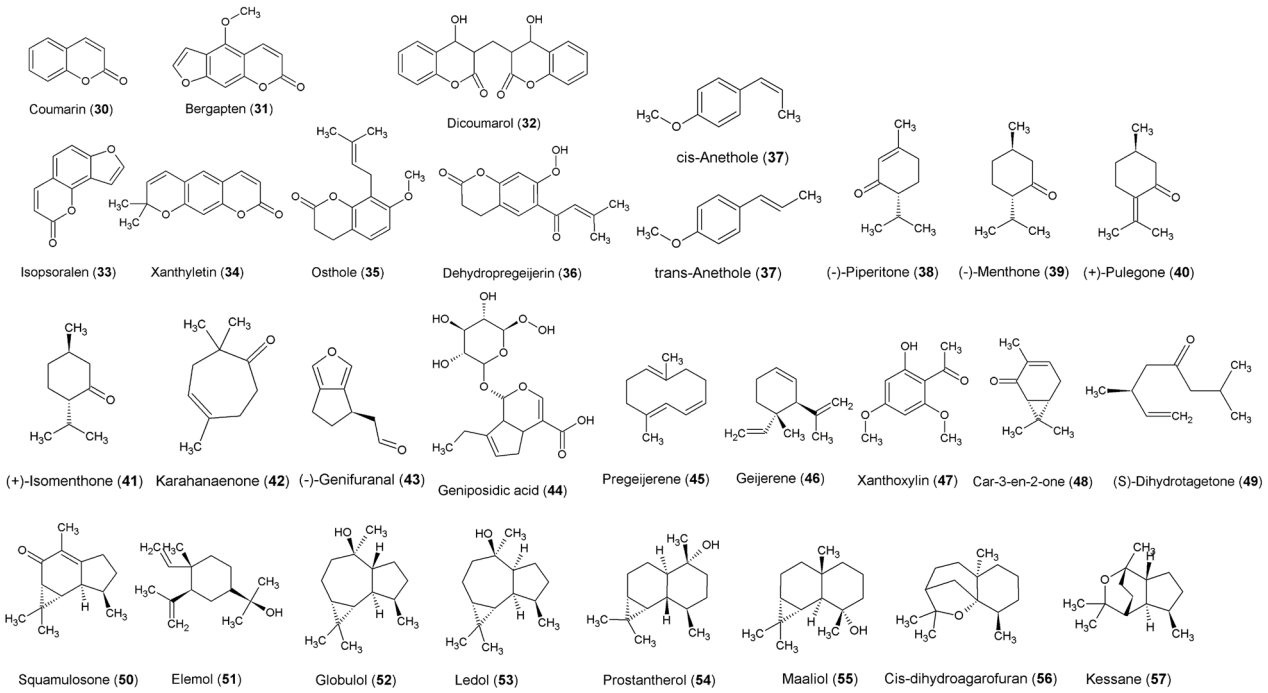

Figure A1. Chemical structure.

Introduction to Line Structures and Chiral Concepts Used in Organic Chemistry

Briefly, the most common atoms occurring in a volatile organic molecule, such as an essential oil molecule, are carbon, hydrogen and oxygen. Throughout this review the molecular structures are represented by line structures, combining 3-D structural formula where necessary (Figure A2, Lesson B) and incorporating chemical formula on the lines where methyl groups (CH_3) or other groups are specified (Figure A2, Lesson C). In a line structure diagram, the lines represent connections between carbons (one line connects two carbons) with hydrogen atoms bonded to them. For convenience (tidiness) the chemical symbols for both carbon and hydrogen (C—carbon, H—hydrogen) are generally replaced with lines, where single lines represent single bonds between carbons and double lines represent double bonds (Figure A2, Lesson D). Because carbons usually only have a maximum of four bonds, hydrogens occupy the remaining (invisible) bonds (Figure A2, Lessons C and E). Where another atomic element is present, such as oxygen (O—oxygen), the chemical symbol is always included. Some chemists like to show methyl groups (CH_3), and that is what we have done here.

In chemical identification the 3D spatial constitution, or stereochemistry, of connective parts of a molecule, as well as the position of a double bond, can significantly influence the chemical behaviour and pharmacological activity of the compound. Usually small differences in the spatial configuration (not to be confused with conformation) result in detectable differences in chemical analysis, such as in gas chromatography (GC) or nuclear magnetic resonance spectroscopy (NMR). However, often a single change in the spatial configuration of one molecule can produce another compound that is its exact mirror image, called an enantiomer. When a molecule is chiral this means it has an enantiomer or a mirror image of itself. Figure 2 depicts the two enantiomers of carvone (**1**), which is a chiral molecule. Although there appears to be four molecules in Figure 2, there are actually only two, with each enantiomer (either S+ or R−) depicted from both front and back.

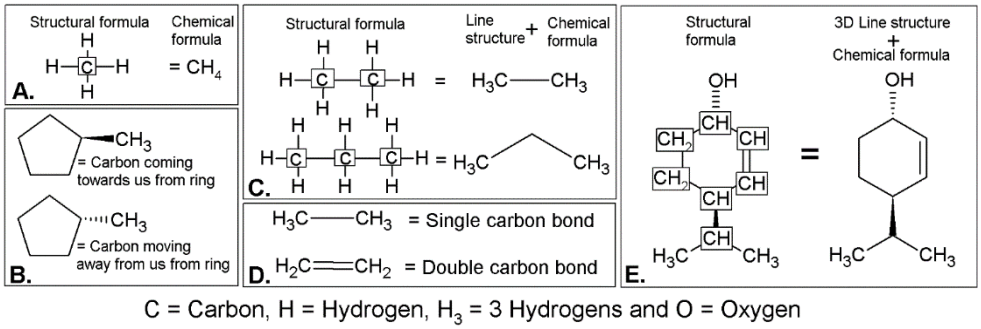

Figure A2. Lessons **A–E** demonstrating how to interpret a line structure representation of an organic molecule. (**A)** The difference between a structural formula and a chemical formula; (**B)** How spatial distribution (stereochemistry) is conveyed; (**C**) The difference between structural formula and line structures that use chemical formula for methyl groups; (**D)** Single and double bonds; (**E)** The structural formula compared to its equivalent line structural diagram utilizing a 3D effect and chemical formula for methyl groups.

Briefly, a chiral centre is identified by a central carbon that is bonded to four different groups (Figure 3A). Often one of those bonds is to a hydrogen atom, but generally not shown in the line structure. Although ρ-cymene (**4**) does not have a chiral centre, one of the two hypothetical compounds depicted in Figure 3 (B**)** does. The compound on the left appears to have a chiral centre, but it does not because two of the bonds are identical and the compound is symmetrical. This means that although there is a 3D spatial constitution, it does not create a new molecule because it is superimposable over its mirror image. However, the compound on the right does have a chiral centre on the same carbon, but with the double bond in the molecule it means that it does not have a plane of symmetry. Therefore the compound on the left is chiral and the other is not (it is achiral).

In the unlikely event that a molecule has both a chiral centre and a plane of symmetry, it is called a "meso" compound, but this can only occur if two chiral centres are in the one molecule, each cancelling the other out by rotating plane polarised light in equal and opposite directions. However, unlike an achiral compound, which rotates 180° about its plane of symmetry, parallel to its mirror image to realise their synonymy, meso compounds rotate about their plane of symmetry 180° perpendicular to their mirror image. Generally meso compounds are not discussed in essential oil chemistry. In a meso compound the two chiral centres must have opposite configurations (*i.e.*, both *S* and *R*) and a plane of symmetry. *S* and *R* configurations are demonstrated in Figure 3.

Conflicts of Interest

The authors declare no conflict of interest.

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