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ABSTRACT

The aim of this thesis was to obtain an overall picture of the chemical profile of the essential oil of two plant species, *Calytrix exstipulata* and *Cymbopogon bombycinus.*

The GC-MS analyses of the whole leaf oil of *Calytrix exstipulata* led to the identification of twenty compounds, predominated by monoterpenes and sesquiterpenes. The major compounds were α–pinene, β–pinene, valencene and globulol, with a relative distribution of 25.2 %, 17.9 %, 7.5 % and 4.4 %, respectively. In comparison the *Calytrix exstipulata* whole stem oil was predominated by globulol with a relative distribution of 17.1 %, followed by the monoterpene α–pinene with a relative distribution of 7.1 %. Fractionation of *Calytrix exstipulata* leaf oil by normal phase column chromatography yielded nine fractions, with the majority of fractions containing monoterpenes and sesquiterpenes. Fraction E gave the highest yield and was further fractionated. Subfractions E_1 and E_6 contained sesquiterpenes, while the rest of the subfractions contained monoterpenes and sesquiterpenes.

Pharmacological assays were performed on the whole oils, on the fractions and subfractions and were therefore screened for antioxidant and cytotoxic activity as well. Antioxidant activity was measured using the ORAC assay and the highest antioxidant activity was observed from subfraction E_4 (1185 ± 240 μmol TE/g), followed by subfraction E_1 (1053 ± 20 μmol TE/g), fraction F (865 ± 124 μmol TE/g), and fraction E (756 ± 24 μmol TE/g). Comparison of the ORAC values with the positive control epicatechin (20,000 μmol TE/g) showed that none of these tested samples showed high antioxidant activity.

Cytotoxic activity was investigated against P388, 3T3 and HS 27 cell lines with chlorambucil as positive control. Highest activity against the P388 mammalian cell line was observed from subfraction E_2 with IC_{50} value of 0.01 μg/mL, followed by fraction C and subfraction E_4 with IC_{50} values of 1.63 μg/mL and 1.81 μg/mL, respectively.

In conclusion, fractions E, F and subfraction E_4 showed the highest pharmacological results, which could be attributed to the monoterpene globulol. Globulol was tested to have cytotoxic activity and it is the most abundant compound in Fractions E, F and also in subfraction E_4.

The essential oils of *Cymbopogon bombycinus* and the *Cymbopogon citratus* oil were both rich in monoterpenes only. The major compound of *C. bombycinus* was geraniol and was present only in a low percentage in the *C. citratus* oil. The predominant compounds geranial and neral in the *C. citratus* oil were not present in the *C. bombycinus* oil. *C. citratus* oil is already well-studied and was used to compare with the less explored species *C. bombycinus*.

Both oils were screened for antioxidant and cytotoxic activity. The ORAC assay determined that the essential oil of *C. bombycinus* had higher antioxidant activity than that of *C. citratus*, which was attributed to its high geraniol content. On all cell lines, the whole oil of *C. citratus* was more cytotoxic than the whole oil of *C. bombycinus,* which was attributed to its high geranial content.

ZUSAMMENFASSUNG

Das Ziel dieser Diplomarbeit war es einen Überblick über die chemische Zusammensetzung der beiden Spezies *Calytrix exstipulata* und *Cymbopogon bombycinus* zu erhalten.

Die GC–MS Analyse des ätherischen Öles aus den Blättern von *Calytrix exstipulata* führte zur Identifikation von 20 Verbindungen, welche von Monterpenen und Sesquiterpenen dominiert werden.

Die Hauptinhaltsstoffe waren α–Pinen, β–Pinen, Valencene und Globulol, mit einer relativen Verteilung von 25.2 %, 17.9 %, 7.5 % bzw. 4.4 %. Im Vergleich dazu war das ätherische Öl aus den Stämmen von *Calytrix exstipulata* vor allem durch Komponenten wie Globulol mit 17.1 % relativer Verteilung; gefolgt von dem Monoterpen α–Pinen mit 7.1 % relativer Verteilung, gekennzeichnet. Die Fraktionierung durch eine Normalphasenchromatographie des ätherischen Öles aus den Blättern von *Calytrix exstipulata* ergab neun Fraktionen, wobei die Mehrheit dieser aus Sesquiterpenen und Monoterpenen zusammengesetzt war. Fraktion E bot die höchste Ausbeute und wurde somit weiterfraktioniert. Unterfraktionen E_1 and E_6 enthielten Sesquiterpene; während die verbleibenden Unterfraktionen sowohl Monoterpene als auch Sesquiterpene enthielten.

Die Durchführung der pharmakologischen Tests erfolgte sowohl an den durch Wasserdampfdestillation gewonnenen Ölen als auch an den Fraktionen und Unterfraktionen. Für diesen Zweck wurden diese sowohl auf die antioxidative als auch auf die zytotoxische Aktivität untersucht. Die antioxidative Aktivität wurde mittles des ORAC Assay analysiert, wobei sich der höchste antioxidative Wert bei der Unterfraktion E_4 (1185 ± 240 µmol TE/g) ergab, gefolgt von der Unterfraktion E_1 (1053 ± 20 µmol TE/g), der Fraktion F (865 ± 124 µmol TE/g) und der Fraktion E (756 ± 24 µmol TE/g). Vergleicht man hingegen die ORAC Werte mit der Kontrollsubstanz Epicatechin (20,000 µmol TE/g) ist ersichtlich, dass keine der getesteten Proben hohe antioxidative Fähigkeiten besaß. Die zytotoxische Aktivität wurde gegen P388, 3T3 und HS 27 Zellen mit Chlorambucil als Kontrollsubstanz erstellt. Die höchste Aktivität gegen P388 Zellen wurde bei der Unterfraktion E_2, mit einem IC_{50} Wert von 0.01 µg/mL, gefolgt von der Fraktion C und der Unterfraktion E_4 mit IC_{50} Werten von 1.63 µg/mL und 1.81 µg/mL beobachtet.

Zusammengefasst zeigten die Fraktionen E, F und die Unterfraktion E_4 die höchsten Ergebnisse in den pharmakologischen Tests. Dies könnte auf das Monoterpen Globulol zurückzuführen sein, denn dieses zeigte hohe zytotoxische Fähigkeiten in den Untersuchungen und war der Hauptinhaltsstoff in den Fraktionen E, F und in der Unterfraktion E_4.

Die flüchtigen Öle von *Cymbopogon bombycinus* und *Cymbopogon citratus* setzten sich beide ausschließlich aus Monoterpenen zusammen. Die Hauptkomponente von *Cymbopogon bombycinus* war Geraniol, welches in einer niedrigen prozentuellen Zusammensetzung im *C. citratus* Öl vorzufinden war. Die dominantesten Inhaltsstoffe Geranial und Neral im *C. citratus* Öl waren nicht im *C. bombycinus* Öl präsent. Das flüchtige Öl von *C. citratus* ist sehr gut erforscht und wurde somit als Vergleich für die weniger gut erforschte Spezie *C. bombycinus* benutzt.
Die flüchtigen Öle beider *Cymbopogon* Spezies wurden auf antioxidative und zytotoxische Fähigkeiten untersucht. Das ORAC- Assay ergab, dass das ätherische Öl von *C. bombycinus* eine höhere antioxidative Aktivität als das ätherische Öl von *C. citratus* besitzt, was wiederum auf den hohen Geraniolgehalt zurückzuführen sein könnte. Auf allen der drei besagten Zellen zeigte das flüchtige Öl von *C. citratus* eine höhere zytotoxische Neigung als das flüchtige Öl von *C. bombycinus,* wobei dies möglicherweise durch den hohen Geranialgehalt begründet sein könnte.

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1 INTRODUCTION

Medicinal plants are one of the oldest remedies. The use of plant medicine has sky-rocketed in the last decade and is still the first choice of many cultures. Until the 19th century herbal medicine could only be explained through traditional usage and through patient's experience. This knowledge was passed on through generations without being able to explain the plant indications. However, we have to consider the positive and negative effects of herbal
medicines and we must be aware of the fact that natural products are not always safe and nontoxic. These untested entities should be more explored to demonstrate their efficacy and
toxicity. The modern phytotherapeutical and phytopharmacological science is trying to approve these traditional experiences and observations by establishing the traditional perceptions through scientific approach [1].

Australia has an extraordinary variety of unique and distinct flora, which is not elsewhere found. Before the arrival of the Europeans, herbal medicine was a vital factor of the aboriginal culture and tradition. Through the aboriginal ethnopharmacology, it was possible to understand the effects of some Australian native plants beforehand. It is important to note that the aboriginal culture is slowly disintegrating which, consequently leads to the disappearing of the traditional knowledge of those unique plants. Considering the loss of handed-down knowledge and the diversity of plants, it is only natural that the majority of Australian plants are left un-researched.

Australian plants are rich in essential oil, a mixture of terpenes. Especially the volatile oils of the members of the Myrtaceae family (*Eucalyptus, Melaleuca, Leptospermum*) are well explored. They consist of terpenes, which are known to be used as food flavoring agents. Some of the essentials oils proved to have antimicrobial activities and thus are used against pathogens [2].

Many plants have been used in the aboriginal traditional treatment without knowing their active constituents. Examples of such are: *Calytrix exstipulata* and *Cymbopogon bombycinus;* which have been used by aboriginal people over a long period of time. Although *Calytrix exstipulata* belongs to the well studied plant family Myrta-

ceae, there is no published research on this particular plant. Today these plants are used in commercial products for external use, and have been referred by aboriginal women, living in the small city Katherine in the Northern Territory of Australia, in order to find out the reason for their activity.

Both of these two genera are cited in the important aboriginal books: "Traditional Aboriginal Medicines in the Northern Territory of Australia" [3] and "Traditional Bush Medicine – An Aboriginal Pharmacopoeia" [4].

1.1 Botany

1.1.1 *Calytrix exstipulata*

Calytrix exstipulata, also known as "Turkey Bush" is a common plant of tropical Australia and there is a wide range from shrubs to trees (ca. 4.5 metres high). The flowering is spectacular (Figure 1) [5], masking the insignificant pine-like leaves (Figure 2) and giving the plants an overall pink appearance [6].

Figure 1. *Calytrix exstipulata* flowers [5].

Figure 2. *Calytrix exstipulata* leaves.

It is found in the Gulf Country of Queensland, from the tropical Northern Territory all the way to Kimberly in Western Australia [7]. Figure 3 shows the distribution of *Calytrix exstipulata* in Australia [5].

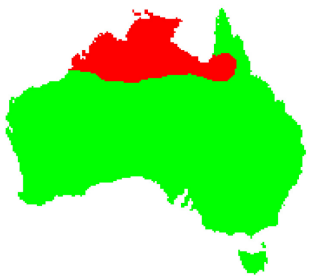


Figure 3. Distribution of *Calytrix exstipulata* in Australia (red-shaded region).

The function and importance of Turkey Bush goes far beyond its medicinal use; this plant is very important in the preservation of habitats. It achieves this by aerating the soil with its roots allowing water into the soil, returning nutrients to the soil when it sheds its leaves extensively across the highly lateritic soil [8].

The wood provides excellent firewood attributes and due to the durable and sharp edged wood, it can be used to make implements, such as fighting sticks, spears, music sticks, digging sticks and other small tools. Aboriginals extracted the essence from the Turkey Bush by boiling it in water; the decoction was then used as a body cleanser for skin related problems such as skin sores. Crushed leaves are used as liniment remedies for the treatment of wounds, aches and pains by applying them locally on the injured place [9].

Calytrix exstipulata belongs to the family Myrtaceae, consisting of trees and shrubs found in the tropical and warm-temperate regions of the world. The recent estimates for this family compromises ca. 5,650 species and ca. 130 - 150 genera. Myrtaceae was divided into two subfamilies, the predominantly Australian Leptospermoideae, having dry fruits with spiral or alternate leaves; and the mainly South American Myrtoideae with fleshy fruits and opposite, entire leaves [10].

Special notable characters of the Myrtaceae family are the presence of aromatic oil glands in leaves, alternate or opposite leaves, flowers with usually five sepals and petals and many conspicuous stamens, and usually a dry fruit, often a woody capsule. This family played an important role to the aboriginal lives as they collected water from the roots of many eucalyptus, edible grubs and insects from the foliage or under the barks of many species, and utilized the wood and bark of *Eucalyptus and Melaleuca* for a variety of purposes [11].

Furthermore plants from this family were used in folk medicine, as an antidiarrheal, antimicrobial, antioxidant, cleanser, anti-rheumatic and anti-inflammatory agent and to control blood cholesterol. It was also used for juices and liqueurs. Sweets are made from some fruits or the fruits are eaten fresh [12].

1.1.2 *Cymbopogon* spp.

Cymbopogon is a group of grass species, which comprises about 140 species [13] and is most common in tropical and sub-tropical climates including Africa, China, India, South America and Australasia [14]. Grass species which belong to the *Cymbopogon* genus have a wide variety of uses, including as liniment for scabies, cramps and sore heads, etc. The preparation and application process was very simple, the dried plant had to be crushed and boiled in water. However, the grass could also be used fresh, whereby it was simply crushed between hands and inhaled to relieve congestion [15].

Cymbopogon bombycinus is not a well-studied plant and therefore *Cymbopogon citratus* was used to compare it with, because there is a lot of research done about the latter. Both belong to the family Poaceae, thus it could be possible that they have similar chromatographic profiles or at least similar activity.

1.1.2.1 Cymbopogon bombycinus

Cymbopogon bombycinus or silky oil grass (Figure 4) is widely distributed from the Central Northern, Barkly Ta blelands, and Victoria River to the Gulf regions of the Northern Territory. It is a perennial grass, which grows up to 1.5 m and is also strongly aromatic. It has narrow and curly leaves, parallel-nerved and strap-like with prominent membranous outgrowths at the junction of the leaf sheath and blade (Figure 5). The callus hair is 3 – 5mm long and its lower leaves are curled. The flowering tops of the *Cymbopogon bombycinus* resemble a mass of silk.

Figure 4. *Cymbopogon bombycinus*.

Figure 5. Leaves of *Cymbopogon bombycinus.*

The Aborigines used to soak the whole plant in water, which was later used to treat sore eyes [4]. A decoction from the leaves as well as from the stems has been utilized to relieve infections of the respiratory tract and for the treatment of post-natal care. Furthermore it can also be used as a pain reliever; simply by mixing the leaves and stems with mound and allowing them to soak in for a few hours [3].

1.1.2.2 Cymbopogon citratus

Cymbopogon citratus is commonly known as lemon grass, West Indian Lemongrass, as well as oil grass (Figure 6). It is a tropical plant which is wide distributed in Southeast Asia, but can also be grown in warm temperate regions [16]. Due to the micro hairs, which are sparsely distributed in the adaxial epidermis and prickle hairs present in both abaxial and adaxial epidermis it is easy to recog nize this species [17].

Figure 6. *Cymbopogon citratus.*

Leaves of the *Cymbopogon citratus* are often used in the form of medicinal tea, which is known to treat stomach and gut problems. Furthermore it is well known for its act as an antidepressant and as a mood enhancer. Indeed the Brazilian folk medicine uses the oil for anxiolytic, hypnotic and anticonvulsant properties, nevertheless a study in humans found no effect [18]. In 2006 great success in the history of science was achieved by the research team from the Ben Gurion University in Israel. They verified that lemon grass caused apoptosis in cancer cells due to their most abundant constituent citral and thus can be used in anti-cancer therapy [14]. The lemon grass oil can aid in repelling mosquitoes due to the active component citral [16].

1.1.2.3 Family - Poaceae

Poaceae, formerly known as Graminae, which is a grass family of monocotyledonous flowering plants which has more than 12,000 species and spread across 700 genera, including

the *Cymbopogon spp* [19]. This family is distributed throughout the world, even in Antarctica; it is estimated that they comprise about 20 per cent of the world's vegetation cover. Most of the members of Poaceae occur as herbs, but a significant number are also shrubs and only few are even trees. The flowers are small and they are known as florets, the perianth is also small and colourless. Stamens are usually three, but can appear also at different numbers and are almost always free [11]. The fruit is usually a caryopsis, rarely a nut or a berry. The leaves are alternate and simple leaves, long, hairy or rough with linear, parallel veins [20].

It is used as a source of food for humans and also grazing animals, which renders Poaceae, economically the most important plant in the family. Furthermore bamboo stems and leaves, such as the leaves of other large grasses are both used as building materials [11].

1.2 Aims

The aim of this thesis is to obtain an overall picture of the chemical profile of the essential oil of two plant species, *Calytrix exstipulata* and *Cymbopogon bombycinus*, which have a history of traditional use; to identify the compounds present; and to attempt to prove the correlation of these compounds with the pharmacological usage through antioxidant and cytotoxic activity assays.

Although *Calytrix exstipulata* has a long history of use in the Australian aboriginal society for treating wounds and aches [9], there are hardly any studies about this species. The essential oil is generally obtained by hydrodistillation and analysed by GC/MS, with the constituents being identified by comparison of their retention indices (RIs) and mass spectra with published data or database library. The amount of each component is given as percentage of the total oil; in general 80 – 90 % of the oil is identified in this way. To isolate and investigate the active compound(s) of the essential oil, bioactivity-guided fractionation by column chromatography was performed to fractionate the mixture of substances. Pharmacological studies are described according to cytotoxicity and antioxidant properties.

Cymbopogon bombycinus have not received significant in-depth research as other species in the genus such as *Cymbopogon citratus*, which have been well studied. As such, the essential oil of *Cymbopogon bombycinus* was studied to determine its chemical composition. The essential oils of *Cymbopogon bombycinus* and *C. citratus* were compared to find out whether there are any similarities in their chromatographic profiles and biological activities.

2 ETHNOBOTANY AND ETHNOPARMACOLOGY

Essential oils are a mixture of lipophylic and volatile substances, which occur particularly as terpenes, a compound class made up of isoprene units.

As chains of isoprene units are built up, the resulting terpenes are classified sequentially by size as monoterpenes, sesquiterpenes, diterpenes, etc. The essential oils consist mainly of the monoterpenes and sesquiterpenes, and often a mixture of both is found. Monoterpenes, which can be acyclic, monocyclic and bicyclic, consist of two isoprene units and have the molecular formula $C_{10}H_{16}$. Sesquiterpenes, which can be acyclic, monocyclic and polycyclic, consist of three isoprene units and have the molecular formula $C_{15}H_{24}$ [21].

Terpenes are effective in three different ways:

1 the pharmacological way; as the oil is reacting with the hormones and enzymes in the bloodstream (antiviral, antimicrobial, cytotoxic, antioxidant)
2 the physiological way, as the oil act as stimulants to the body
3 the psychological way, as the inhalated oil has individual impacts

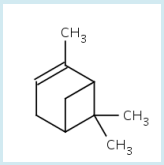
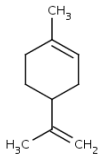
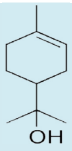
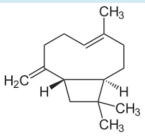
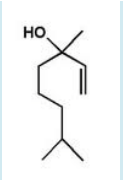
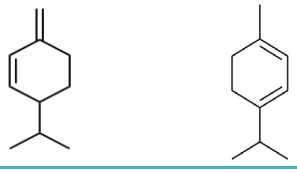
Although essential oils have been used therapeutically for centuries, there is little published research on many of them and as such their biological functions are incompletely investigated [22].

2.1 *Calytrix exstipulata* – Compounds and pharmacological effects

There is a huge lack of knowledge about the phytochemistry or pharmacology of *Calytrix exstipulata,* therefore its indication cannot be further explained. Whereas on another species, *Calytrix brownii,* the indication of its use for symptoms of respiratory tract problems is reported. Limonene, β–phellandrene and α–pinene are one of the major compounds found in this oil; limonene and pinene are both anti–spasmodic, which could explain the use as an inhalation to relieve congestion of the nasal and bronchial passages [3]. *Calytrix brownii* is also used to treat the symptoms of colds and flu [4]. Furthermore compounds such as linalool, myrcene, β–pinene, α–terpineol and caryophyllene were minor components in the essential oil, which are an interest-

ing group of components with various therapeutic properties, as shown in Table 1. Many peaks in the chromatogram of this essential oil were unidentified [4]. Table 1 shows the activity of chemical constituents of *Calytrix brownii*. The therapeutic effects of some of the compounds are well-known.

Table 1. Activity of chemical constituents obtained in *Calytrix* sp. leaf oil.

Compound	Activity of chemical constituent
Pinene 	Anti-inflammatory; Anti-spasmodic and spasmogene; anti-septic; Pesticidal and herbicidal; Flavor, fragrance and perfumery [9]
Limonene 	Anticancer, chemopreventive, detoxicant and antimutagenetic Antibacterial, antiseptic Antifungal and anti-candidal Antiviral and anti-influenzal Anti-acetylcholinesterease Anti-inflammatory Antiasthmatic and antispasmodic Expectorant Sedative Muscle relaxant Immunomodulatory Flavoring and fragrance[9] Antioxidative [23] Cytotoxic [24]
α–Terpineol 	Antioxidative [23, 25, 26]
Caryophyllene 	Preservative agent for food, cosmetics and drugs, antifungal [9] Cytotoxic (β–caryophyllene [24])
Linalool 	Antimicrobial (antiseptic, antibacterial, antifungal), antiviral, anticancer, anti-spasmodic and sedative [9], Antioxidative [23]
Phellandrene, Terpinene 	Flavour and fragrance industry [9]

2.2 *Cymbopogon* spp. – Compounds and pharmacological effects

The monoterpenes that have been reported in several members of the genus *Cymbopogon* are predominantly monocyclic (e.g. limonene) and acyclic monoterpenes (e.g. citral, citronellol, geraniol). Apart from these monoterpenes, some sesquiterpenes, e.g. caryophyllene, elemol, have also been detected in the volatile oils of different *Cymbopogon* species. All components are highly valued as flavouring agents and in the pharmaceutical industry [27].

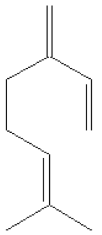
The Aboriginal Pharmacopoeia [4] reported that the major component in the *Cymbopogon bombycinus* oil might be the nerolidol isomer, followed by the minor components α–pinene, camphene, limonene, β-ocimene, trans-ocimene, linalool, camphor, (*Z*)-β-farnesene, 2-tridecanone, α-farnesene and some trace components as borneol, tricyclene, 4-terpineol and α-terpineol. It has to be mentioned that some peaks could not be identified. The book on Traditional Aboriginal Medicines [3] reported two similar results of the chemical analysis of the volatile oil. In the lower oil yield the components of the oil have been similar to the components as reported in the Aboriginal Pharmacopoeia. In the higher oil yield, which was 1.5 %, the questionable major component was identified as geraniol, followed by minor components as camphene, myrcene, borneol, β- and cis-ocimene, geranyl acetate, limonene, geranyl formate, linalool, geranial, α-terpineol and other trace components with many not identifiable peaks.

The usage of the essential oil of *Cymbopogon bombycinus* for cold or flu [3] could be explained by the compounds identified in the oil, which are found to have antimicrobial or antibacterial activities. Examples are nerolidol, linalool, limonene, citral, geraniol and citronellal, as shown in Table 2. Furthermore this plant was rich in antitussive and expectorant compounds, such as limonene, terpinene-4-ol and citronellal. Due to this fact its usage for respiratory tract infections is plausible [3].

In 2011, Bassolé reported that the whole oil of *Cymbopogon citratus* was predominated by geranial, neral, myrcene, geraniol, linalool and some other components [28]. As mentioned in section 1.1.2.2. this plant was used as an antidepressant, mood

enhancer and had also anticancer and anxiolytic properties. The antidepressant and anxiolytic usage could be explained through the sedative components such as limonene and linalool, as shown in Table 2. Furthermore the anticancer properties were reported in 2006 and it was found that the compound citral was responsible for this antitumor efficacy [14]. In Table 2 it is noted that other compounds such as linalool and geraniol have cytotoxic properties.

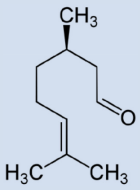
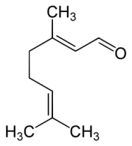
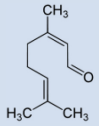
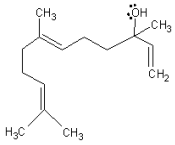
In *Cymbopogon citrates*, active compounds such as myrcene, an antibacterial and pain reliever, citronellol and geranial make this plant special [18].

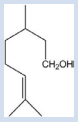
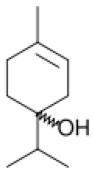
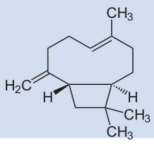
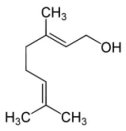
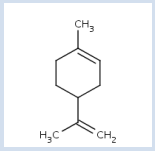
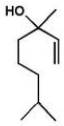
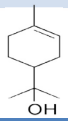


Myrcene

Two terpenoids, a ketone named cymbopogone and an alcohol cymbopogonol were isolated from *C. citratus* [27].

Table 2. Activity of chemical constituents, obtained in *Cymbopogon* spp. volatile oil.

Compound		**Activity of chemical constituent**
Citronellal		Antimicrobial (antiseptic, antifungal, antibacterial) Antiviral Analgesic Expectorant Insecticidal, insect repellent [29]
Geranial (β-Citral)		Antimicrobial (antiseptic, antifungal, antibacterial) Anticancer Fragrance [29] Antioxidative [23]
Neral (α-Citral)		Antimicrobial (antiseptic, antifungal, antibacterial) Aromatic (perfumery) [29] Antibacterial [30]
Nerolidol		Aromatic (perfumery) Anticancer activity [29] Antibacterial and antiprotozoal [30, 31]

Citronellol		Antioxidative [23, 32] Antitumor [30]
Terpinen-4-ol		Anti-inflammatory Anti-allergic Anti-asthmatic Antimicrobial (antiseptic, antifungal, antibacterial) Antitussive, expectorant [29]
Caryophyllene		Preservative agent for food, cosmetics and drugs [31] Antioxidative (β–caryophyllene [24])
Geraniol		Antibacterial [30] Antitumor [30]
Limonene		Anticancer, chemopreventive, detoxicant and antimutagenetic Antibacterial, antiseptic Antifungal and anti-candidal Antiviral and anti-influenzal Acetylcholinesterase-antagonistic Anti-inflammatory Antiasthmatic and antispasmodic Expectorant Sedative Muscle relaxant Immunomodulatory Flavoring and fragrance [9] Antioxidative [23]
Linalool		Antimicrobial (antiseptic, antibacterial, antifungal), antiviral, anticancer, anti-spasmodic and sedative [9], Antioxidative [23]
Camphene		Cytotoxic [24]
A–terpineol		Antioxidative [23, 25, 26]

3 MATERIALS AND METHODS

3.1 Sample preparation

The plant materials, leaves and/or stems, have been collected by Greg. J. Leach in March 2011 from the Northern Territory State of Australia (Howard Springs, Darwin). *Cymbopogon citratus* was collected from the Herbal Garden of Southern Cross University in April 2011. Detailed description about the location of the collection is given in Table 3.

Table 3. Sample plant material details.

Code	**Plant Name**	**Date collected**	**Location**	**GPS Coordinate**
Cal1L1 + Cal1S1	*Calytrix exstipulata* (part: leaves & stems)	1st collection: 21 March 2011	5 m away from 30 Barker Rd, Howard Springs, Northern Territory	12°30'14.57S 131°02'50.70E
		2nd collection: 27 March 2011	Adjacent to Darwin Airport, near MacMillans Road, Northern Territory	
		3rd collection: 11 April 2011	Purchased from: Greening Australia NT, 125 Thorak Rd, Knuckey Lagoon, GPO BOX 1604, Darwin 0801	
Cymb1	*Cymbopogon bombycinus* (part: leaves)	1st collection: 21 March 2011 (3 potted plants)	Purchased from: Greening Australia NT, 125 Thorak Rd, Knuckey Lagoon, GPO BOX 1604, Darwin 0801	131°02'50.70E
Cymb2	*Cymbopogon citratus* (part:leaves)	April 2011	Herbal Garden (Southern Cross university)	

The plant material was cut finely and then put into a weighed round bottom flask and the essential oils were obtained by steam distillation. A setup of the water steam distillation apparatus can be seen in Figure 7. Table 4 summarises which part of the plant was snipped and how much plant material was used. After approximately 20 hours, the obtained essential oil was transferred into a weighed brown vial and the yield was calculated. The amount of essential oils found in these plants ranged from 0.1 percent to 1.3 percent of the plant material.

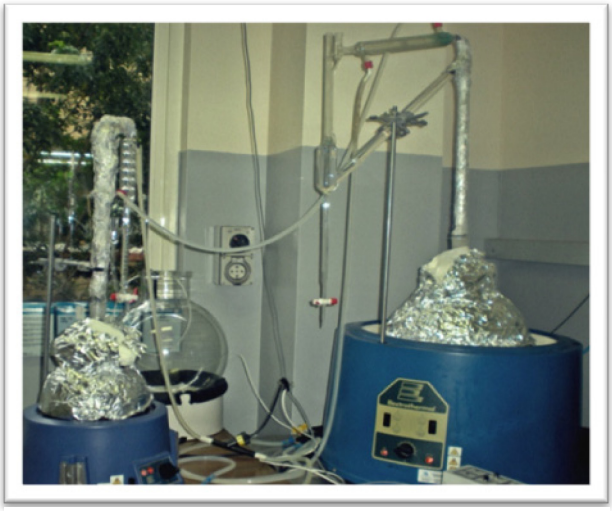


Figure 7. Water steam distillation apparatus.

Table 4. Oil yields, colours and duration of the distillation of *Calytrix exstipulata* and *Cymbopogon* sp.

Code	**Scientific name**	**Plant material (g)**	**Length of distillation (hrs)**	**Yield (g)**	**Yield (%)**	**Colour of essential oil**
Cymb1	*Cymbopogon bombycinus*	39.4	21	0.496	1.3	yellow
Cymb2	*Cymbopogon citratus*	828.9	19	0.6885	0.1	yellow
Cal1L1	*Calytrix exstipulata* LEAVES	956.3	21	12.43	1.3	green-yellow
Cal1S1	*Calytrix exstipulata* STEMS	340.5	21	-	-	cloudy white

3.2 Gas Chromatography – Mass Spectroscopy (GC-MS)

The chemical profile of the essential oil was determined by GC-MS, which provided detailed mass spectra and retention indices of the individual peaks in the Total Ion Chromatogram (TIC). One or two drops of the essential oil were transferred into a 2ml glass autosampler vial and dissolved in 1mL of acetone.

The GC-MS system used was an Agilent 6890 GC system fitted with an Agilent 7673 series auto sampler/injector and Electro Spray Mass Spectrometer, SGE BPX5 capillary column (50.0m x 0.22mm ID x 1μm film thickness) using the following acquisition parameters.

MASS SELECTIVE DETECTOR (MSD)

Transfer Temperature:	240°C
Source Temperature:	230°C
Quadrupole Temperature:	150°C
Ionisation Mode:	Electrospray (ESI)
Ionisation Voltage:	Average 1500 eV
Scanning mass range:	35-350 m/z

INJECTOR PARAMETERS:		*COLUMN PARAMETERS:*
Injection volume:	1.0μL	Gas: He
Gas type:	He	Pressure: 23.86 psi
Inlet Temperature:	220°C	Flow: 1.0 mL/min
Inlet Pressure:	23.85 psi	Average velocity: 28 cm/sec
Total flow:	53.8 mL/min	
Split Ratio:	50:1	
Split Flow:	49.4 mL/min	

The oven temperature program of the GC–MS run is shown in Table 5.

Table 5. Oven temperature program of the GC-MS run.

OVEN TEMPERATURE PROGRAM			
Time (min)	Temp	Ramp (°C/min)	Flow (cm/sec)
0.0	60	3.0	28
73.33	280	0.0	28
78.33	280	0.0	28

Data were processed using MSD ChemStation Software (Version D.00.00.38, Agilent Technologies).

The identification of peaks in the chromatographic profile was initially carried out using database libraries (ADAMS, WILEY275 and Nist98). Kovats index (KI) values were calculated for each peak and compared with known literature values in Adams book referred to as Adams Index (AI) [33]. Some reference standards were available and peak identification was further confirmed by comparison of the retention time and fragmentation patterns of the individual peaks with that of the reference standards. The reference standards used are listed in Table 6, which were prepared in acetone at a concentration of 10 mg/mL. By comparing the mass spectra and retention times of the individual peaks in the oils with the reference compounds, more information about the compounds of the whole oil was obtained.

Table 6. Reference standards.

Reference Standard	**Retention Time (Adams)**	**Adams Index (AI)**
Geraniol	16.97	1255
Borneol	14.29	1165
Camphene	5.67	953
α–Pinene	5.85	932
β–Pinene	7.04	974
α–Terpineol	15.21	1186
Citronellol	16.8	1223
Globulol	32.5	1590
Butylated hydroxytoluene	29.43	1514
γ–Gurjunene	27.8	1475
Terpinolene	10.98	1086

The Kovats Index (KI) for all of the compounds was calculated. The formula used for calculation of KI is as follows [33].

$$KI(x) = 100\,Pz + [(\log RT(x) - \log RT(Pz)) / (\log RT(P_{z+1}) - \log RT(Pz))]$$

KI = Kovats retention index

Pz = the number of carbon atoms in the smaller alkane

P_{z+1} = the number of carbon atoms in the larger alkane

RT = the adjusted retention time

3.3 Column chromatography

Column chromatography is a purification technique to isolate a compound from a mixture of substances. Two column sizes were used: (1) glass column with a diameter of 4.5 cm and a height of 50.6 cm (Figure 8); and (2) a glass burette with a diameter of 1.2 cm and length of 63 cm (Figure 9).

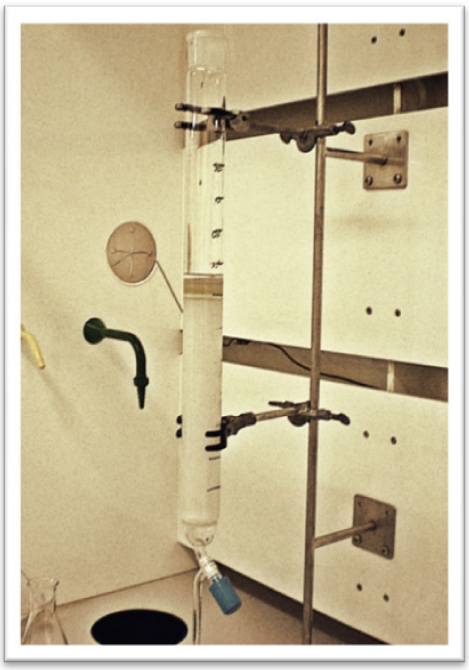


Figure 8. Glass column.

Figure 9. Burette column.

Silica gel (SiO_2) was used as stationary phase and was packed as slurry (in hexane) into the glass column. The essential oil was dissolved in a minimum amount of n-hexane and was applied at the top of the column. The mobile phase was added at the top and allowed to flow through the column under gravity.

Due to the interactions of the compounds with the stationary and mobile phases, the individual compounds were carried down the column with the mobile phase at variable rates and a separation had been achieved. From the bottom of the column the eluent was collected in a series of fractions. Since silica gel is a polar sorbent, the least polar molecules were eluted first, because polar compounds adsorbed more strongly with the stationary phase and the less polar molecules were retained less by the stationary phase and quickly pushed through the column by the mobile phase.

Figure 10 shows the column after adding the whole oil and Figure 11 shows some coloured bands, which is indicative of separation occurring.

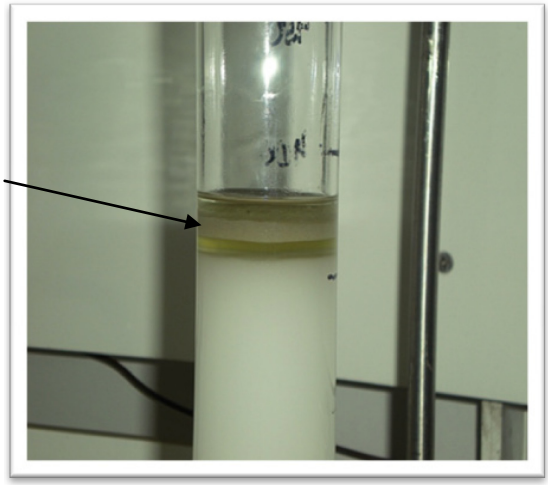


Figure 10. Column with the added Cal1L1 oil.

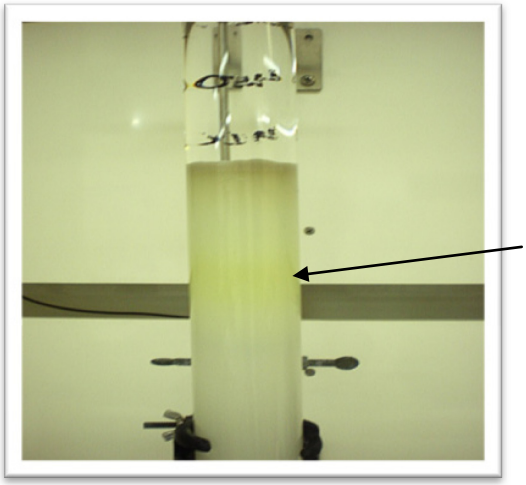


Figure 11. Separation of the Cal1L1 oil in the column, with the arrow pointing at the yellow-coloured band.

3.3.1 First fractionation

The first fractionation made use of the larger-sized column (4.5 cm diameter x 50.6 cm H) using a gradient of solvents (hexane, diethyl ether, ethyl acetate and methanol) as mobile phase. Table 7 shows the proportion of solvents used and their elution order. Ten grams of the whole oil of Cal1L1 (Turkey bush leaves) was loaded onto the column and elution was carried out using the mobile phases shown in Table 7. Eleven fractions were collected (Fractions A to K). The fractions were concentrated to dryness and the yields were obtained.

Table 7. Solvent systems used for the fractionation of the Cal1L1 oil.

Elution Order	Volume (mL)	Mobile Phase (Solvent)
1	500	90% Hexane - 10% Diethylether
2	500	80% Hexane - 20% Diethylether
3	500	50% Hexane - 50% Diethylether
4	500	100% Diethylether
5	500	50% Diethylehter - 50% Ethylacetate
6	500	100% Ethylacetate
7	500	50% Ethylacetate - 50% Methanol
8	500	100% Methanol

3.3.2 Second fractionation

Fraction E (621 mg) was fractionated using a glass burette as the column. The solvents used as mobile phase is summarised in Table 8, which also shows the elution order. Fractions were collected into 20 mL vials and 24 subfractions were obtained. The fractions were dried under nitrogen gas and the yields were recorded.

Table 8. Solvent systems used for the fractionation of Fraction E.

Elution Order	Volume (mL)	Mobile Phase (Solvent)
1	500	75% Hexane - 25% Diethylether
2	300	60% Hexane - 40% Diethylether
3	300	50% Hexane - 50% Diethylether

3.4 Biological activity testing

3.4.1 Antioxidant Activity

Oxygen radical absorbance capacity (ORAC) presents an innovative test to determine the antioxidant activity of many compounds and food samples [34].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed under physiological conditions in the human body and are neutralized by cellular antioxidant defense. Under different circumstances, such as stress and environmental

toxins, these reactive species start acting as strong oxidizing agents or free radicals. This in return leads to tissue damage and cell death and finally to degenerative diseases such as: cancer, heart disease, Alzheimer's and Parkinson's [35]. Therefore, antioxidants are required to remove all those dangerous radicals, oxygen ions and peroxides by counteracting the damaging effects of oxidation and breaking down radical chain reactions through an autoxidation between oxygen and the substrates [36]. Antioxidants have gained increasing importance in recent years; therefore, nutraceutical manufactures are going to include ORAC values on product labels [34].

The ORAC assay provides a good linear relationship between concentration and fluorescence by measuring antioxidant scavenging activity against peroxyl radical induced by AAPH (2,2'-azobis-(2-amidinoporpane)-dihydrochloride) at 37°C. The damage from its reaction with the peroxyl radical is visible through the loss of fluorescence of every sample. The net integrated areas under the fluorescence decay curves (area under the curve, AUC), calculates the protective effects of an antioxidant. Trolox, a water-soluble derivative of vitamin E, is used as a calibration standard and ORAC values are reported as µmole Trolox equivalent (TE) per g of sample [37]. Epicatechin was used as a positive control. The assay was carried out in a 96-well microplates and the fluorescence was measured in Wallac Victor 2 reader (Perkin Elmer).

3.4.1.1 Sample preparation

Different dilutions of Trolox and sample compounds were dissolved in DMSO and then diluted in phosphate buffer working solution (PBS, 75mM, pH 7.4), as shown in Figure 12.

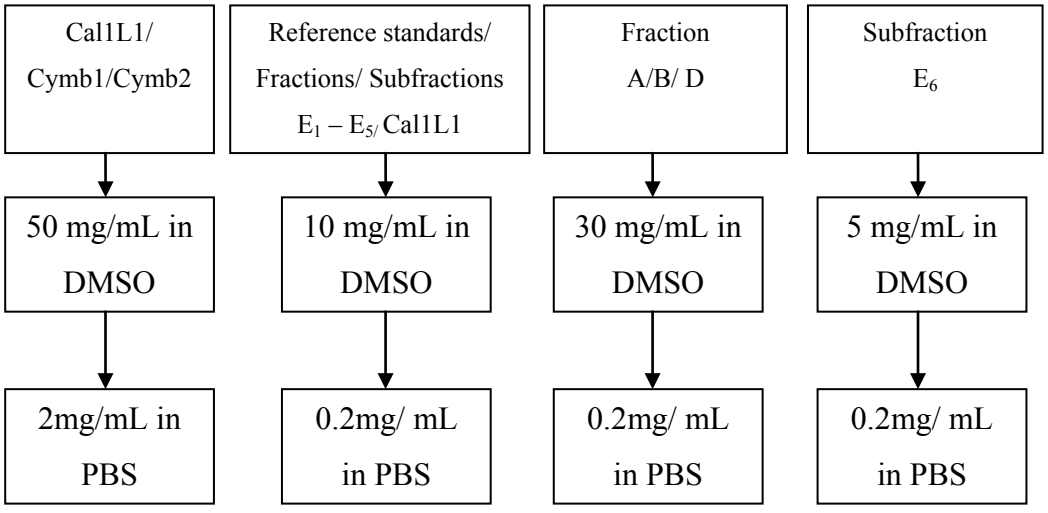


Figure 12. Sample preparation for the ORAC assay.

3.4.1.2 Reagent preparation

Phosphate Buffer Stock Solution (750mM).

Phosphate salts (17.25 g NaH_2PO_4 and 86.25 g Na_2HPO_4) were dissolved in 1L H_2O; pH was adjusted to 7.4 with KOH. Due to the pH sensitivity of fluorescein, maintaining the pH at 7.4 is important.

Phosphate Buffer Working Solution, PBS (75mM).

One hundred mL of the phosphate buffer stock solution (750mM) was added to 900mL "Milli-Q water" and the pH was adjusted to 7.4.

Phosphate Buffer plus 2% DMSO.

One mL of DMSO was added to 49 mL 75mM phosphate buffer.

Fluorescein Stock Solution (5mM) Mol. Wt 376.28g/mol.

Fluorescein (47 mg) was dissolved in 25mL of 75mM phosphate buffer (pH 7.4).

Fluorescein Working Solution.

Fifteen µL of fluorescein stock solution (5mM) was added to 16.5mL of the 75mM phosphate buffer (pH 7.4).

Trolox Standard Stock (20mM) Mol. Wt 250.32 g/mol.

Trolox (0.25 g) was dissolved in 50 mL of 75mM phosphate buffer (pH 7.4).

AAPH Working Solution (20mM).

AAPH (0.2 g) was dissolved in 25 mL of 75mM phosphate buffer (pH 7.4). The rate of peroxyl radical generation from AAPH is temperature-sensitive so this should be freshly prepared just prior to use. Moreover, new AAPH solution was prepared per each run.

Epicatechin.

The standard was prepared at a concentration of 20 mg/mL in PBS and then diluted to 0.5 mg/mL in PBS.

3.4.1.3 Procedure

The first step was to turn on the Wallac Victor 2 plate reader and the temperature was set to 37℃ to warm up and equilibrate the plate reader.

All samples were dissolved in DMSO and then diluted in PBS. Each well was filled with 100μL PBS buffer containing DMSO and 100 μL of each sample and was added to the first column. All samples were serially diluted to give a 1:1 concentration; for example, Trolox was diluted three times to the following concentrations: 50; 25; 12.5 mol/l, all other samples have been diluted seven times. In a fluorescence 96-well assay plate (black) – Perkin Elmer Optiplate, 10 μL fluorescein working solution were added into each well furthermore, all diluted samples at different concentrations were transferred at an amount of 20 μL to the assay plate.

As a final step 170 μL freshly prepared AAPH was added to each well of the assay plate, except the fluorescein control column, which was filled with PBS instead of AAPH. In the end, on the assay plate: a fluorescein control column containing 10 μL fluorescein working solution, 20 μL solvent, plus 170 μL 75 mM phosphate buffer (pH 7.4) and a blank/background column containing 10 μL fluorescein working solution, 20 μL solvent plus 170 μL AAPH must be included.

3.4.1.4 Analysis

The assay plate was read in a pre-warmed 37^{o}C Wallac Victor 2. The plate was automatically shaken for 10 seconds in a slowly orbital manner, before the first reading. Measurement was carried out 35 times at 1 min intervals. To calculate the AUC for each sample, the raw AUC data from each well was exported to Excel.

3.4.2 Cytotoxicity

Cytotoxicity is a substance's quality of being poisonous to cells. A cytotoxic compound treatment can be fatal to a cell in two ways: necrosis and apoptosis. Necrosis causes the cell to rapidly swell and lose membrane integrity. Apoptosis on the other hand, is a controlled cell death and is activated after a genetic program which releases apoptotic markers such as adenosine troposphere, LDH (Lactate Dehydrogenase).

Cytotoxicity is a subject of heavy pharmaceutical study, particularly, in the area of cancer research. The desire is to develop a therapeutic treatment that targets dividing cancer cells; with the ultimate goal of creating chemotherapeutic drugs which will give low cytotoxicity to healthy cells and high cytotoxicity to cancerous cells. Unfortunately, in most cases that is not possible [38].

The most common way to measure cytotoxic substances and cell viability is by assessing cell membrane integrity by using the adenosine triphosphate (ATP) assay system based on firefly (*Photinus pyralis*) luciferase. All cells require ATP to remain alive and carry out their specialized function. ATP is present in all metabolically active cells and as such ATP is responsible for cell viability. Damaged cells *in vivo*, which are undergoing necrosis or apoptosis, usually activate their apoptotic machinery by releasing contents like protease biomarkers into the environment. The difference between healthy and damaged cells is that damaged cells *in vitro* do not have enough time to activate their apoptotic machinery; the concentration of biomarkers, such as ATP, declines very rapidly as the cells undergo necrosis or apoptosis.

The "ATP-lite" assay system is based on the production of light, catalysed by the enzyme luciferase; as shown below:

$$ATP + \text{D-luciferin} + O_2 \xrightarrow{\text{LUCIFERASE/}Mg^{2+}} \text{Oxyluciferin} + AMP + PPi + \text{Light}$$

Under optimum conditions the emitted light is proportional to the ATP concentration [39].

Due to the need of assessing the cytostatic effects of every substance, the whole oils, all oil fractions and sub-fractions of the Turkey Bush essential oil and reference standards, and a positive control chlorambucil were subjected to cytotoxicity testing using 3 different cell lines: P388 cells (mouse lymphoblast); HS27 cells (human fibroblast); and 3T3 cells (mouse). All samples (whole oil, fractions, subfractions and standards) were tested against the P388 cells. All samples, except the subfractions, were tested on HS27 cells; and the whole oils and the first fractions (A – I) were tested against 3T3 cells.

In the following pages the assay procedure is explained specific for the P388 cell line. For the other two cell lines, HS 27 and 3T3, the procedure is almost similar except at the step where the cells were removed from the flask wall, which made use of 0.5 mL trypsin. This step was not necessary for the P388 cell line because these cells are not adherent cells. For HS27 and 3T3 culture media, bovine sera was used instead of horse sera and the volumes used were different. All other ingredients were the same.

3.4.2.1 Sample preparation

Chlorambucil, a cytostatic drug, was the positive control, and was used at different concentrations (60; 30; 15; 7.5; 3.75; 1.875 mg/mL). The other samples were also dissolved in DMSO, at concentrations between 5 mg/mL in DMSO and 50 mg/mL in DMSO, which is illustrated in Table 9.

Table 9. Sample preparation for the cytotoxicity testing.

Samples diluted in DMSO at different concentrations					
60 mg/mL	50 mg/mL	30 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL
Chloramb ucil	Cal1L1 Cymb1 Cymb2	Fraction A Fraction B Fraction D	Standard 3-14	Cal1S1 Subfraction E_1 - E_5	Subfraction E_6

3.4.2.2 Reagent preparation

Stock solution: (for 80 mL, approximately 15 mL per 96-well plate is required for each plate)

INGREDIENTS:	AMOUNT:
o DMEM (highglucose)	68 mL
o Horse Sera	8 mL
o L-Glutamine (200mM)	1.6 ml
o Pen/strep (5000 U/mL & 5000 µg/mL)	1.6mL
o Na Pyruvate (100mM)	0.8 mL

Cell line: P388

In a 50 mL centrifuge tube cells, 0.6 mL of the tested sample added to the required amount culture medium (80 mL) to give a final concentration of 4,500 cells/well.

3.4.2.3 Procedure

In another 96-well clear dilution plate, each well was filled with 80 µL media, 2 and 20 µl of every sample at different diluted concentrations from plate 1, to give a final dilution of 1:4. Using a multichannel pipette 95 µL of the media with cells and 5 µL of each sample from the second dilution plate were dispensed into each well of the assay plate to a final volume of 100 µL/ well. Each concentration was replicated, on each plate included a solvent control and a media control (5 µL of media) and these five plates were incubated for 24 – 48 hours.

To develop the plate, 50 µL of mammalian cell lysis solution and 50 µL luciferin dissolved in its buffer solution were added to 100 µL of cell suspension per well of a microplate. Then the plates were shaken for 5 minutes in an orbital shaker (700 rpm) to lyse the cells and stabilize the ATP. At the final step, 50 µL substrate solution was added to each well and the plates were incubated for 5 minutes on the plate shaker. The microplates were dark adapted for ten minutes.

3.4.2.4 Analysis

The luminescence was measured on the PerkinElmer TopCount Microplate Scintilation and Luminescence Counter at 22°C.

GraphPad Prism was used to calculate the IC_{50} values and the 95% confidence intervals for each of the estimated parameters [40].

4 ESSENTIAL OIL OF *CALYTRIX EXSTIPULATA*

4.1 Comparison of *Calytrix exstipulata* leaf oil (Cal1L1) and *Calytrix exstipulata* stem oil (Cal1S1)

The *Calytrix exstipulata* leaf and stem essential oils were both dissolved in acetone at a concentration of approximately 10 mg/mL and profiled by GC-MS. The compound identification was based on comparison with mass spectra and retention indices from GC-MS-library (Adams, Wiley275 and Nist98 library), references to Kovats index in the literature and authentic reference compounds, if available (detailed description in section 3.2).

4.1.1 Phytochemical analysis on the whole oil of *Calytrix exstipulata*

The freshly distilled leaf oil appeared as green-yellowish color and the GC-MS analysis confirmed the predominant peaks as the monoterpenes, α–pinene and β–pinene, with a relative distribution of 25.2 % and 17.9 %, respectively. Further on, the compounds valencene and globulol were shown in the region of sesquiterpenes with a relative distribution of 7.5 % and 4.4 %, respectively. One unknown peak was observed at the retention time 52.37 min. The chemical profiles of the essential oils, the identification and the amount (%) of the individual components are summarized in Figure 13 and Table 10.

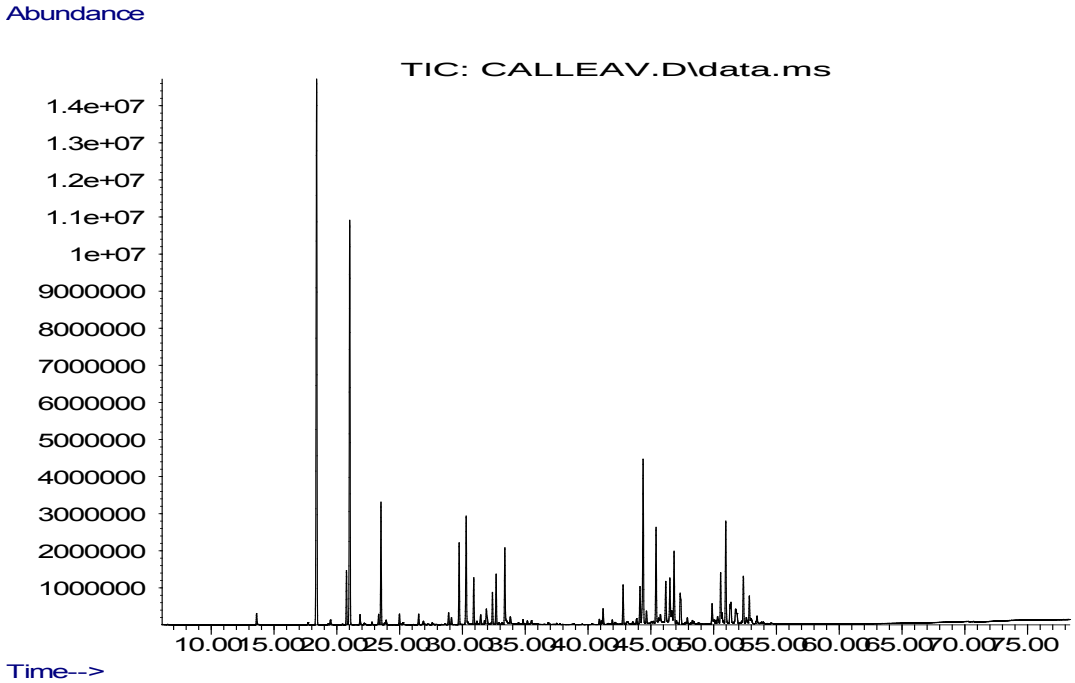


Figure 13. Total Ion Chromatogram of Calytrix exstipulata leaf oil.

Table 10. Chemical composition of *Calytrix exstipulata* leaf oil (Cal1L1).

Compound	RT (min)	Area (%)	MF	MW (g/ mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
α–Pinene	18.39	25.2	$C_{10}H_{16}$	136	94	932	5.85	954.18	A,B
Myrcene	20.77	1.9	$C_{10}H_{16}$	136	94	988	7.43	996.64	A
β-Pinene	21.03	17.9	$C_{10}H_{16}$	136	97	974	7.04	1001.23	A, B
Limonene	23.54	4.5	$C_{10}H_{16}$	136	96	1024	8.69	1051.01	A
Citronellal	29.74	3.0	$C_{10}H_{18}O$	154	96	1148	13.58	1170.19	A
neo-Isopulegol	30.3	4.6	$C_{10}H_{18}O$	154	98	1144	13.37	1180.68	A
iso-Isopulegol	30.90	1.8	$C_{10}H_{18}O$	154	98	1155	13.86	1191.77	A
n-Decanal	32.39	1.1	$C_{10}H_{20}O$	156	91	1201	15.83	1222.50	A
α-Terpineol	32.69	2.0	$C_{10}H_{18}O$	154	91	1186	15.21	1228.73	A, B
Citronellol	33.38	2.9	$C_{10}H_{20}O$	156	83	1223	16.8	1243.32	A, B
α-Gurjunene	42.79	1.6	$C_{15}H_{24}$	204	99	1409	25	1436.21	A
β-Gurjunene	44.14	1.6	$C_{15}H_{24}$	204	99	1431	25.95	1466.95	A
Valencene	44.39	7.5	$C_{15}H_{24}$	204	93	1496	28.66	1472.47	A
9-epi-(*E*)-Caryophyllene	45.42	3.9	$C_{15}H_{24}$	204	99	1464	27.33	1507.27	A
Germacrene D	46.20	1.9	$C_{15}H_{24}$	204	97	1484	28.15	1526.85	A
Viridiflorene	46.52	2.1	$C_{15}H_{24}$	204	99	1496	28.68	1534.94	A
Bicyclo-germacrene	46.85	3.2	$C_{15}H_{24}$	204	99	1500	28.83	1543.18	A
γ-Cadinene	47.34	2.0	$C_{15}H_{24}$	204	91	1374	23.49	1555.14	A
Spathulenol	50.56	1.8	$C_{15}H_{24}O$	220	99	1577	31.96	1624.67	A
Globulol	50.97	4.4	$C_{15}H_{26}O$	222	98	1590	32.5	1635.34	A, B
Unidentified	52.37	2.0	-	164	-	-	-	1671.24	-

"A" – Identification based on comparison with the GC-MS database library

"B" – Identification based on comparison with the reference standard

The color of the stem oil showed a different color than that of the leaf oil; as it appeared cloudy white unlike the color of the leaf which was green-yellowish. The major compound was globulol with a relative distribution of 17.1 % followed by the monoterpene α–pinene with a relative distribution of 7.1 %. Nineteen compounds were detected by the GC-MS method, as shown in Figure 14 and eleven compounds were identified, which are listed in Table 11.

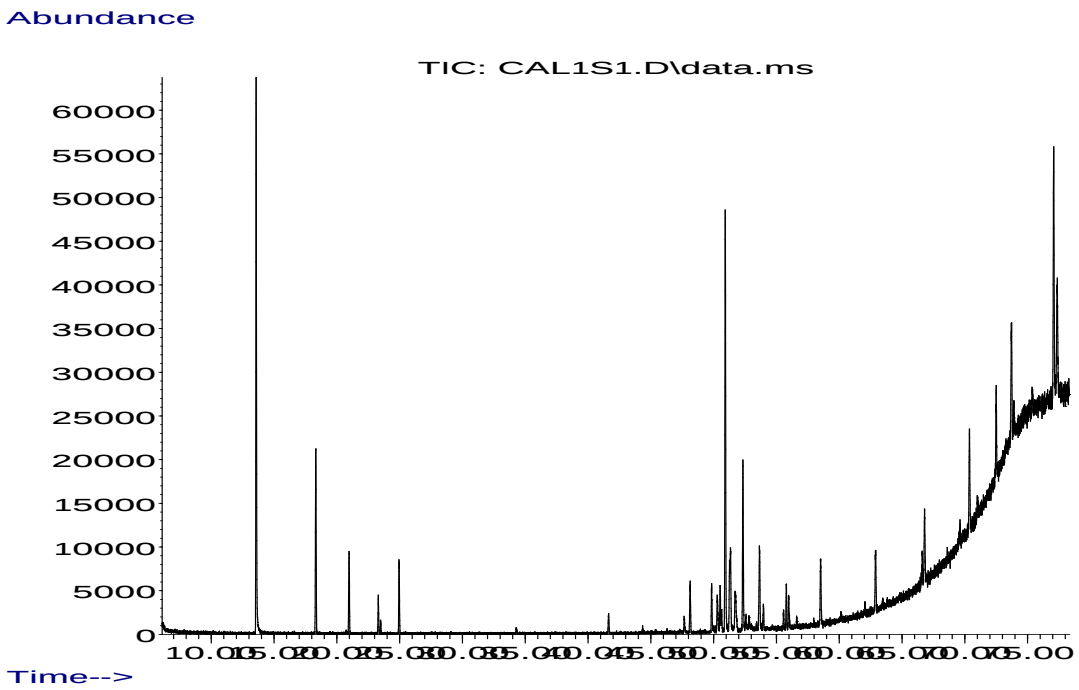


Figure 14. Total Ion Chromatogram of *Calytrix exstipulata* stem oil.

Table 11. Chemical composition of *Calytrix exstipulata* stem oil (Cal1S1).

Compound	RT (min)	Area (%)	MF	MW (g/ mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
Solvent peak	13.58	19.3	-	-	-	-	-	-	-
α–Pinene	18.33	7.1	$C_{10}H_{16}$	136	94	932	5.85	953.71	A, B
β-Pinene	20.98	3.3	$C_{10}H_{16}$	136	90	974	7.04	1001.00	A, B
ortho-Cymene	23.30	1.2	$C_{10}H_{14}$	134	86	1022	8.59	1047.73	A
Terpinolene	24.95	2.7	$C_{10}H_{16}$	136	91	10.98	1086	1078.19	A, B
Unidentified	48.14	2.2	$C_{15}H_{24}$	204	-	-	-	1575.48	-
Valencene	49.845	1.7	$C_{15}H_{24}$	204	92	1496	28.66	1607.03	A
Unidentified	50.29	1.9	$C_{15}H_{24}O$	222	-	-	-	1618.86	-
Spathulenol	50.52	1.9	$C_{15}H_{26}O$	220	93	1577	31.96	1624.84	A
Globulol	50.93	17.1	$C_{15}H_{26}O$	222	91	1590	32.5	1635.47	A, B
Cubeban-11-ol	51.27	2.3	$C_{15}H_{24}$	222	93	1595	32.7	1644.35	A
α-Guaiene	51.35	3.8	$C_{15}H_{24}$	204	91	1437	26.2	1646.26	A
Unidentified	51.71	2.2	-	-	-	-	-	1655.67	-
Unidentified	52.33	7.1	-	204	-	-	-	1671.46	-
Unidentified	53.51	4.5	-	-	-	-	-	1717.37	-
Unidentified	53.97	1.0	-	212	-	-	-	1726.17	-
Unidentified	55.79	1.8	-	212	-	-	-	1776.03	-
Unidentified	55.98	1.5	-	212	-	-	-	1781.05	-
Unidentified	58.52	3.5	-	-	-	-	-	1853.37	-
Unidentified	62.91	3.0	-	-	-	-	-	1983.45	-

"A" – Identification based on comparison with the GC-MS database library
"B" – Identification based on comparison with the reference standard

Comparison of the volatile compounds of *C. extipulata* leaf oil with those in stem oil shows that there are some differences in the relative distribution between the two essential oils, as seen in Figure 15. In the distilled stem oil the major compound was globulol with a relative distribution of 17.1 % and in comparison to the leaf oil globulol was also present but in a lower relative distribution of 4.4 %. In addition, in the leaf oil the monoterpenes α–pinene and β–pinene showed the highest percentage; both compounds were also in the stem oil, but at 25.2 % to 7.1%. Spathulenol, a sesquiterpene alcohol, was present in both essential oils at a similar percentage distribution. Other components in the stem oil were not identified, especially in the region of sesquiterpenes. In the leaf oil only one compound was not identified.

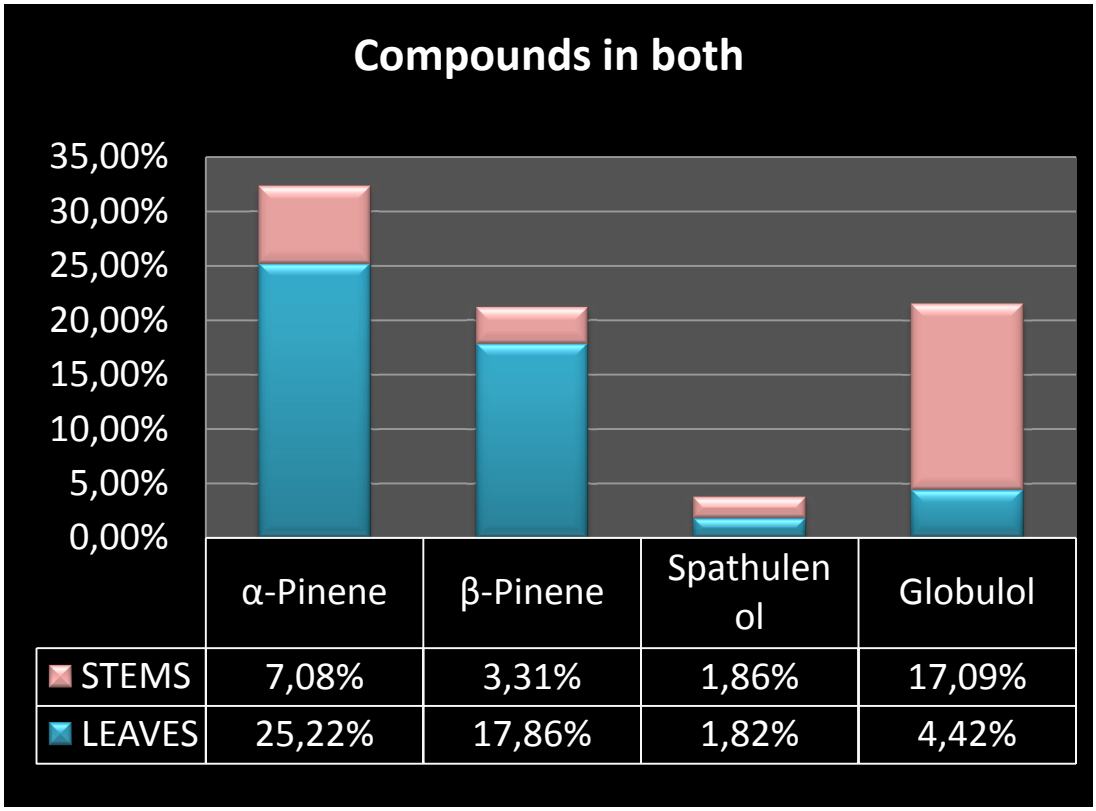


Figure 15. Comparison of the chemical profile of Cal1L1 and Cal1S1.

4.1.2 Pharmacological analysis on the whole oil of *Calytrix exstipulata*

4.1.2.1 Antioxidant activity

The *Calytrix exstipulata* leaf oil showed a low antioxidant activity of 66 ± 4 μmol TE/g compared to the positive control epicatechin with an ORAC value of 20,000 μmol TE/g. The trolox equivalent values are all listed in Figure 16. Some reference standards such as α–terpineol, citronellol and globulol were subjected to ORAC assay and gave ORAC values of 1343, 586, 574 μmol TE/g, respectively. These compounds were present at low concentrations in the oil (Table 10).

The major component in the whole oil was α–pinene, which when tested did not show any activity. While α–pinene did not show any antioxidant activity in this assay, it has been reported to act as an antioxidant [24, 25]. To the best of my knowledge, there are no literature reports regarding the chemical analyses and antioxidant activity of the essential oil from this species. α–Terpineol has already been reported to have antioxidant activity [26]. Furthermore it has been reported that terpenes such as α–terpineol, α–pinene and globulol were found to act as antioxidants [25]. Citronellol was also found to have scavenging activity as an antioxidant [23, 32].

Assigning the activity of a complex mixture to a single or particular constituent can be difficult because, major or minor compounds might give rise to the biological activities exhibited. In comparison to the leaf oil, the stem oil did not show any antioxidant activity. The major component of the essential oil was globulol, but it seems that the percentage of its distribution alone (17.1 %) was not enough to show a positive ORAC score in the whole oil of the stem distillation.

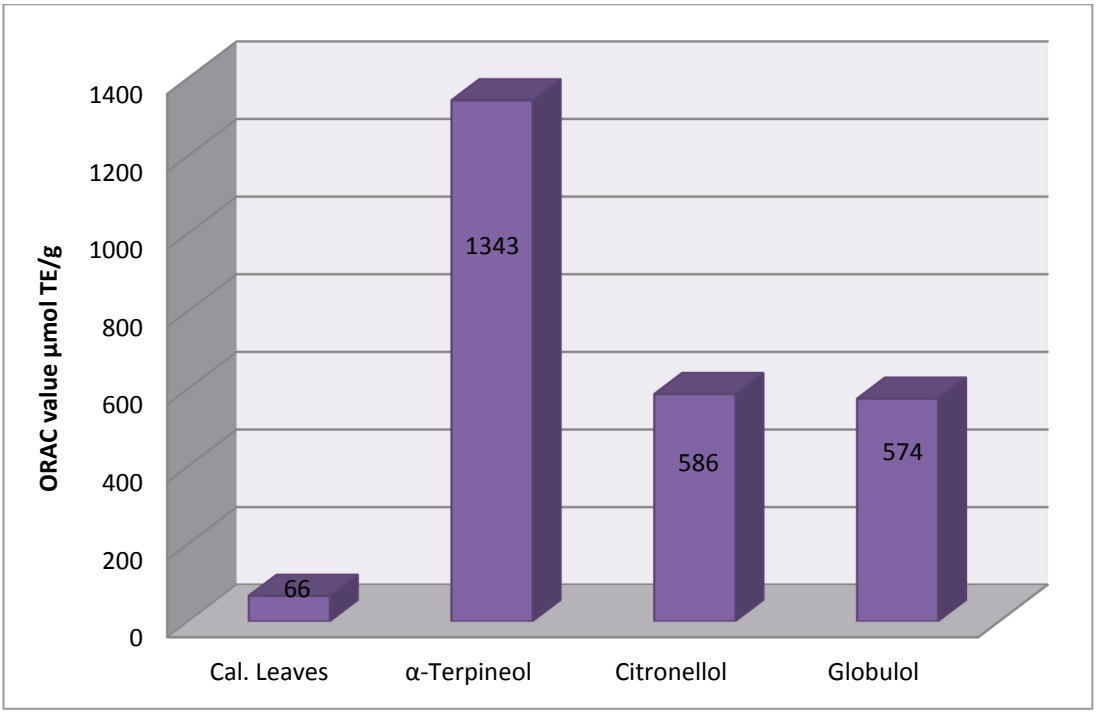


Figure 16. ORAC values (µmol TE/g) of *Calytrix exstipulata* leaf oil (Cal1L1) and some reference standards (α–terpineol, citronellol and globulol).

4.1.2.2 Cytotoxicity study

The cytotoxic activity of the leaf oil on all cell lines is illustrated in Figure 17. The leaf oil showed the highest inhibition against 3T3 cells, followed by P388 cells and HS 27 cells. Detailed cytotoxic results are shown in Appendix 1. High cytotoxic activity of the whole might be due to the presence of the monoterpenes β–pinene, citronellol and other compounds, as shown in Figure 17, since they are relevant components in the whole oil. α–Pinene and β–pinene were the most abundant components in the leaf oil and reference compounds showed high cytotoxicity. In previous studies pinene has been reported to be cytotoxic against HEP G2 cells [24]. In another study pinene and citronellol were described to have antitumor properties [9].

The leaf oil was found to be active against all cell lines. The reference standards (α–pinene, β–pinene, citronellol α-terpineol and globulol) were also active against P388 and 3T3 cells. Activity of the reference standards were also observed against HS27 cells, except for α-terpineol and globulol, which did show any results.

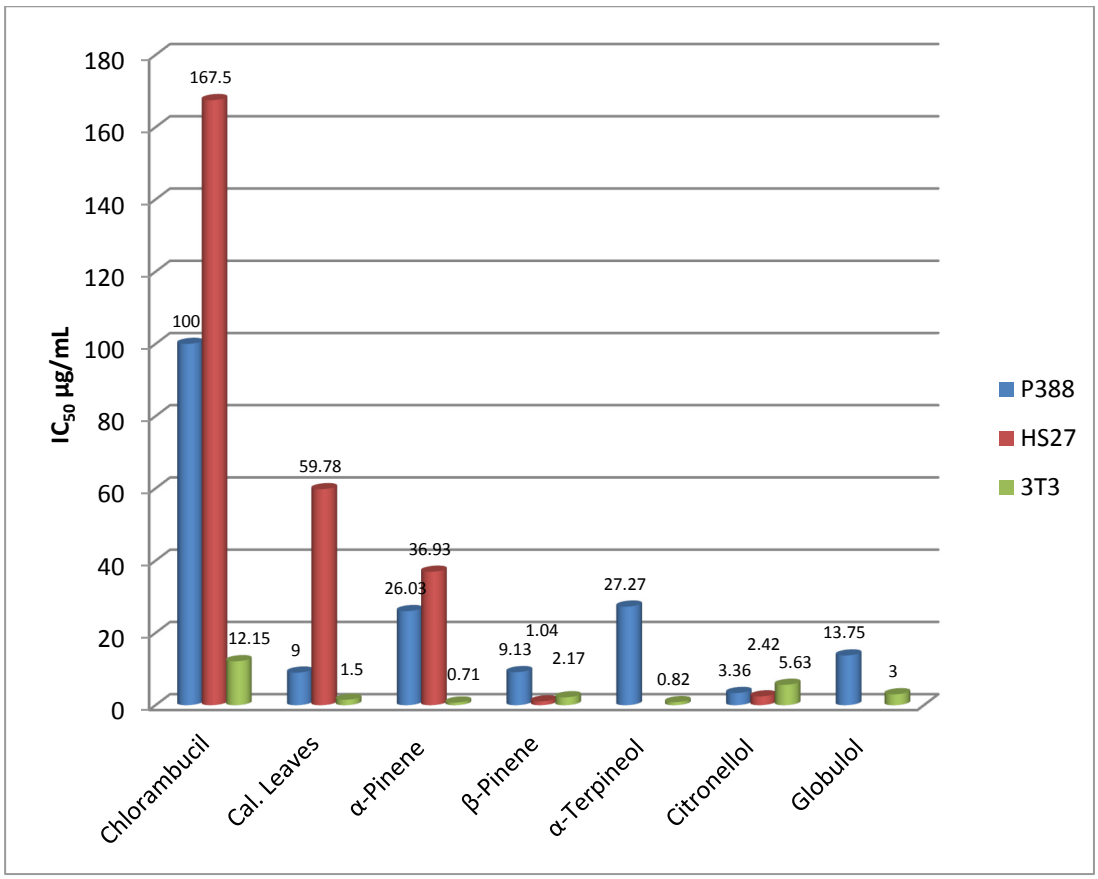


Figure 17. Cytotoxicity results (µg/mL) of *Calytrix exstipulata* leaf oil and some reference. standards (α–pinene, β-pinene, α–terpineol, citronellol, and globulol).

The stem oil showed the highest cytotoxic activity on 3T3 cells followed by the P 388 cells (Figure 18). On HS27 cells no toxicity was observed. The cytotoxic activity generally could be due to the compounds α–pinene, β–pinene and globulol, since they were tested to be cytotoxic.

The stem oil was more active against the 3T3 cells than the leaf oil whereas, the leaf oil showed on the P388 cell higher cytotoxic results than the stem oil (Figure 19). They both contained cytotoxic compounds as α–pinene, β–pinene and globulol. Furthermore the stem oil contained many unidentified compounds, which might be more cytotoxic on 3T3 cells.

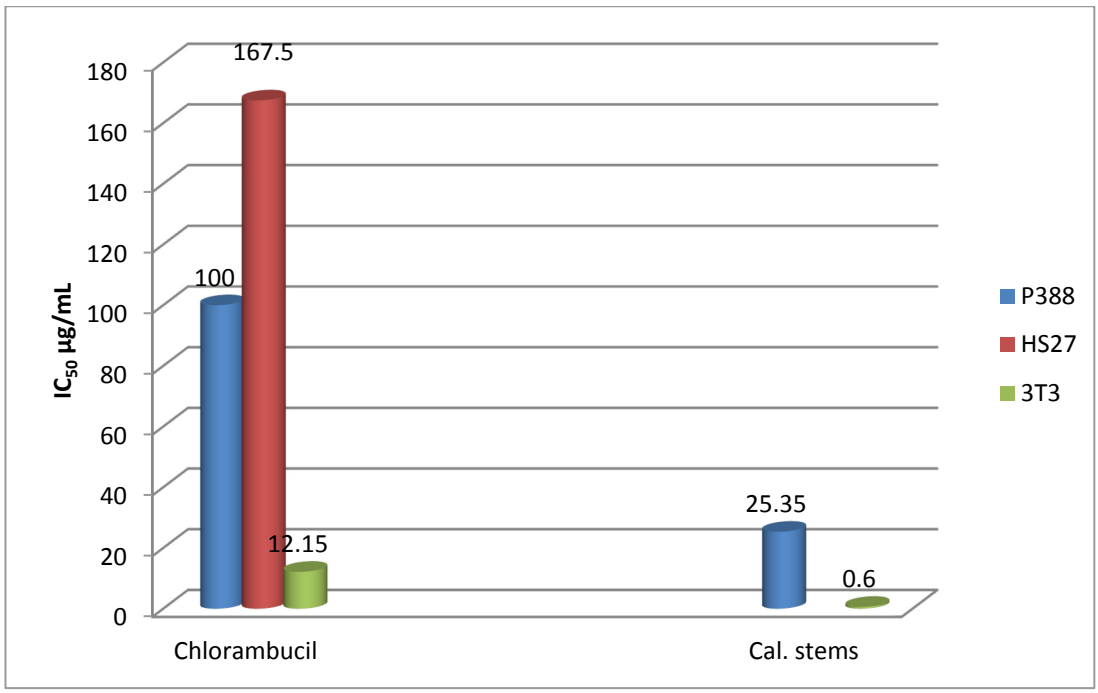


Figure 18. Cytotoxicity results (µg/mL) of *Calytrix exstipulata* stem oil.

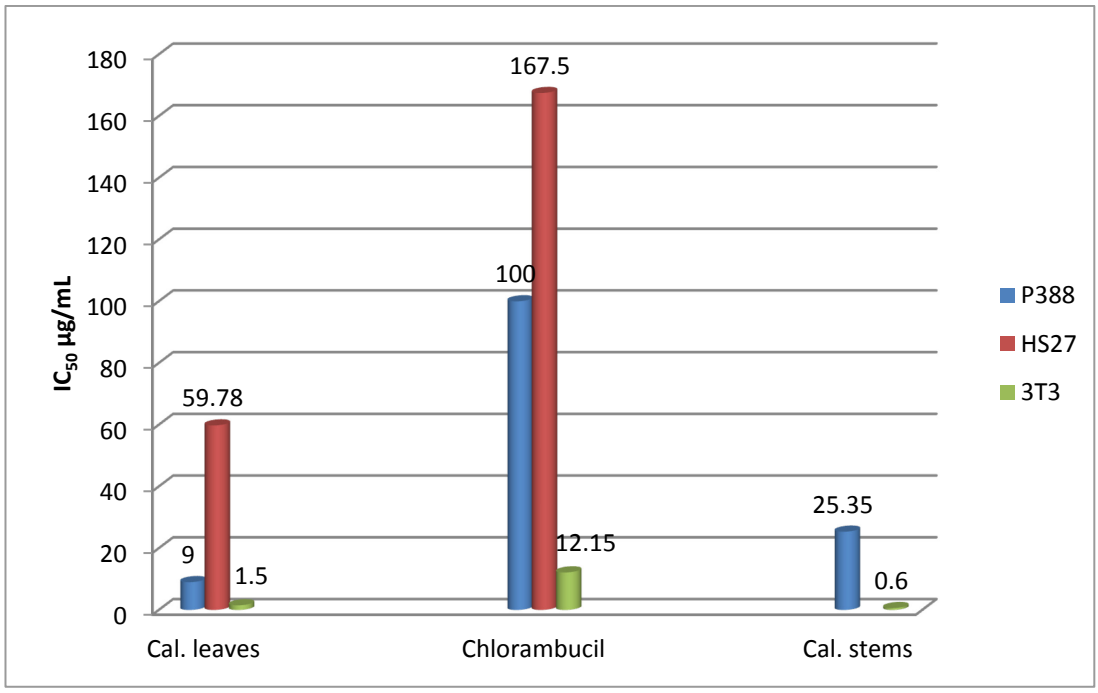


Figure 19. Comparison of IC_{50} values (µg/mL) of Cal1L1 and Cal1S1

4.2 Fractionation of *Calytrix exstipulata* leaf oil (Cal1L1)

In the 1st fractionation of the whole oil 9 fractions were obtained and in the 2nd fractionation of fraction E 24 subfractions were collected.

The fractionation of the leaf oil is shown in Figure 20 and the yields obtained are shown in Table 12.

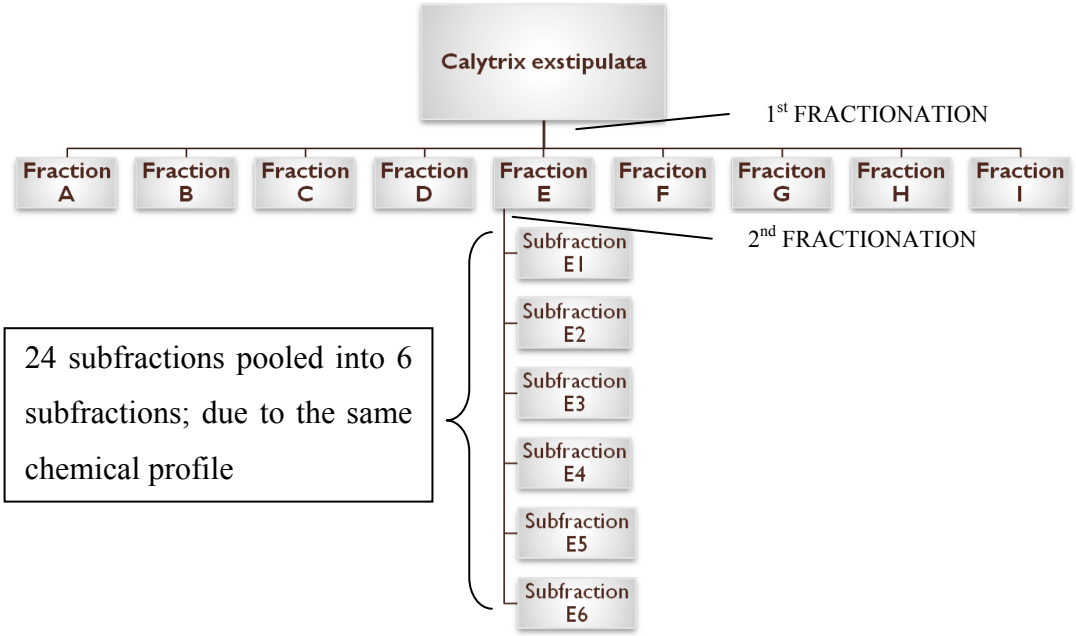


Figure 20. Fractionation scheme for the isolation of compounds from *Calytrix exstipulata* leaf oil.

Table 12. Column fractionation strategy, solvents and yields of *Calytrix exstipulata* leaf oil (Cal1L1).

Fraction	Solvent	Volume (mL)	Yield (mg)	Yield (%)
A	100% Hexane	500	860.4	8.6
B	90% Hexane – 10% Diethylether	500	268	2.68
C	80% Hexane – 20% Diethylether	300	21	0.21
D	80% Hexane – 20% Diethylether & 50% Hexane – 50% Diethylether	200 + 300	529	5.29
E	50% Hexane – 50% Diethylether	300	641	6.41
F	100% Diethylether	500	100	1
G	50% Diethylether – 50% Ethylacetate	500	39	0.39
H	100% Ethylacetate	500	20	0.20
I	50% Ethylacetate – 50% Methanol	500	29	0.29

4.2.1 Phytochemical analysis on fractions obtained from the whole oil of Cal1L1

The essential oil of fraction A, which was colorless, consisted of sesquiterpenes, with valencene as the most abundant compound (29.9 %), followed by 9-epi-(*E*)-caryophyllene (12.2 %), γ-cadinene (7.0 %) and some others, as shown in Table 13. The chemical profile of the whole oil is shown in Figure 21. The sesquiterpenes β-gurjunene, valencene, 9-epi-(*E*)-caryophyllene, viridiflorene and gamma- cadinene were also detected in the whole leaf oil at the same retention time.

The green-yellowish fraction B oil like the fraction A oil was rich in sesquiterpenes; but the major compound with a relative distribution of 11.7 % could not be identified. The second highest compound with a relative distribution of 9.5 % was bicyclogermacrene, followed by germacrene D and other sesquiterpenes, which are all listed in Table 14 and the chromatogram is shown in Figure 22. Valencene, germacrene D, bicyclogermacrene and spathulenol were also detected in the whole leaf oil.

Table 13. Chemical composition of Fraction A.

Compound	RT (min)	Area (%)	MF	MW (g/ mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
Solvent peak	6.2	19.3	-	-	-	-	-	-	-
α-Copaene	41.2	1.5	$C_{15}H_{24}$	204	98	1374	23.49	1398.63	A
α-Gurjunene	42.8	2.4	$C_{15}H_{24}$	204	99	1409	25	1436.23	A
β-Gurjunene	44.15	5.3	$C_{15}H_{24}$	204	99	1409	25	1467.04	A
Valencene	44.43	29.9	$C_{15}H_{24}$	204	91	1496	28.66	1473.36	A
cis-Eudesma-6,11-diene	44.66	1.5	$C_{15}H_{24}$	204	99	1489	28.36	1478.55	A
9-epi-(E)-Caryophyllene	45.43	12.2	$C_{15}H_{24}$	204	99	1464	27.33	1507.67	A
γ-Muuorolene	45.62	1.2	$C_{15}H_{24}$	204	99	1478	27.91	1512.44	A
γ-Gurjunene	45.77	1.1	$C_{15}H_{24}$	204	99	1475	27.8	1516.15	A, B
Virdiflorene	46.53	6.2	$C_{15}H_{24}$	204	99	1496	28.68	1535.12	A
β-Selinene	46.7	2.4	$C_{15}H_{24}$	204	99	1489	28.37	1539.50	A
γ-Cadinene	47.41	7.0	$C_{15}H_{24}$	204	98	1513	29.35	1556.72	A
Unidentified	55.08	1.2	-	209	-	-	-	-	-
Unidentified	59.78	1.4	-	218	-	-	-	-	-

"A" – Identification based on comparison with the GC-MS database library
"B" – Identification based on comparison with the reference standard

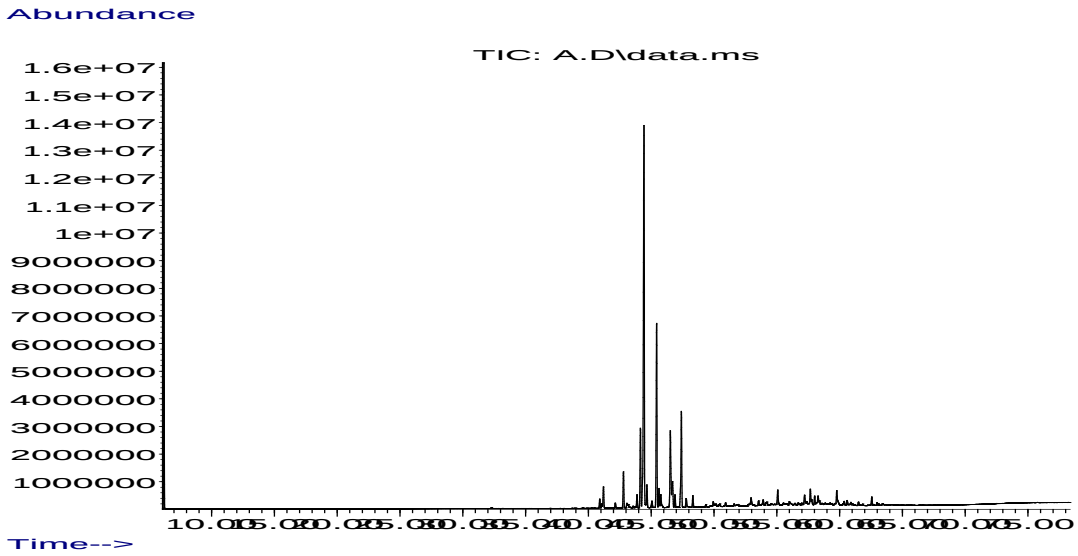


Figure 21. Total Ion Chromatogram of Fraction F of *Calytrix exstipulata* leaf oil.

Table 14. Chemical composition of Fraction B.

Compound	RT (min)	Area (%)	MF	MW (g/ mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
Solvent peak	6.20	33.9	-	-	-	-	-	-	-
Unidentified	41.93	2.1	-	204	-	-	-	1416.00	-
Valencene	44.38	2.5	$C_{15}H_{24}$	204	99	1439	26.27	1472.24	A
α-Humulene	45.20	1.2	$C_{15}H_{24}$	204	93	1452	26.82	1501.61	A
Germacrene D	46.20	6.7	$C_{15}H_{24}$	204	97	1484	28.15	1526.85	A
Viridiflorene	46.52	4.0	$C_{15}H_{24}$	204	99	1496	28.68	1534.82	A
Unidentified	46.70	2.4	-	204	-	-	-	1539.30	-
Bicyclo-germacrene	46.85	9.5	$C_{15}H_{24}$	204	99	1500	28.83	1543.08	A
α-Copaene	47.39	4.7	$C_{15}H_{24}$	204	95	1374	23.49	1556.36	A
trans-Calamenene	47.79	2.4	$C_{15}H_{22}$	202	97	1521	29.69	1565.91	A
α-Cadinene	48.32	1.2	$C_{15}H_{24}$	204	99	1537	30.33	1578.65	A
Citronellyl pentanoate	48.50	1.6	$C_{15}H_{28}O_2$	240	91	1624	33.82	1583.11	A
β-Calacorene	48.79	2.6	$C_{15}H_{20}$	200	94	1564	31.43	1589.94	A
Spathulenol	50.56	1.8	$C_{15}H_{24}O$	220	99	1577	31.96	1624.54	A
Unidentified	50.68	11.7	-	220	90	-	-	1627.79	-
1-epi-Cubenol	51.86	6.6	$C_{15}H_{26}O$	222	93	1439	26.27	1658.26	A
Unidentified	52.62	1.2	-	220	-	-	-	1677.50	-
epi-α-Cadinol	52.93	0.9	$C_{15}H_{26}O$	222	83	1374	23.49	1685.25	A
Unidentified	58.06	1.0	-	172	-	-	-	1627.69	-
Unidentified	62.09	2.3	-	190	-	-	-	1646.39	-

“A” – Identification based on comparison with the GC-MS database library
“B” – Identification based on comparison with the reference standard

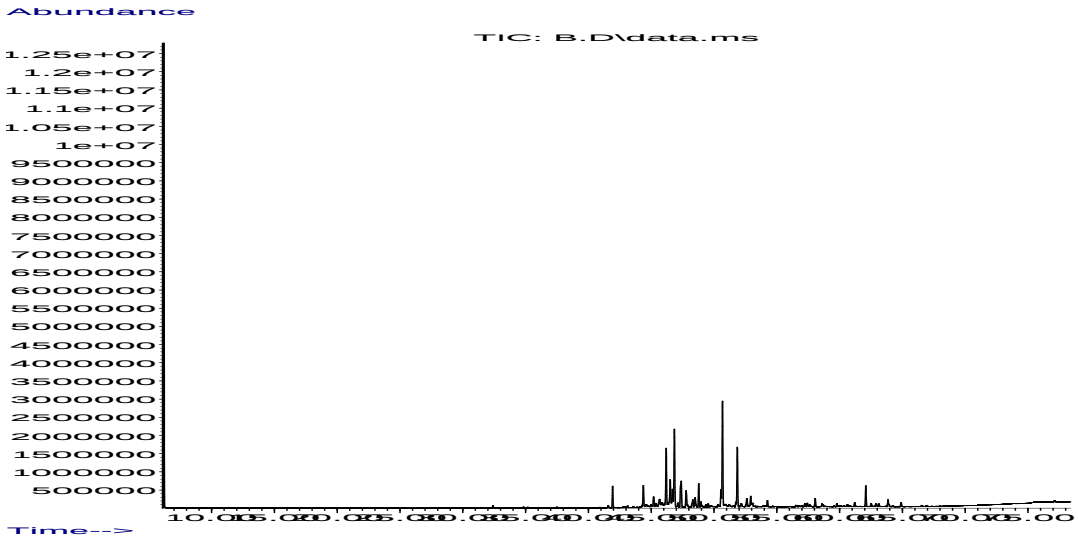


Figure 22. Total Ion Chromatogram of Fraction B of *Calytrix exstipulata* leaf oil.

In the yellow colored fraction C, eighteen peaks were detected by the GC-MS method (Figure 23) and fourteen compounds were identified by the database library (Table 15). This fraction like the first two fractions A and B, was characterized to contain only sesquiterpenes. The major compound of fraction C was palustrol with a relative distribution of 25.7 %, followed by 1-epi-cubenol with a relative distribution of 11.3 % and some other sesquiterpenes.

Of the compounds identified in this fraction, only spathulenol and globulol were detectable in the whole oil.

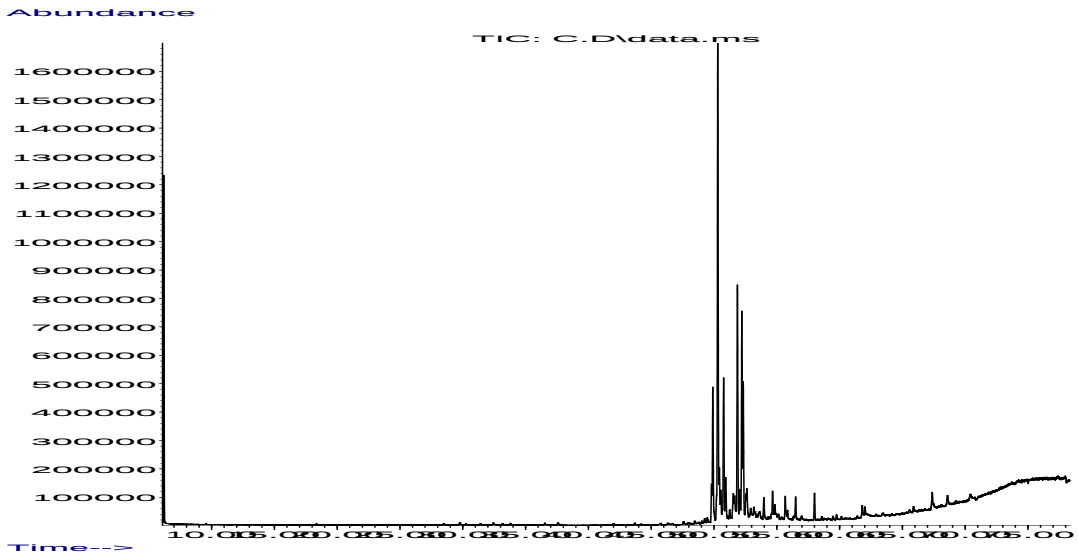


Figure 23. Total Ion Chromatogram of Fraction C of *Calytrix exstipulata* leaf oil.

Table 15. Chemical composition of Fraction C.

Compound	RT (min)	Area (%)	MF	MW (g/ mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
Solvent peak	6.20	10.3	-	-	-	-	-	-	-
Unidentified	49.82	1.7	-	163	-	-	-	-	-
α-Guaiene	49.92	7.6	$C_{15}H_{24}$	204	80	1437	26.2	1607.86	A
Palustrol	50.32	25.7	$C_{15}H_{26}O$	204	99	1567	31.56	1618.27	A
Gleenol	50.44	2.6	$C_{15}H_{26}O$	222	98	1586	32.33	1621.61	A
Spathulenol	50.56	1.6	$C_{15}H_{24}O$	220	97	1577	31.96	1624.54	A
Unidentified	50.79	7.9	-	204	-	-	-	1630.53	-
Globulol	50.95	2.5	$C_{15}H_{26}O$	222	99	1475	27.8	1634.69	A, B
Unidentified	51.56	1.4	-	220	-	-	-	1650.50	-
1-epi-Cubenol	51.88	11.3	$C_{15}H_{26}O$	222	93	1618	33.6	1658.80	A
Unidentified	52.07	1.2	-	220	-	-	-	1663.51	-
Muurola-4,10(14)-dien-1-β-ol	52.24	9.9	$C_{15}H_{24}O$	220	94	1630	34.06	1667.80	A
Unidentified	52.35	6.5	$C_{15}H_{26}O$	222	-	-	-	1670.59	-
Germacrene B	52.55	1.1	$C_{15}H_{24}$	204	86	1559	31.24	1675.69	A
Junenol	52.64	1.7	$C_{15}H_{26}O$	222	99	1618	33.61	1677.93	A
Unidentified	53.99	1.2	-	220	-	-	-	1725.45	-
2*E*, 6*Z*-Farnesol	54.68	1.4	$C_{15}H_{26}O$	220	92	1714	37.27	1744.65	A
Zierone	56.52	1.3	$C_{15}H_{22}O$	218	83	1574	31.86	1794.10	A
Squamulosone	58.02	1.4	$C_{15}H_{22}O$	218	94	1770	39.31	1837.59	A

"A" – Identification based on comparison with the GC-MS database library

"B" – Identification based on comparison with the reference standard

Fraction D was found to contain fourteen compounds (Figure 24), of which were twelve identified as monoterpenes and sesquiterpenes (Table 16). The major component with a relative distribution of 22.7 % was not identifiable, followed by other components spathulenol, epi-α-cadinol and α–guaiene with a relative distribution of 17.8 %, 11.1 % and 9.0%, respectively. The color of the essential oil fraction was yellow. Three compounds present in fraction D were detectable in the whole oil, namely iso-isopulegol, spathulenol and globulol.

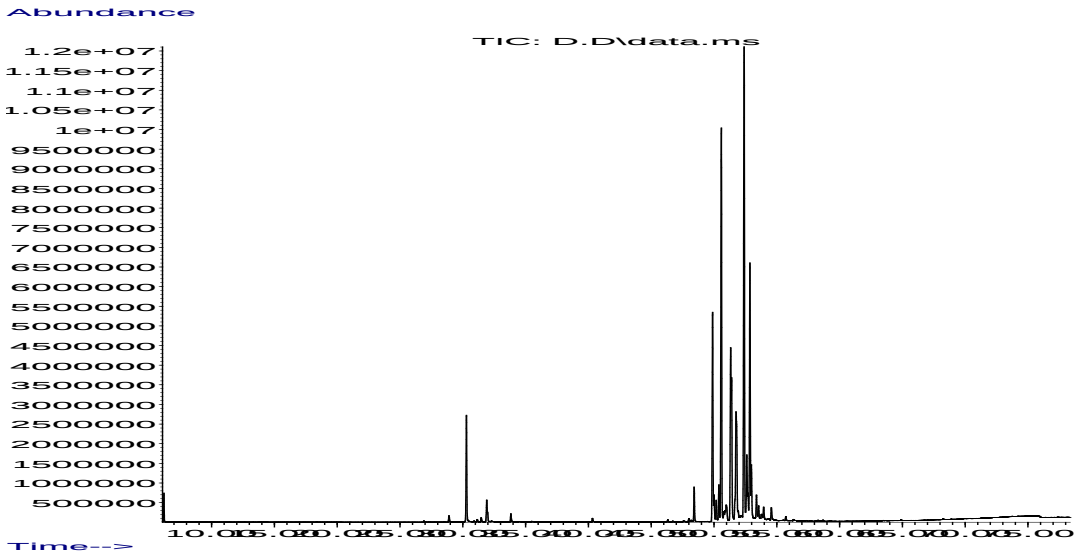


Figure 24. Total Ion Chromatogram of Fraction D of *Calytrix exstipulata* leaf oil.

Table 16. Chemical composition of Fraction D.

Compound	RT (min)	Area (%)	MF	MW (g/ mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
iso-Isopulegol	30.29	4.3	$C_{10}H_{18}O$	154	80	1155	13.86	1180.51	A
Borneol	31.91	1.3	$C_{10}H_{18}O$	154	99	1165	14.29	1212.03	A, B
E-Nerolidol	48.42	1.3	$C_{15}H_{26}O$	222	98	1564	29.93	1581.22	A
α-Guaiene	49.89	9.0	$C_{15}H_{24}$	204	97	1475	27.8	1606.98	A
Unidentified	50.01	1.1	-	222	-	-	-	1610.11	-
Maaliol	50.39	1.4	$C_{15}H_{26}O$	222	99	1566	31.53	1620.29	A
Spathulenol	50.59	17.8	$C_{15}H_{24}O$	222	60	1577	31.96	1625.35	A
Cubeban-11-ol	51.33	8.0	$C_{15}H_{26}O$	222	93	1595	32.7	1644.69	A
Globulol	51.41	5.5	$C_{15}H_{26}O$	222	98	1590	32.5	1646.59	A, B
Rosifoliol	51.76	7.8	$C_{15}H_{26}O$	222	94	1600	32.89	1655.71	A
Unidentified	52.40	22.7	-	164	99	-	-	1672.05	-
Cyperene	52.61	3.0	$C_{15}H_{24}$	204	86	1398	24.54	1677.37	A
epi-α-Cadinol	52.86	11.1	$C_{15}H_{26}O$	222	99	1638	34.38	1683.63	A
epi-α-Murrolol	52.98	2.3	$C_{15}H_{26}O$	222	95	1640	34.46	1686.55	A

"A" – Identification based on comparison with the GC-MS database library

"B" – Identification based on comparison with the reference standard

Like fraction D and the leaf oil, the yellow fraction E oil was characterized by monoterpenes and sesquiterpene alcohols. The major component was globulol (49.5 %), a sesquiterpene. Other components present in appreciable contents were the monoterpenes citronellol (16.2 %) and α–terpineol (8.5 %). The results are given and Table 17 and represented in Figure 25. Two monoterpenes in fraction D, α–terpineol and citronellol, and one sesquiterpene, globulol, were detectable in the whole oil as well as in fraction D.

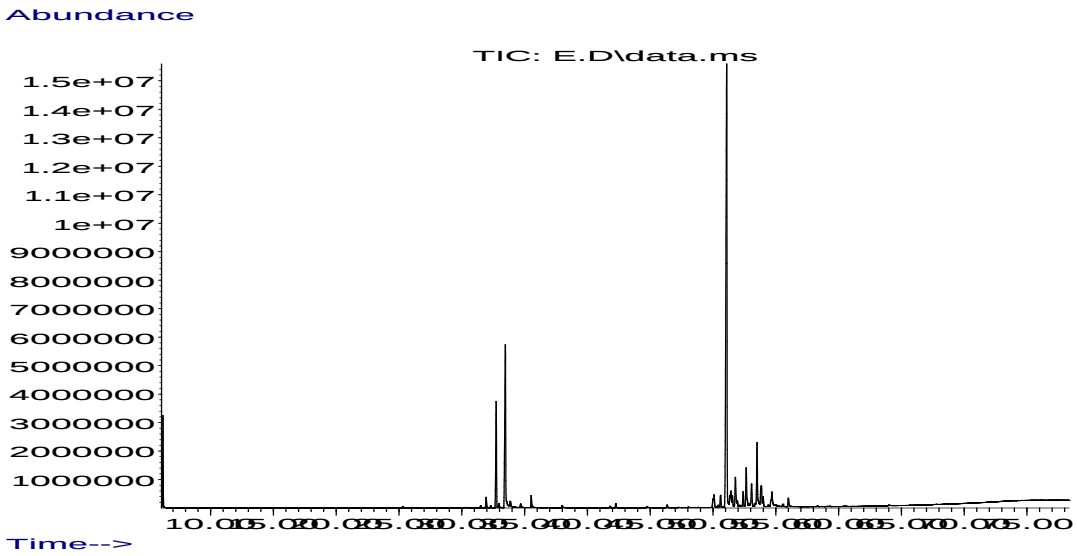


Figure 25. Total Ion Chromatogram of Fraction E of *Calytrix exstipulata* leaf oil.

Table 17. Chemical composition of Fraction E.

Compound	**RT (min)**	**Area (%)**	**MF**	**MW (g/ mol)**	**Match (%)**	**Adams Index (AI)**	**RT-Adams**	**Kovats Index (KI)**	**Identification**
Solvent peak	6.21	4.5	-	-	-	-	-	-	-
α-Terpineol	32.73	8.5	$C_{10}H_{18}O$	154	90	1186	15.21	1229.71	A, B
Citronellol	33.46	16.2	$C_{10}H_{20}O$	156	97	1223	16.8	1245.05	A, B
Globulol	51.08	49.9	$C_{15}H_{26}O$	222	98	1590	32.5	1638.17	A, B
Rosifoliol	51.77	2.4	$C_{15}H_{26}O$	222	90	1600	32.89	1656.04	A
Unidentified	52.39	1.2	-	164	-	-	-	1671.78	-
Cyperene	52.63	3.7	$C_{15}H_{24}$	204	90	1398	24.54	1677.85	A
α-Muurolol	53.06	2.2	$C_{15}H_{26}O$	222	94	1644	34.61	1688.58	A
α-Cadinol	53.50	5.7	$C_{15}H_{26}O$	222	99	1652	34.93	1711.73	A
α-Eudesmol	53.84	2.2	$C_{15}H_{26}O$	222	99	1652	34.91	1721.39	A
2*Z*, 6*E*-Farnesol	54.68	1.8	$C_{15}H_{26}O$	222	93	1714	37.27	1744.51	A

"A" – Identification based on comparison with the GC-MS database library

"B" – Identification based on comparison with the reference standard

In the yellow oil of fraction F only two sesquiterpenes were identified, as shown in Table 18 and Figure 26. The major component was globulol with a relative distribution of 10.5 %, followed by cuparene with a relative distribution of 2.2 %.

Fraction G, H and I did not exhibit any peaks in the chromatographic profile.

Table 18. Chemical composition of Fraction F.

Compound	**RT (min)**	**Area (%)**	**MF**	**MW (g/ mol)**	**Match (%)**	**Adams Index (AI)**	**RT-Adams**	**Kovats Index (KI)**	**Identification**
Solvent peak	6.20	70.0	-	-	-	-	-	-	-
Globulol	50.96	10.5	C15H26O	222	98	1590	32.5	1635.18	A, B
Cuparene	51.51	2.2	C15H22	202	78	1504	29	1649.22	A
Unidentified	53.63	1.7	-	212	90	-	-	1715.62	A
Unidentified	54.00	2.9	-	212	90	-	-	1725.87	A
Unidentified	55.61	1.8	-	212	-	-	-	1769.79	-
Unidentified	55.83	4.7	-	212	90	-	-	1775.63	A
Unidentified	56.01	4.1	-	212	-	-	-	1780.54	-

"A" – Identification based on comparison with the GC-MS database library

"B" – Identification based on comparison with the reference standard

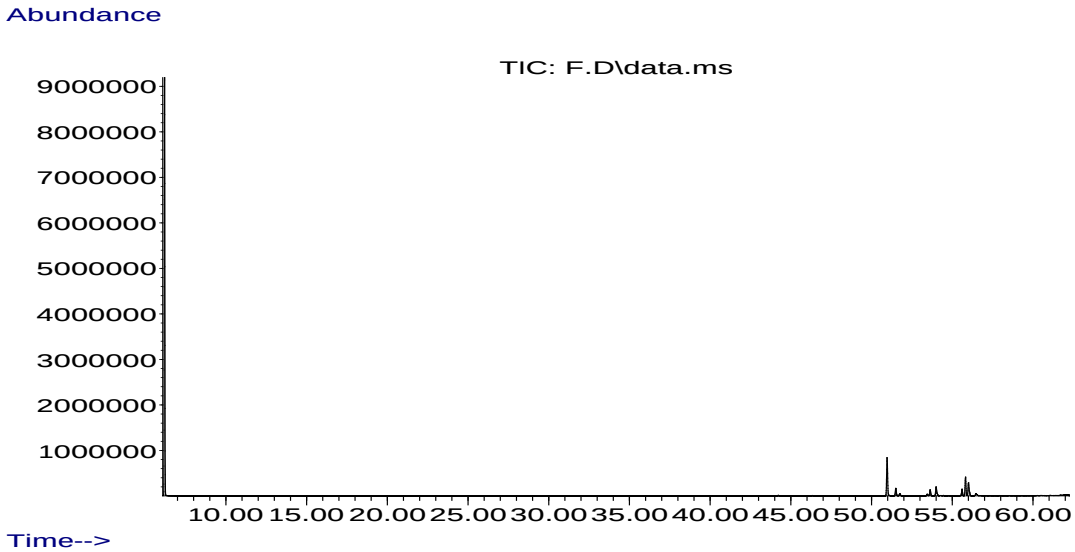


Figure 26. Total Ion Chromatogram of Fraction F of *Calytrix exstipulata* leaf oil.

4.2.2 Pharmacological analysis on fractions obtained from the whole oil of Cal1L1

4.2.2.1 Antioxidant activity

All fractions obtained from the leaf oil were tested for antioxidant activity (Figure 27). Fraction F showed the highest antioxidant activity (ORAC value: 865 ± 124 µmol TE/g) followed by fraction E (ORAC value: 756 ± 24 µmol TE/g) and fraction G (ORAC value: 416 ± 93 µmol TE/g). Fraction D (ORAC value: 209 ± 30 µmol TE/g) and fraction C (ORAC value: 59 ± 32µmol TE/g) showed lower antioxidant activity. Fractions A, B, H and I did not show any response in the antioxidant assay. Interestingly, fractions D, E, F, and G showed even higher ORAC values than the activity of the whole oil. The high activity in fraction E could probably be due to the high amounts of α-terpineol (8.5 %), globulol (49.9 %) and citronellol (36.4 %), which showed high antioxidant activity. Previous work reported these compounds to have antioxidant activities [25, 32].

It was not obvious why fraction F was the most active fraction. In this fraction two compounds; globulol and cuparene were identified. Although globulol alone showed antioxidant activity (574 µmol TE/g), it was not as high as the observed antioxidant activity of fraction F.
The presence of the compound cuparene, although only present in this fraction E (2.8 %) however, could have contributed to the antioxidant activity of the fraction. Due to the low match with the database library its identification is doubtful. Some compounds could not be identified and maybe these compounds are highly active and could be an explanation. However there was not enough time for another fractionation, which could explain fraction F better.

In fraction G, no peaks could be detected by the GC-MS method and so no compounds were identified; due to this fact, its antioxidant activity cannot be explained.

In fraction D, the major compound with a percent distribution of 22.7 % could not be identified, which might be responsible for its activity. Other compounds were spathulenol, α-guaiene, cubeban-11-ol and rosifoliol. Rosifoliol was described to be antioxidant in previous studies [41].

In fraction C, the major compounds were palustrol (25.7 %), muurola-4,10(14)-dien-1-β-ol, α-guaiene, 1-epicubenol and the minor ones were globulol and spathulenol. As mentioned before, globulol has antioxidant activity and a reference standard was tested in this study. It has also been reported to be antioxidant in former studies [25].

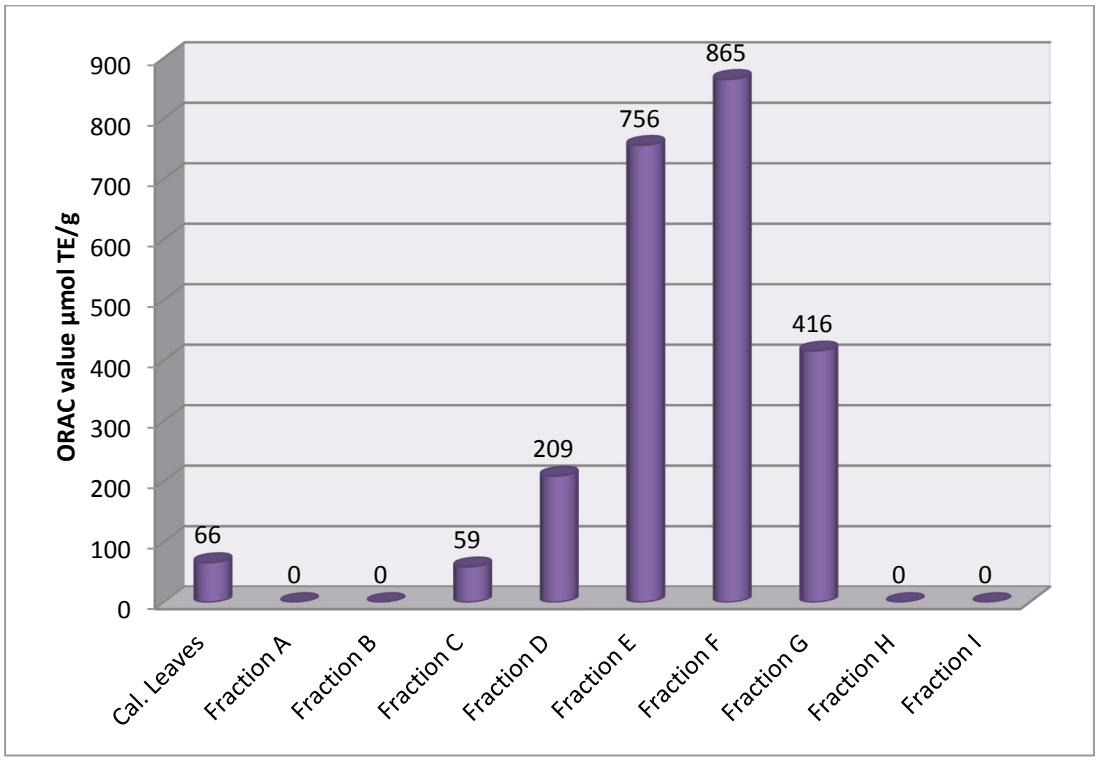


Figure 27. ORAC values (µmol TE/g) of all fractions obtained from Cal1L1.

4.2.2.2 Cytotoxicity study

The fractions were tested against these three different cell lines. The IC_{50} values for P388 cells are presented in Figure 28; the values for HS27 cells are shown in Figure 29 and the values for 3T3 cells are shown in Figure 30. In general, the first six fractions A to F had all cytotoxic properties against these three cell lines, as did the whole oil. Interestingly fraction D was found to be the most cytotoxic against the HS 27 and 3T3 cells with IC_{50} 0.003 and 2.05 µgl/mL, respectively. The major compound in this fraction could not be identified and therefore its cytotoxic activity cannot be explained. Spathulenol which was the second major compound has already been reported as cytotoxic [42].

Fraction C was the most cytotoxic oil on P388 cells, with IC_{50} 1.62 µg/mL, and also showed high cell inhibition against HS 27 cell and against 3T3 cells. Fraction I

showed high cell inhibition against 3T3 cell lines, but since no peaks were detected on the GC-MS profile, the activity could not be explained. Fractions G, H and I did show exhibit cytotoxic activity on the three cell lines.

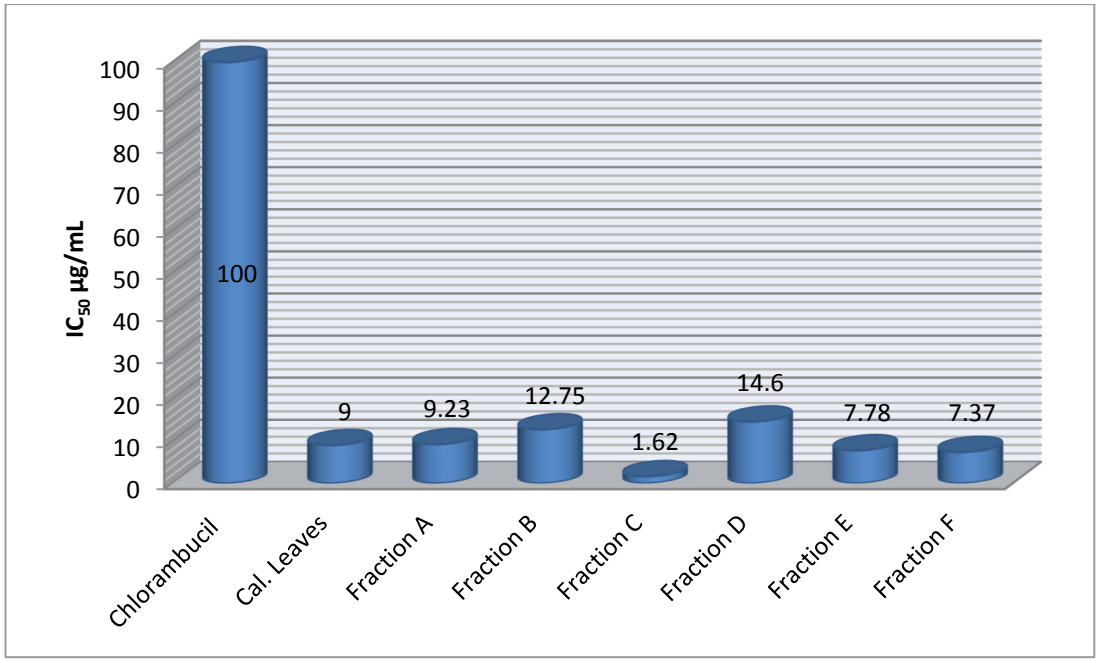


Figure 28. Cytotoxicity results on P388 cells (µg/mL) of all fractions obtained from Cal1L1.

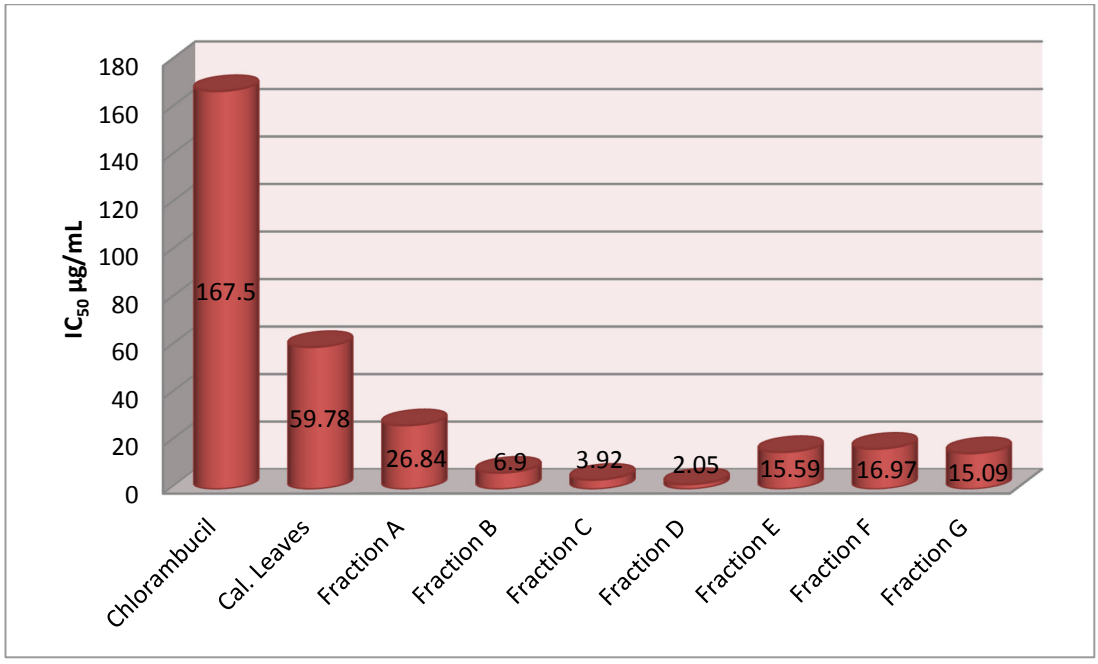


Figure 29. Cytotoxicity results on HS27 cells (µg/mL) of all fractions obtained from Cal1L1.

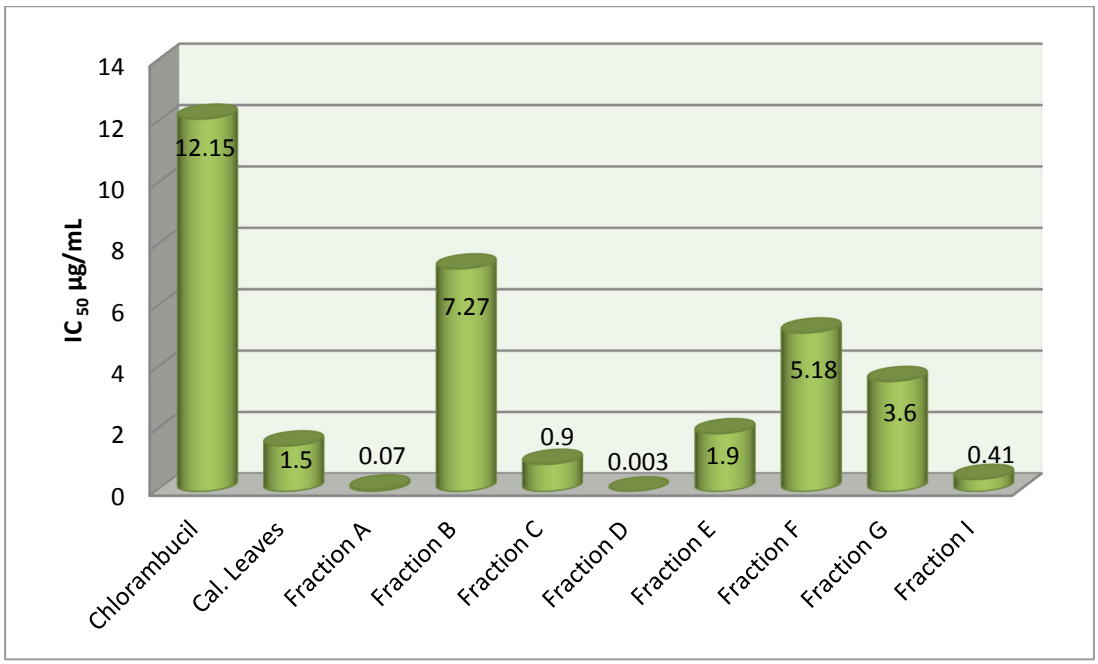


Figure 30. Cytotoxicity results on 3T3 cells (µg/mL) of all fractions obtained from Cal1L1.

4.3 Further fractionation of Fraction E

Fraction E had the highest yield and was further fractionated. The initial fractionation was done by column chromatography, as described in the Materials and Methods section. The solvents hexane and diethylether, which were used at the first fractionation to obtain fraction E, achieved a separation of components and 24 subfractions were collected. After GC-MS analysis some of fractions showed similar profiles and so they were combined, resulting in six subfractions, after pooling. The detailed fractionation scheme is illustrated in Figure 31.

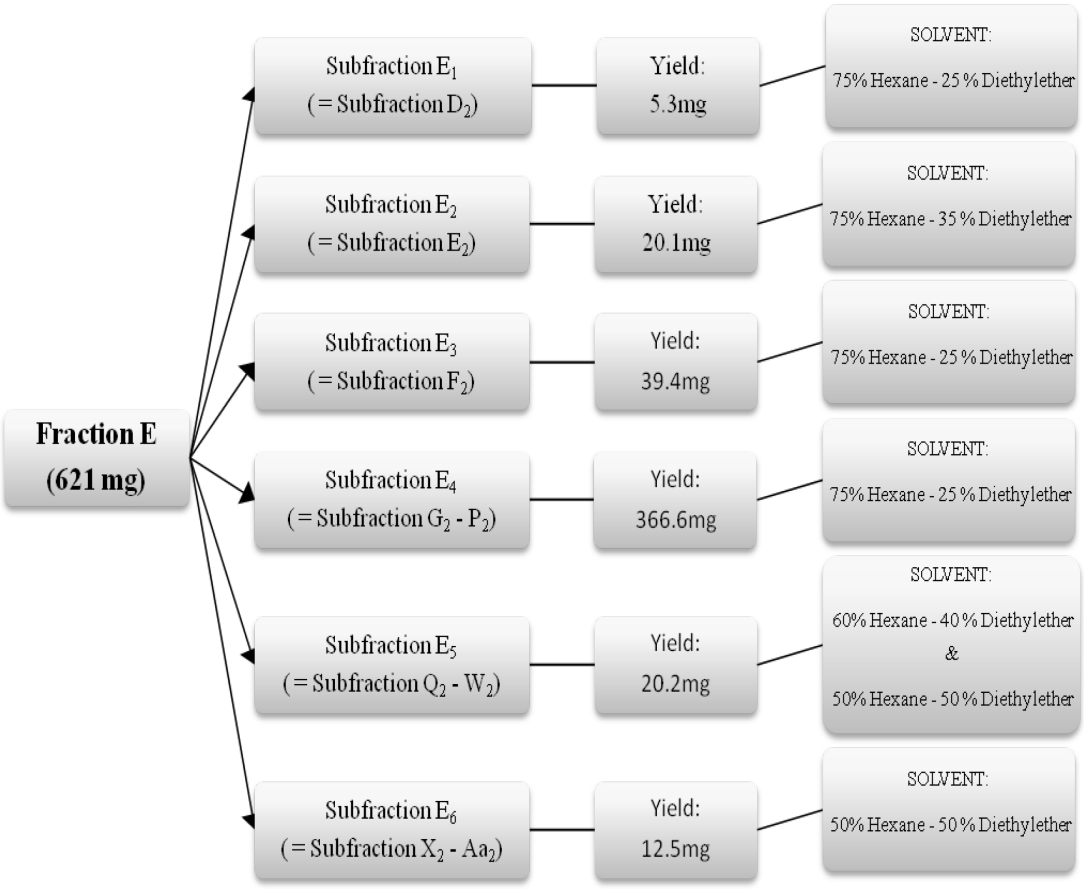


Figure 31. Fractionation scheme for the fractionation of Fraction E.

4.3.1 Phytochemical analysis on subfractions obtained from fraction E

Subfraction E_1 was found to be rich only in sesquiterpenes, representing spathulenol, an unidentified compound and viridiflorol with a relative distribution of 28.9%, 26.1 % and 10.5 %, respectively. The chemical composition of the essential of this subfraction is shown in Figure 32 and Table 19. Interestingly subfraction E_1 did not have any identified common compounds with fraction E, which suggested that these compounds were present at low proportions in fraction E. Only one unidentified peak at the retention time 52.38 min was observed in both oils and the percentage distribution of this unidentified compound was higher in the subfraction. Spathulenol was present in both the whole oil as well as in the subfraction E_1.

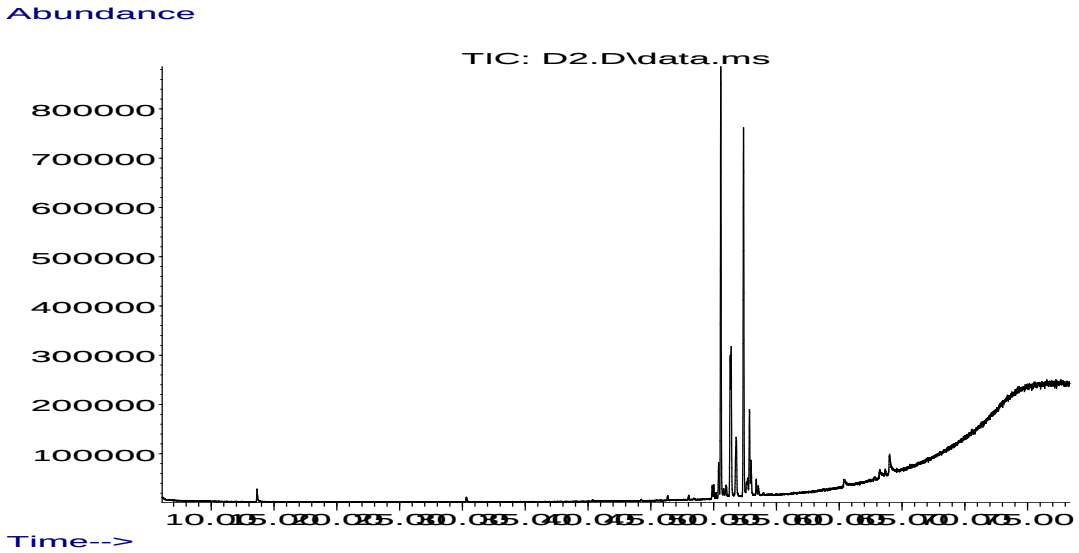


Figure 32. Total Ion Chromatogram of Subfraction E_1 of *Calytrix exstipulata* leaf oil.

Table 19. Chemical composition of Subfraction E_1.

Compound	RT (min)	Area (%)	MF	MW (g/ mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
Maaliol	50.41	2.4	$C_{15}H_{26}O$	222	91	1566	31.53	1620.58	A
Spathulenol	50.57	28.9	$C_{15}H_{24}O$	220	99	1577	31.96	1625.01	A
Cubeban-11-ol	51.32	9.0	$C_{15}H_{26}O$	222	98	1595	32.7	1644.43	A
Viridiflorol	51.40	10.5	$C_{15}H_{26}O$	222	99	1592	32.58	1646.41	A
Selina-3,7,(11)-diene	51.80	6.8	$C_{15}H_{24}$	204	89	1545	30.66	1656.81	A
Unidentified	52.38	26.1	-	164	-	-	-	1671.55	-
cis-β-Guaiene	52.62	1.1	$C_{15}H_{24}$	204	58	1492	28.51	1677.50	A
β-Gurjunene	52.72	1.3	$C_{15}H_{24}$	204	91	1431	25.95	1679.91	A
epi-α-Cadinol	52.86	6.0	$C_{15}H_{26}O$	204	90	1638	34.38	1683.50	A
epi-α-Muurolol	52.99	2.6	$C_{15}H_{26}O$	222	98	1640	34.46	1686.70	A
Unidentified	64.01	2.5	-	-	-	-	-	-	-

"A" – Identification based on comparison with the GC-MS database library

"B" – Identification based on comparison with the reference standard

Subfraction E_2 was characterized by monoterpenes and diterpenes. The major component was cyperene (18.4 %). Other predominant constituents were identified as rosifoliol (16.9 %), viridiflorol (8.6 %), alpha-eudesmol (7.4 %) and spathulenol (6.9 %). The chemical profile is shown in Figure 33 and the results are listed in Table 20.

Only globulol was detectable in the whole oil as well as in the fraction E and in the subfraction E_2. The unidentified compound at the retention time 52.37 min was still unidentified. Rosifoliol, cyperene and α-eudesmol was present in fraction E as well as in subfraction E_2.

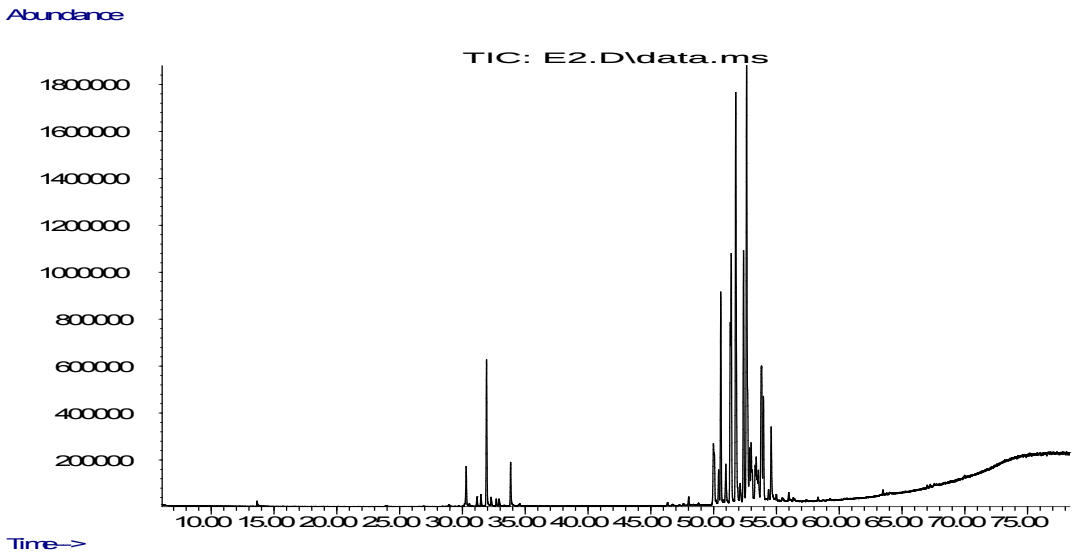


Figure 33. Total Ion Chromatogram of Subfraction E_2 of *Calytrix exstipulata* leaf oil.

Table 20. Chemical composition of Subfraction E_2.

Compound	RT (min)	Area (%)	MF	MW (g/ mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
neo-Isopulegol	30.30	1.4	$C_{10}H_{18}O$	154	96	1144	13.37	1180.62	A
Borneol	31.92	4.8	$C_{10}H_{18}O$	154	92	1165	14.29	1212.31	A, B
trans-Carveol	33.84	1.3	$C_{10}H_{16}O$	152	97	1215	16.44	1252.87	A
Unidentified	49.99	3.7	-	222	-	-	-	1609.56	-
Maaliol	50.40	1.1	$C_{15}H_{26}O$	222	91	1566	31.53	1620.56	A
Spathulenol	50.57	6.9	$C_{15}H_{24}O$	220	99	1577	31.96	1624.99	A
Globulol	50.98	1.6	$C_{15}H_{26}O$	222	98	1505	29.05	1635.52	A, B
Cubeban-11-ol	51.33	5.2	$C_{15}H_{26}O$	222	98	1595	32.7	1644.63	A
Viridiflorol	51.40	8.6	$C_{15}H_{26}O$	222	99	1592	32.58	1646.57	A
Rosifoliol	51.76	16.9	$C_{15}H_{26}O$	222	86	1600	32.89	1655.81	A
Unidentified	52.39	8.4	-	164	-	-	-	1671.60	-
Cyperene	52.63	18.4	$C_{15}H_{24}$	204	93	1398	24.54	1677.78	A
α-Cadinene	52.87	2.1	$C_{15}H_{24}$	204	96	1537	30.33	1683.85	A
epi-α-Muurolol	52.99	2.0	$C_{15}H_{26}O$	220	99	1640	34.46	1686.80	A
Unidentified	53.39	1.4	-	220	-	-	-	1708.90	-
α-Eudesmol	53.82	7.4	$C_{15}H_{26}O$	222	96	1652	34.91	1720.84	A
neo-Intermedeol	53.97	3.5	$C_{15}H_{26}O$	222	99	1658	35.15	1724.92	A
Unidentified	54.59	2.8	-	-	-	-	-	1741.99	-

"A" – Identification based on comparison with the GC-MS database library
"B" – Identification based on comparison with the reference standard

Subfraction E_3 contained twenty different compounds (Figure 34) and fifteen were identified (Table 21). The most abundant compound was globulol with a relative distribution of 27.7 %, followed by α-gurjunene (10.3 %), α-eudesmol (8.0 %) and some other minor components. Similar to subfractions E_1, E_2, fraction E and in the whole oil the peak in the region of 52.37 min was unidentified. α–Terpineol, citronellol and globulol were in the whole oil, in the fraction E and also in this subfraction present. Furthermore rosifoliol, α-muurolol, α-cadinol, and α-eudesmol were found to be the common compounds in the fraction E as well as in the subfraction E_3.

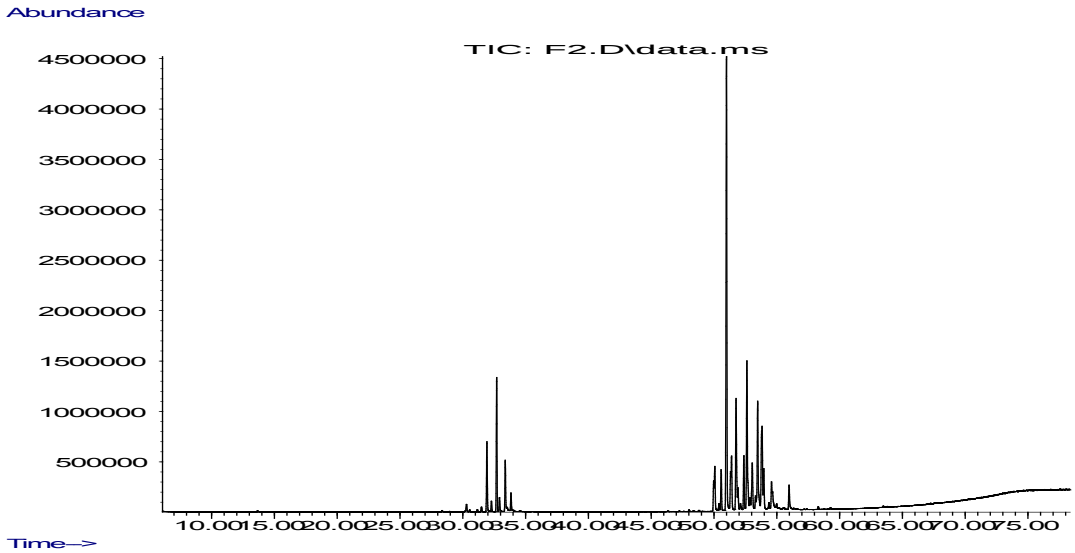


Figure 34. Total Ion Chromatogram of Subfraction E_3 of *Calytrix exstipulata* leaf oil.

Table 21. Chemical composition of Subfraction E_3.

Compound	RT (min)	Area (%)	MF	MW (g/ mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
Borneol	31.92	4.0	$C_{10}H_{18}O$	154	97	1165	14.29	1094.12	A, B
α-Terpineol	32.70	6.9	$C_{10}H_{18}O$	154	90	1186	15.21	1114.26	A, B
Citronellol	33.38	2.5	$C_{10}H_{20}O$	156	87	1223	16.8	1131.60	A, B
trans-Carveol	33.84	0.9	$C_{10}H_{16}O$	152	94	1215	16.44	1143.02	A
Unidentified	49.98	1.7	-	220	-	-	-	1609.34	-
Unidentified	50.07	2.6	-	202	-	-	-	1611.67	-
Spathulenol	50.57	2.4	$C_{15}H_{24}O$	220	99	1577	31.96	1624.99	A
Globulol	51.00	27.7	$C_{15}H_{26}O$	222	98	1590	32.5	1636.07	A, B
Cubeban-11-ol	51.32	2.1	$C_{15}H_{26}O$	222	98	1595	32.7	1644.48	A
Viridiflorol	51.40	3.2	$C_{15}H_{26}O$	222	99	1592	32.58	1646.46	A
Rosifoliol	51.76	7.5	$C_{15}H_{26}O$	222	83	1600	32.89	1655.65	A
Spathulenol	51.91	1.2	$C_{15}H_{24}O$	220	91	1577	31.96	1659.56	A
Unidentified	52.38	3.1	-	164	-	-	-	1671.52	-
α-Gurjunene	52.62	10.3	$C_{15}H_{24}$	204	92	1409	25	1677.60	A
α-Muurolol	53.05	3.5	$C_{15}H_{26}O$	222	94	1644	34.61	1688.28	A
α-Cadinol	53.48	7.7	$C_{15}H_{26}O$	222	99	1652	34.93	1711.31	A
α-Eudesmol	53.83	8.0	$C_{15}H_{26}O$	222	96	1652	34.91	1721.06	A
neo-Intermedeol	53.97	2.5	$C_{15}H_{26}O$	222	99	1658	35.15	1724.87	A
Unidentified	54.58	0.7	-	204	-	-	-	1741.94	-
Unidentified	55.98	1.6	-	220	-	-	-	1779.79	-

"A" – Identification based on comparison with the GC-MS database library

"B" – Identification based on comparison with the reference standard

Subfraction E_4 was found to be rich in monoterpenes and sesquiterpenes, eleven of which were identified. The major compound was globulol, representing 65.1 % of the total oil. Results are listed in Table 22 and the chemical profile is shown in Figure 35. Subfraction E_4 showed similar chemical profile to Fraction E, as it consisted of the same compounds with an exception of n-decanol and α-copaene, which were only present in this subfraction. Furthermore α-terpineol, citronellol and globulol were in this subfraction as well as in the whole oil.

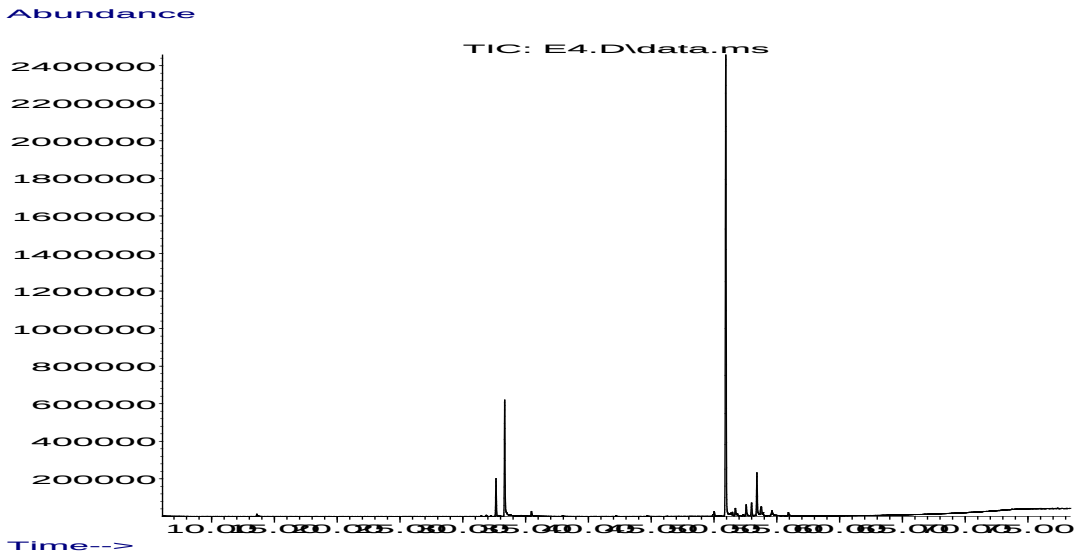


Figure 35. Total Ion Chromatogram of Subfraction E_4 of *Calytrix exstipulata* leaf oil.

Table 22. Chemical composition of Subfraction E_4.

Compound	RT (min)	Area (%)	MF	MW (g/mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
α-Terpineol	32.65	4.7	$C_{10}H_{18}O$	154	90	1186	15.21	1228.81	A, B
Citronellol	33.34	15.1	$C_{10}H_{20}O$	156	80	1223	16.8	1243.34	A, B
n-Decanol	35.47	0.7	$C_{10}H_{20}O$	158	70	1201	15.83	1286.29	A
Globulol	50.94	65.1	$C_{15}H_{26}O$	222	96	1590	32.5	1635.90	A, B
Rosifoliol	51.71	1.0	$C_{15}H_{26}O$	222	64	1600	32.89	1551.27	A
Cyperene	52.57	1.8	$C_{15}H_{24}$	204	94	1398	24.54	1462.04	A
α-Copaene	53.00	1.9	$C_{15}H_{24}$	204	86	1374	23.49	1351.82	A
α-Cadinol	53.42	6.1	$C_{15}H_{26}O$	222	99	1652	34.93	1711.06	A
β-Eudesmol	53.77	1.5	$C_{15}H_{26}O$	222	98	1649	34.79	1720.79	A
2*E*, 6*E*-Farnesol	54.62	1.2	$C_{15}H_{26}O$	222	91	1742	38.3	1744.36	A

"A" – Identification based on comparison with the GC-MS database library

"B" – Identification based on comparison with the reference standard

Subfraction $E_{5,}$ as in subfraction E_4 showed globulol as the major compound with relative distribution of 78.8 %. The chemical composition of the oil is presented in Figure 36 and Table 23. Citronellol and globulol appeared in this subfraction as well as in fraction E and in the whole oil. Cuparene may be present but the low match in the database search makes this identification doubtful.

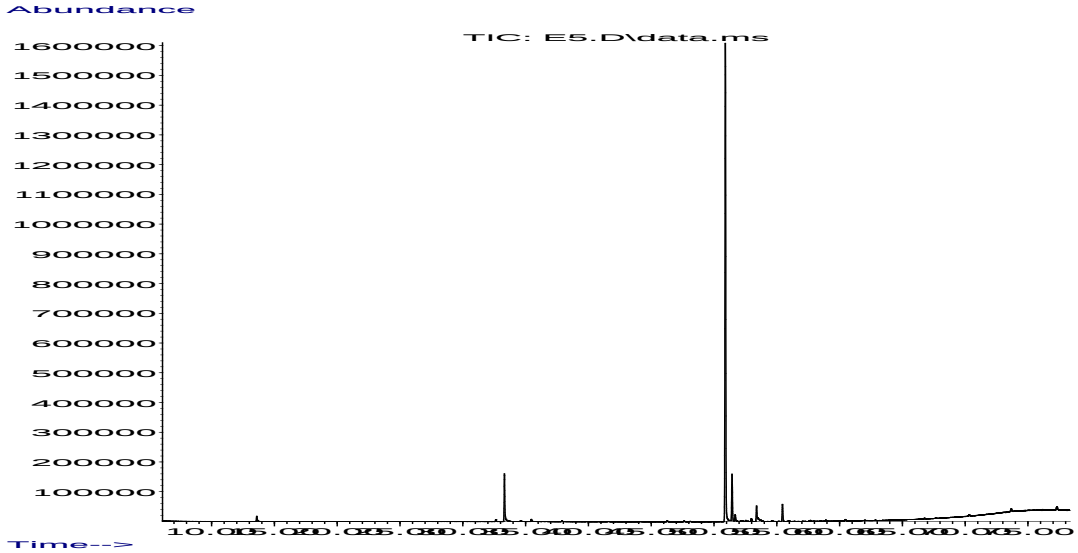


Figure 36. Total Ion Chromatogram of Subfraction E_5 of *Calytrix exstipulata* leaf oil.

Table 23. Chemical composition of Subfraction E_5.

Compound	**RT (min)**	**Area (%)**	**MF**	**MW (g/ mol)**	**Match (%)**	**Adams Index (AI)**	**RT-Adams**	**Kovats Index (KI)**	**Identification**
Citronellol	33.33	7.3	$C_{10}H_{20}O$	156	96	156	1223	1243.26	A, B
Globulol	50.93	78.8	$C_{15}H_{26}O$	222	98	1590	32.5	1635.66	A, B
Cuparene	51.47	7.4	$C_{15}H_{22}$	202	33	1504	29	1649.44	A
Unidentified	51.72	1.1	-	202	-	-	-	1655.88	-
α-Cadinol	53.42	2.6	$C_{15}H_{26}O$	222	91	1652	34.93	1711.06	A
Unidentified	55.49	2.8	-	-	-	-	-	1767.84	-

"A" – Identification based on comparison with the GC-MS database library

"B" – Identification based on comparison with the reference standard

In subfraction E_6, only a few peaks were detected by the GC-MS method (Figure 37) and two of those were identified, as shown in Table 24. In comparison to earlier subfractions E_3, E_4 and E_5, where globulol appeared as the major compound with remarkable values of relative distribution, in subfraction E_6 globulol was only present with a relative distribution of 1.2 %. Moreover another compound, namely butylated hydroxytoulene, appeared at the retention time of 46.34 min. The sesquiterpene globulol is proved to be an important and common compound in the whole oil as well as in the fraction E or in this subfraction.

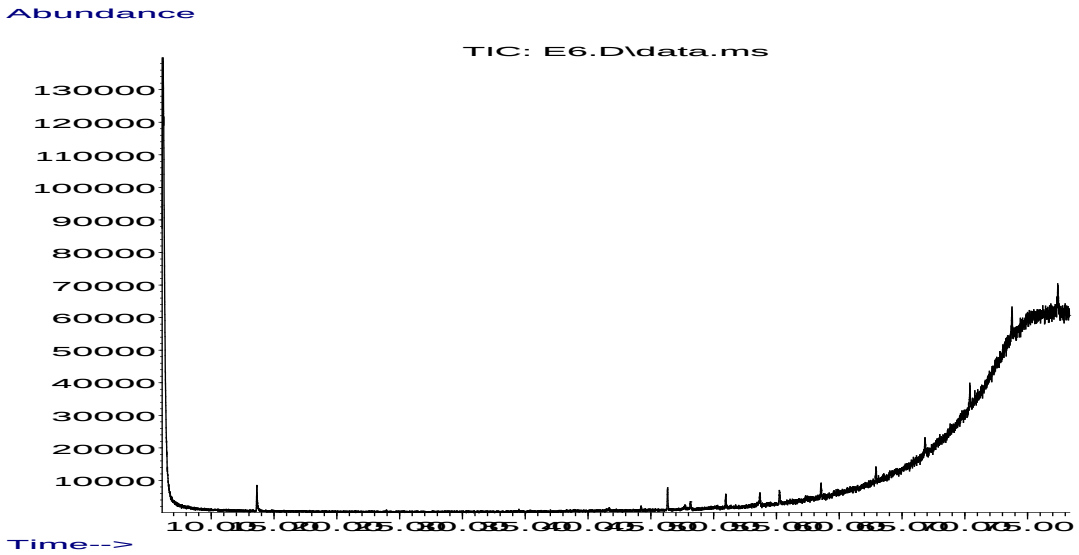


Figure 37. Total Ion Chromatogram of Subfraction E_6 of *Calytrix exstipulata* leaf oil.

Table 24. Chemical composition of Subfraction E_6.

Compound	RT (min)	Area (%)	MF	MW (g/ mol)	Match (%)	Adams Index (AI)	RT-Adams	Kovats Index (KI)	Identification
Solvent peak	6.17	91.1 %	-	-	-	-	-	-	-
Solvent peak	13.64	2.1%	-	-	-	-	-	-	-
Butylated hydroxytoluene	46.34	1.6%	$C_{15}H_{24}O$	220	72	1514	29.43	1531.70	A, B
Globulol	50.97	1.2%	$C_{15}H_{26}O$	222	46	1590	32.5	1636.60	A, B
Unidentified	53.69	1.3%	-	-	-	-	-	1718.50	-
Unidentified	55.24	1.2%	-	-	-	-	-	1861.27	-
Unidentified	58.56	1.6%	-	-	-	-	-	1948.69	-

"A" – Identification based on comparison with the GC-MS database library
"B" – Identification based on comparison with the reference standard

4.3.2 Pharmacological analysis on subfractions obtained from fraction E

4.3.2.1 Antioxidant activity

After fractionating the antioxidant active fraction E, five antioxidant active subfractions and one non active subfraction were obtained, as presented in Figure 38. All of these five antioxidant active subfractions were higher than the values obtained for the whole oil. Subfraction E_1 and subfraction E_4 showed even higher ORAC values than the fraction E.

The most active fraction was subfraction E_4 (ORAC value: 1185 $\pm$ 240 µmol TE/g), which obtained the active compound globulol (65.1 % in subfraction E_4) and citronellol (15.1% in subfraction E_4). Citronellol and globulol were both tested as standards and showed antioxidant activity in this work, they have also been reported in previous studies to have antioxidant properties [23, 25, 32]; this fact could probably be an explanation for the high ORAC scores in subfraction E_4.

In subfraction E_5 globulol existed in an even higher percentage (78.8 %) than E_4 and citronellol was lower (7.3 %). Interestingly subfraction E_5 was less active (ORAC value: 597 ± 62 µmol TE/g) than fraction E4 (Figure 38). The only difference between these two subfractions was the presence of cuparene, and two other unidentified compounds in subfraction E_5. Cuparane's identification is doubtful due to the low percentage match (33 %) and a reference standard of cuparene was not available for comparison. As mentioned before citronellol and globulol have already been described as antioxidants [23, 25, 32].

The second most active fraction was subfraction E_1 with the most abundant compound spathulenol (percentage distribution: 28.9 %), followed by viridiflorol (10.5 %) and cubeban-11-ol (9.0 %). To the best of my knowledge nothing has been reported about the antioxidant properties of these principal compounds, and due to this fact the activity of this subfraction will require further investigation.

In subfraction E_2 cyperene and rosifoliol were the major compounds. Rosifoliol was found to be antioxidant in previous studies [41].

In subfraction E_3, as in the whole oil and in fraction E, the compounds globulol, α-terpineol and citronellol were present. As mentioned before these compounds have been tested in this study and they proved to have antioxidant activity. Previous studies, also report that they have antioxidant properties; that might be an explanation for the antioxidant activity of these different oils [23, 25, 32].

Subfraction E_6 did not show any activity, only two compounds in a very low percentage were identified, in fact the compound globulol and butylated hydroxytoluene.

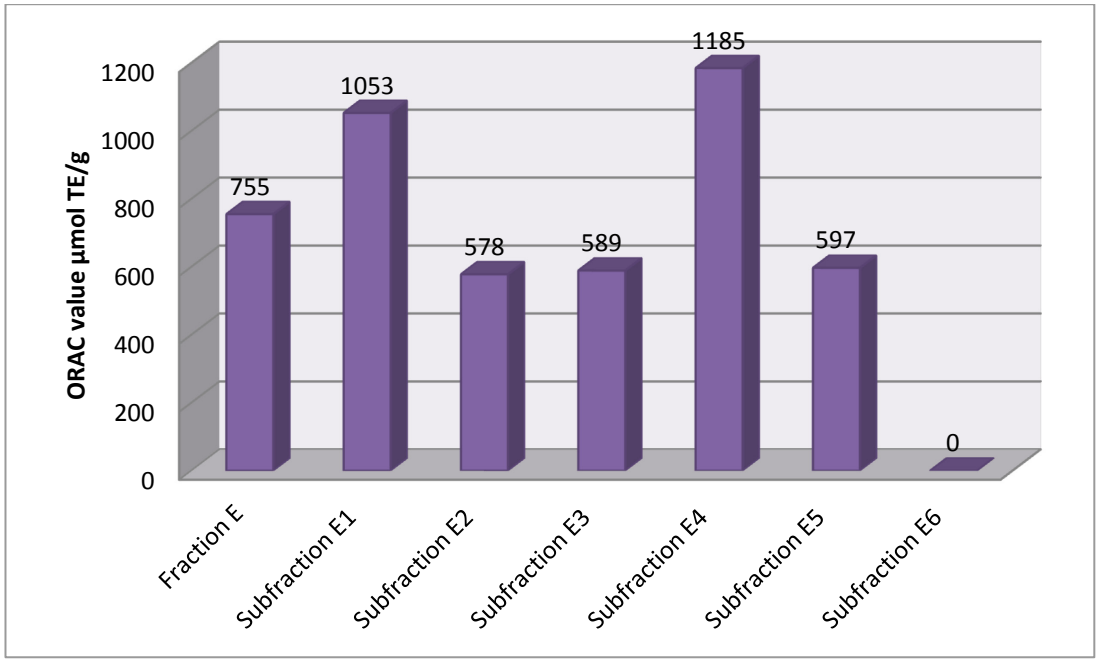


Figure 38. ORAC values (µmol TE/g) of all subfractions obtained from Fraction E.

4.3.2.2 Cytotoxicity study

Due to the low yield and the lack of time, the cytotoxicity assay for the six subfractions was only done on P388 cells and all subfractions showed an inhibition against these cells. In Figure 39 the IC_{50} values of the whole oil and of the fraction E, with IC_{50} 9 and 7.78 µmol/mL It is obvious that all subfractions, except subfraction E_5, are more active than the whole oil or the fractionated fraction E itself.

Subfraction E_2 showed the highest inhibition with IC_{50} 0.01 µg/mL, followed by subfraction E_4 (IC50 1.81 µg/mL) and subfraction E_3 (IC50 2.71 µg/mL).

An explanation for the cytotoxic properties in fraction E and subfraction E_4 and E_3 might be the compound globulol, since it existed predominantly in these subfraction oils. Globulol has been tested pure as a reference standard and showed high activity; (see cytotoxic results in Appendix 1).

Eudesmol was present in subfraction E_2, E_3, E_4 and in fraction E. This compound has already been described to have cytotoxic activity [24]; which might be an explanation for the high activity in these subfractions and in fraction E. In the most active subfraction E2 cyperene, rosifoliol and viridiflorol were the major components. There does not appear to be any reports about these compounds' cytotoxic properties and, therefore, the reason for the high inhibition in this subfraction cannot be explained.

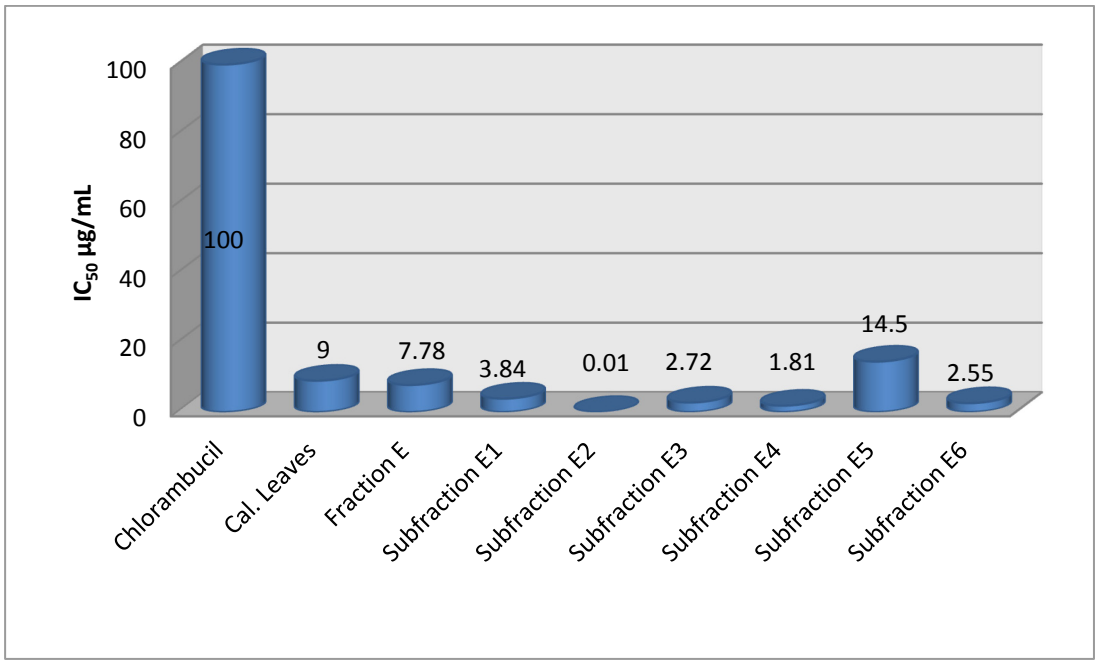


Figure 39. Cytotoxicity results on P388 cells (µg/mL) of all subfractions obtained from Fraction E.

5 ESSENTIAL OIL OF *CYMBOPOGON BOMBYCINUS* AND *C. CITRATUS*

5.1 Phytochemical analysis of *Cymbopogon bombycinus* and *Cymbopogon citratus*

The major component of *Cymbopogon bombycinus* essential oil was geraniol, with a relative distribution of 32.3 %. Other predominant constituents were 2-methylisoborneol and limonene with a lower relative distribution of 4.7 % and 4.1 %, respectively, followed by other minor components, as shown in Figure 40 and Table 25. Geraniol has already been reported to be the principal compound in former studies in "Traditional Bush Medicines" [4]. Two compounds were not identified.

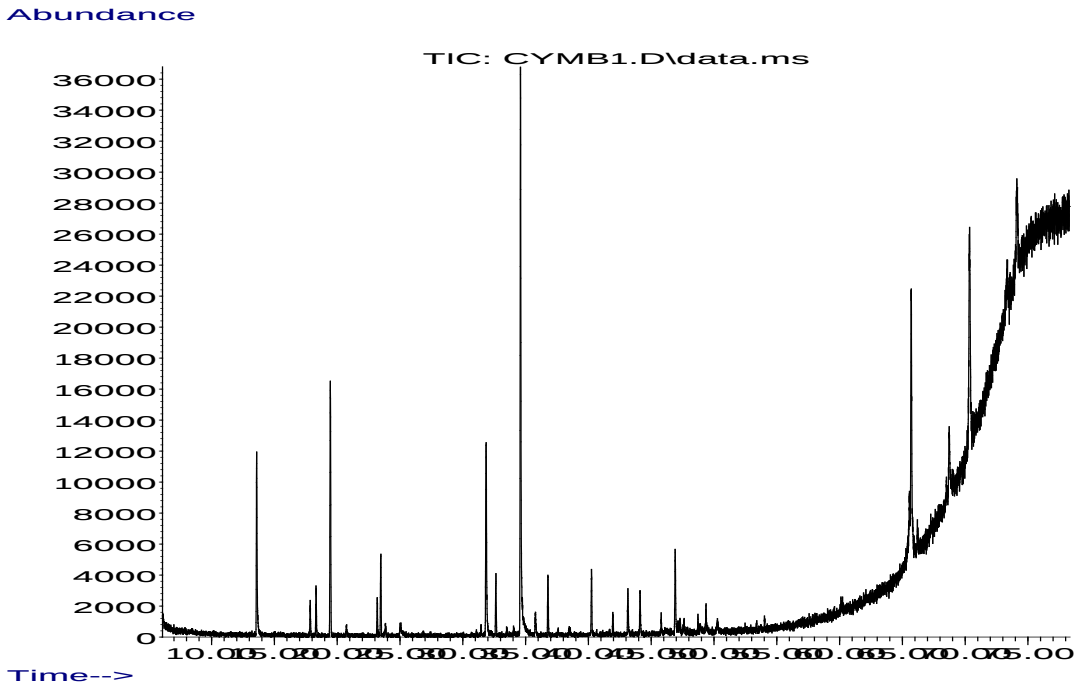


Figure 40. Total Ion Chromatogram of *Cymbopogon bombycinus* essential oil.

Table 25. Chemical composition of *Cymbopogon bombycinus* essential oil.

Compound	**RT (min)**	**Area (%)**	**MF**	**MW (g/ mol)**	**Match (%)**	**Adams Index (AI)**	**RT-Adams**	**Kovats Index (KI)**	**Identification**
pollution	13.59	9.7	-	-	-	-	-	-	-
α-Tujene	17.86	1.6	$C_{10}H_{16}$	136	83	931	5.12	944.75	A
α–Pinene	18.32	2.6	$C_{10}H_{16}$	136	94	932	5.85	953.61	A, B
Camphene	19.46	13.9	$C_{10}H_{16}$	136	89	953	5.67	974.65	A, B
α–Pinene	23.20	1.8	$C_{10}H_{16}$	136	90	932	5.85	1045.78	A
Limonene	23.48	4.1	$C_{10}H_{16}$	136	91	1024	8.69	1051.21	A
Borneol	31.87	13.0	$C_{10}H_{18}O$	154	78	1165	14.29	1387.04	A, B
α-Terpineol	32.66	3.0	$C_{10}H_{18}O$	154	78	1186	15.21	1199.67	A, B
Geraniol	34.61	32.3	$C_{10}H_{18}O$	154	87	1255	16.97	1201.29	A, B
Bornyl acetate	36.80	3.2	$C_{10}H_{20}O_2$	196	91	1285	18.32	1298.90	A
Geranyl propanoate	40.27	3.1	$C_{13}H_{22}O_2$	210	90	1475	26.42	1301.34	A
Unidentified	43.17	3.0	-	110	-	-	-	1388.89	-
Carvone hydrate	44.14	2.6	$C_{10}H_{18}O_2$	168	64	25.54	1422	1389.48	A
2-Methylisoborneol	46.94	4.7	$C_{10}H_{20}O$	168	78	14.85	1178	1546.35	A
Unidentified	49.39	1.4	-	168	-	-	-	1605.20	-

"A" – Identification based on comparison with the GC-MS database library

"B" – Identification based on comparison with the reference standard

Geranial dominated *Cymbopogon citratus* essential oil, constituting 47.0 %. Other components present in appreciable contents were neral (35.4 %), alpha-thujene (11.2 %) and geraniol (1.9 %). The results are listed in Table 26 and represented in Figure 41. A previous study of the composition of the volatile oil, obtained from *Cymbopogon citratus*, showed the same four compounds as the most prominent [28].

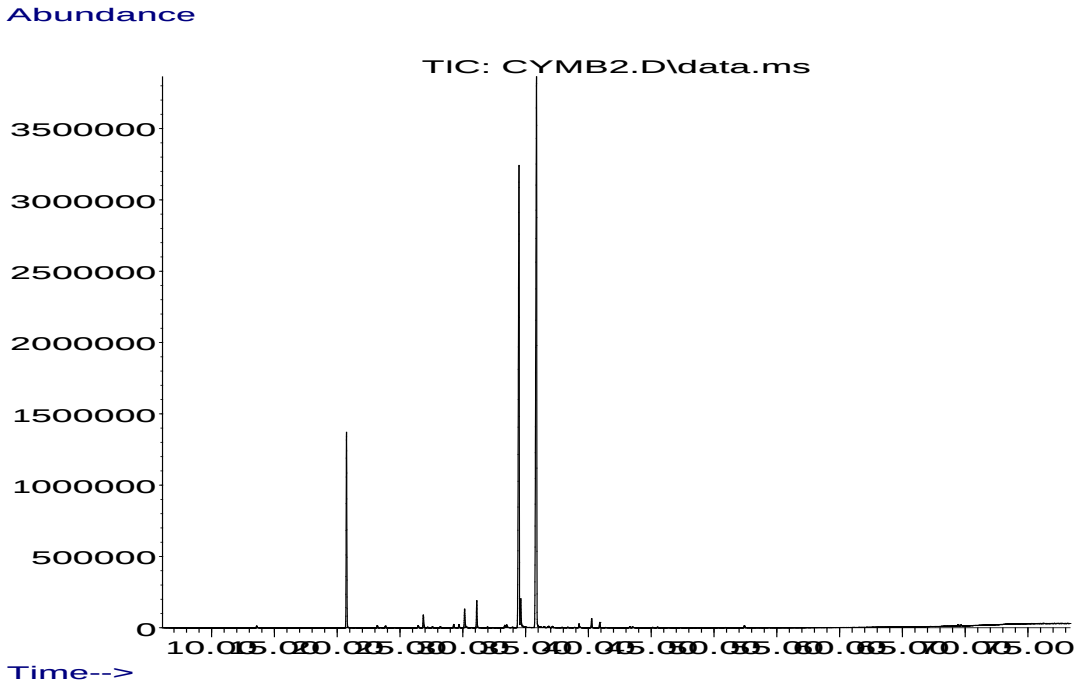


Figure 41. Total Ion Chromatogram of *Cymbopogon citratus* essential oil.

Table 26. Chemical composition of *Cymbopogon citratus* essential oil.

Compound	**RT (min)**	**Area (%)**	**MF**	**MW (g/ mol)**	**Match (%)**	**Adams Index (AI)**	**RT-Adams**	**Kovats Index (KI)**	**Identification**
α-Thujene	20.74	11.2	$C_{10}H_{16}$	136	95	931	5.12	907.94	A
α–Pinene	23.20	0.1	$C_{10}H_{16}$	136	93	932	5.85	1001.83	A
Limonene	23.48	0.02	$C_{10}H_{16}$	136	87	1024	8.69	1002.20	A
Linalool	26.86	0.8	$C_{10}H_{18}O$	154	96	1098	10.53	1113.82	A
Unidentified	30.15	1.2	-	-	-	-	-	1178.63	-
Unidentified	31.11	1.6	-	-	-	-	-	1196.35	-
Neral (=Citral B)	34.48	35.4	$C_{10}H_{16}O$	152	97	1240	16.33	1266.62	A
Geraniol	34.62	1.9	$C_{10}H_{18}O$	154	93	1255	16.97	1269.49	A
Geranial (=Citral A)	35.87	47.0	$C_{10}H_{16}O$	152	95	1270	17.62	1294.13	A
Unidentified	39.26	0.2	-	-	-	-	-	1368.57	-
Geranyl propanoate	40.26	0.5	$C_{13}H_{22}O_2$	210	91	27.82	1476	1389.66	A
Unidentified	40.91	0.3	-	-	-	-	-	1387.48	-

"A" – Identification based on comparison with the GC-MS database library
"B" – Identification based on comparison with the reference standard

Comparison of the volatile compounds of *Cymbopogon bombycinus* oil with those in *Cymbopogon citratus* oil shows that there are differences between the two oils. The major compound of *Cymbopogon bombycinus* whole oil was geraniol (32.3 %); geraniol was present in *Cymbopogon citratus* whole oil but at a decreased relative distribution of only 1.9 %. Whereas in the oil of *Cymbopogon citratus*, the most abundant compound was geranial (47.0 %); which was not at all present in the oil of the *Cymbopogon bombycinus*. Other similarities in the chemical composition of the two oils are presented in Figure 42. The monoterpenes limonene and geraniol are reported to be typical compounds in the essential oils of different *Cymbopogon* species [27].

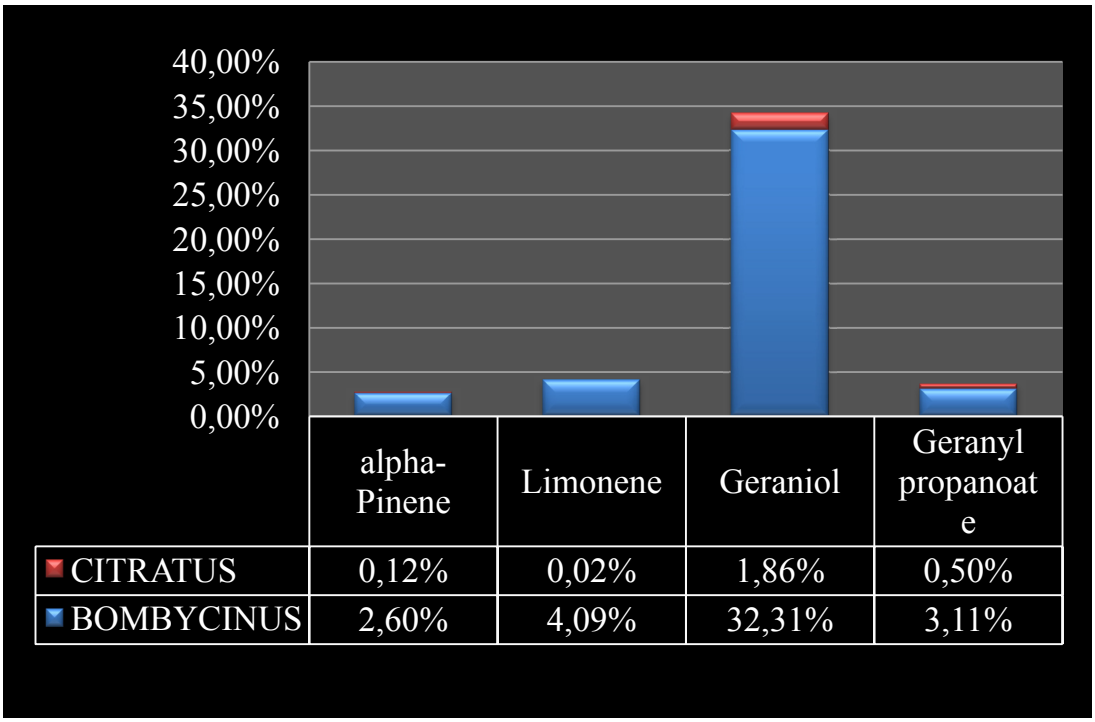


Figure 42. Comparison of the chemical profile of Cymb1 and Cymb2.

5.2 Pharmacological analysis of *Cymbopogon bombycinus* and *Cymbopogon citratus*

5.2.1 Antioxidant activity

In order to investigate what the active components in the oil were, an ORAC assay was conducted with the whole oil of both *Cymbopogon* species. The highest antioxidant activity was found in the tested reference standard geraniol, the most abundant compound in *Cymbopogon bombycinus*. The ORAC activity of the oils from *C. bombycinus* and *C. citratus* are shown in Table 27.

Comparing the antioxidant activity of *Cymbopogon bombycinus* and *Cymbopogon citratus*, the oil of *C. bombycinus* showed a higher ORAC value. This high activity is probably due to its high percentage of geraniol that has already been reported as an antioxidant [23]. The *Cymbopogon citratus* essential oil consisted also of geraniol but at a significant lower percent distribution. In the *C. bombycinus* essential oil, other major compounds such as limonene and camphene were present and in previous studies they were reported to be antioxidants [23, 43].

The activity of *C. citratus* could be explained by the predominant compounds geranial, neral and geraniol; these compounds were described as antioxidants [23]. Moreover in former studies geraniol, geranial, neral and limonene were found to have antibacterial activity [9, 29, 30].

Table 27. ORAC values (µmol TE/g) of Cymb1 and Cymb2 and geraniol (reference standard).

Sample	**ORAC value (µmol TE/g)**
Cymbopogon bombycinus	356 ± 35
Geraniol	969 ± 220
Cymbopogon citratus	298 ± 42

5.2.2 Cytotoxicity study

Figure 43 shows that on all cell lines, the whole oil of *C. citratus* was more cytotoxic than the whole oil of *C. bombycinus*. The reference standards geraniol and camphene did not show any cytotoxicity on HS27 and 3T3 cell lines.

The essential oil of *C. bombycinus* was dominated by compounds such as: geraniol, camphene and borneol. All of these compounds showed cytototoxic activity when reference standards were tested. Geraniol was the major compound in the whole oil of *C. bombycinus* but showed the lowest values in the cytotoxicity assay, whereas geraniol was a minor component in the whole oil of *C. citratus*. That might be a reason in this study why the oil from *C. bombycinus* was less cytotoxic than that of *C. citratus*.

The major compound geranial present in the whole oil of *C. citratus* was not available as a reference standard but it has already been reported to have anticancer activity [29]. In 2006 a research team from the Ben Gurion University in Israel reported that lemon grass caused apoptosis in cancer cells due to their most abundant constituent citral [14]. This could be the reason for the high cytotoxic activity of the *C. citratus* whole oil in this study. Citral was not present in the essential oil of *C. bombycinus* and thus this oil was less cytotoxic.

Limonene, which was in both oils at a low percentage, 4.1 % in *C. bombycinus* and 0.02 % in *C. citratus*, has been reported to have anticancer and antimutagenetic properties [7]. In another study it has been reported that compounds such as: limonene, linalool, citronellol and geraniol are cytotoxic [9, 30].

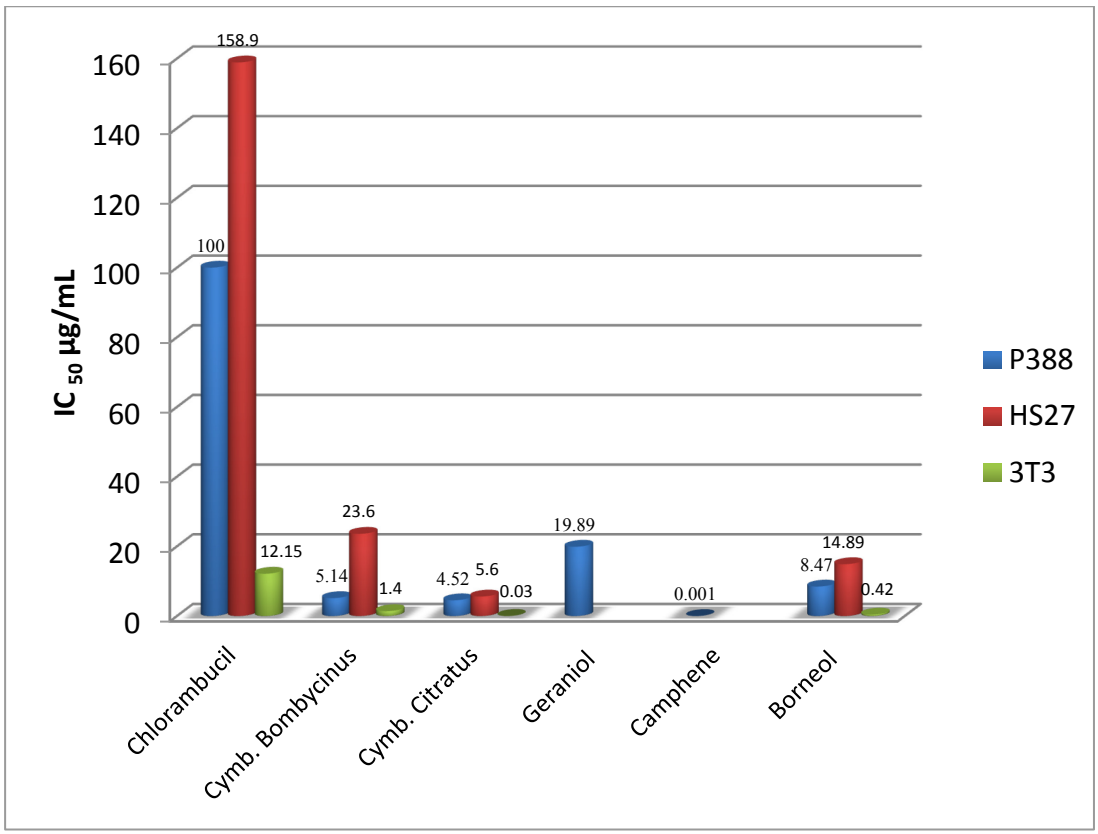


Figure 43. Cytotoxicity results (µg/mL) of Cymb1 and Cymb2.

6 CONCLUSION

6.1 Calytrix exstipulata

Calytrix exstipulata leaf and stem oils were rich in monoterpenes as well as in sesquiterpenes. Pinene, an antiseptic and anti-inflammatory monoterpene, was the major compound of the leaf oil and could probably explain its use as a liniment for aches and wounds [9].

The leaf oil exhibited an ORAC value of 66 μmol TE/g and in contrast, the stem oil did not show any antioxidant activity. Comparison of the ORAC values with the positive control epicatechin (20,000 μmol TE/g) showed that none of these tested samples showed high antioxidant activity. The *Calytrix exstipulata* stem oil seemed to be more cytototoxic than the leaf oil against HS27 and 3T3 cells, as summarized in Table 28.

Fractionation of *Calytrix exstipulata* leaf oil by normal phase column chromatography yielded nine fractions, with the majority of fractions containing monoterpenes and sesquiterpenes, with the exception of fractions A, B, C, and F, which consisted only of sesquiterpenes. Fraction E gave the highest yield and was further fractionated. Subfractions E_1 and E_6 contained sesquiterpenes while the rest of the subfractions contained monoterpenes and sesquiterpenes.
The summary of pharmacological testing is summarized in Figure 44. Fractions E, F and the subfraction E_4 and E_1 had the highest antioxidant activity. Fractions C, E, F and subfractions E_2 and E_4 showed the highest cytotoxic activity against P388 cells (Figure 45). In conclusion, fractions E, F and subfraction E_4 showed the highest pharmacological results, which could be attributed to the monoterpene globulol. Globulol was tested to have cytotoxic activity and it is the most abundant compound in Fractions E, F and also in subfraction E_4.

Table 28. Summary of cytotoxic activity of *Calytrix exstipulata* leaf and stem oil.

Sample	IC_{50}, µg/mL		
	HS 27	3T3	P388
***Calytrix exstipulata* leaf oil**	59.78	1.5	9
***Calytrix exstipulata* stem oil**	25.35	0.6	-
Chlorambucil	167.5	12.15	100

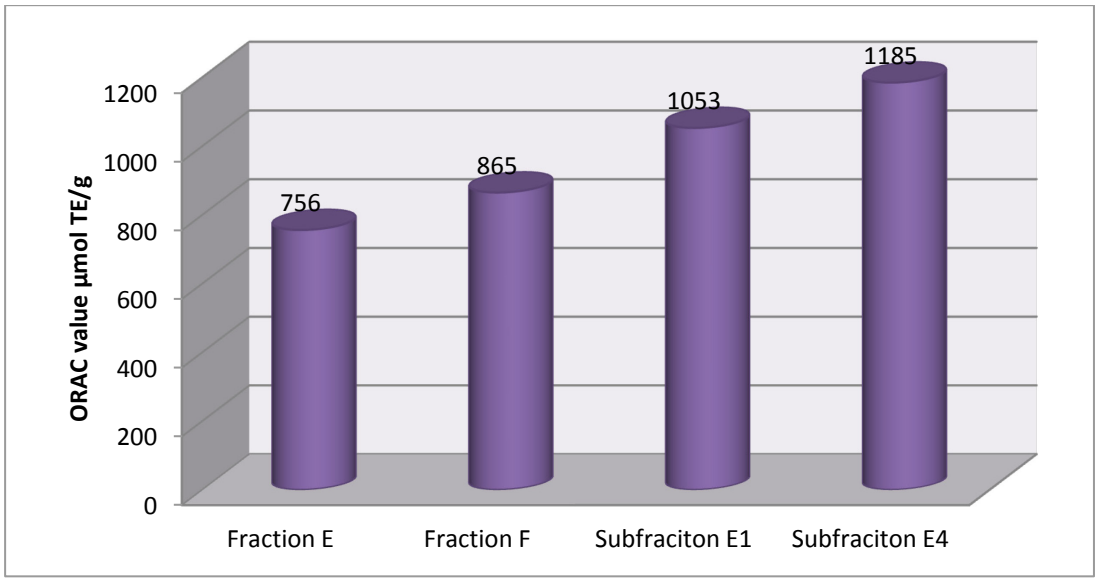


Figure 44. Antioxidant activity of Fractions E and F, and subfractions E_1 and E_4.

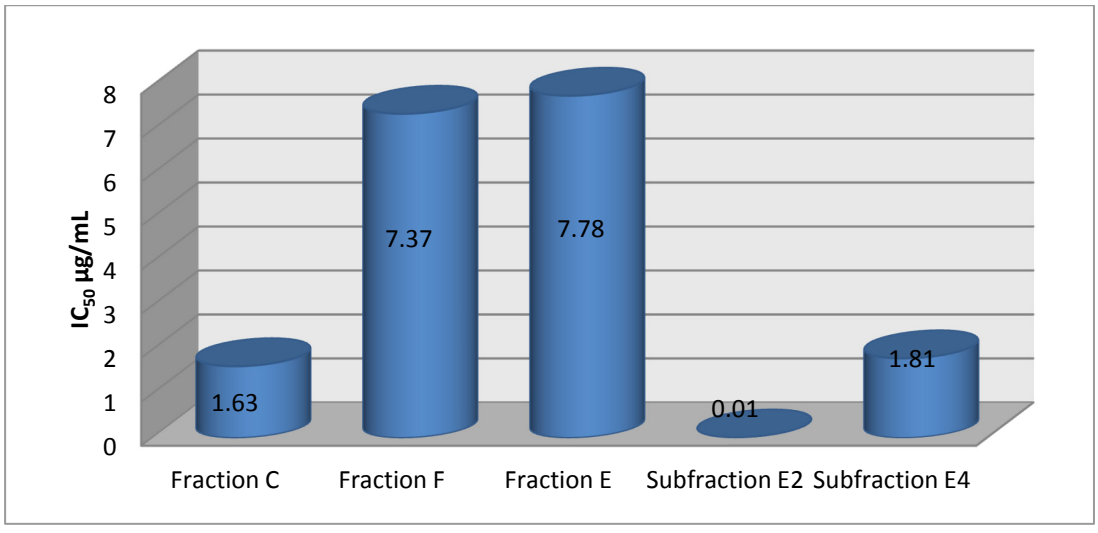


Figure 45. Cytotoxic activity of Fractions C, E and F, and subfractions E_2 and E_4.

6.2 *Cymbopogon* spp.

The essential oils of *Cymbopogon bombycinus* and the *Cymbopogon citratus* oil were both rich in monoterpenes only. The major compound of *Cymbopogon bombycinus* was geraniol and was present only in a low percentage in the *C. citratus* oil. The predominant compounds geranial and neral in the *C. citratus oil* were not present in the *C. bombycinus* oil. *C. citratus* oil is already well-studied and was used to compare with the less explored species *C. bombycinus*.

The essential oil of *C. bombycinus* had higher antioxidant activity than that of *C. citratus* as summarized in Table 29. This could be explained by the presence of geraniol as a major component, the reference standard of which was tested and exhibited antioxidant activity. Additionally geraniol is reported to possess antibacterial properties and could be a reason for its use as a remedy for colds, flu and infections [30].

Furthermore *C. citratus* showed to be more cytotoxic than *C. bombycinus* (Table 29), which could be due to its major compound geranial (*syn.* citral A). In previous studies it has been reported that geranial has antioxidant [23] and cytotoxic [29] properties and is thus used in anticancer therapy [14]. Nevertheless, the essential oil of *C. bombycinus* also exhibited cytotoxic activity and further work needs to be done in order to prove if there are anticancer properties.

In conclusion, this study explored the potential antioxidant and cytotoxicity activities of the essential oils of *Calytrix exstipulata* and *Cymbopogon bombycinus* but further work need to be done to confirm their pharmacological activities. Due to the lack of time only a preliminary work was carried out on these plants, which provided baseline information on the phytochemical composition of their essential oils.

Table 29. Pharmacological results of the *Cymbopogon genus.*

Sample	ORAC (µmol TE/g)	P388 cells (IC_{50} µg/mL)	HS27 cells (IC_{50} µg/mL)	3T3 cells (IC_{50} µg/mL)
C. bombycinus	356 ± 35	5.14	23.6	1.4
C. citratus	298 ± 42	4.52	5.6	0.03
Chlorambucil	-	100	158.9	12.15

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Appendix 1

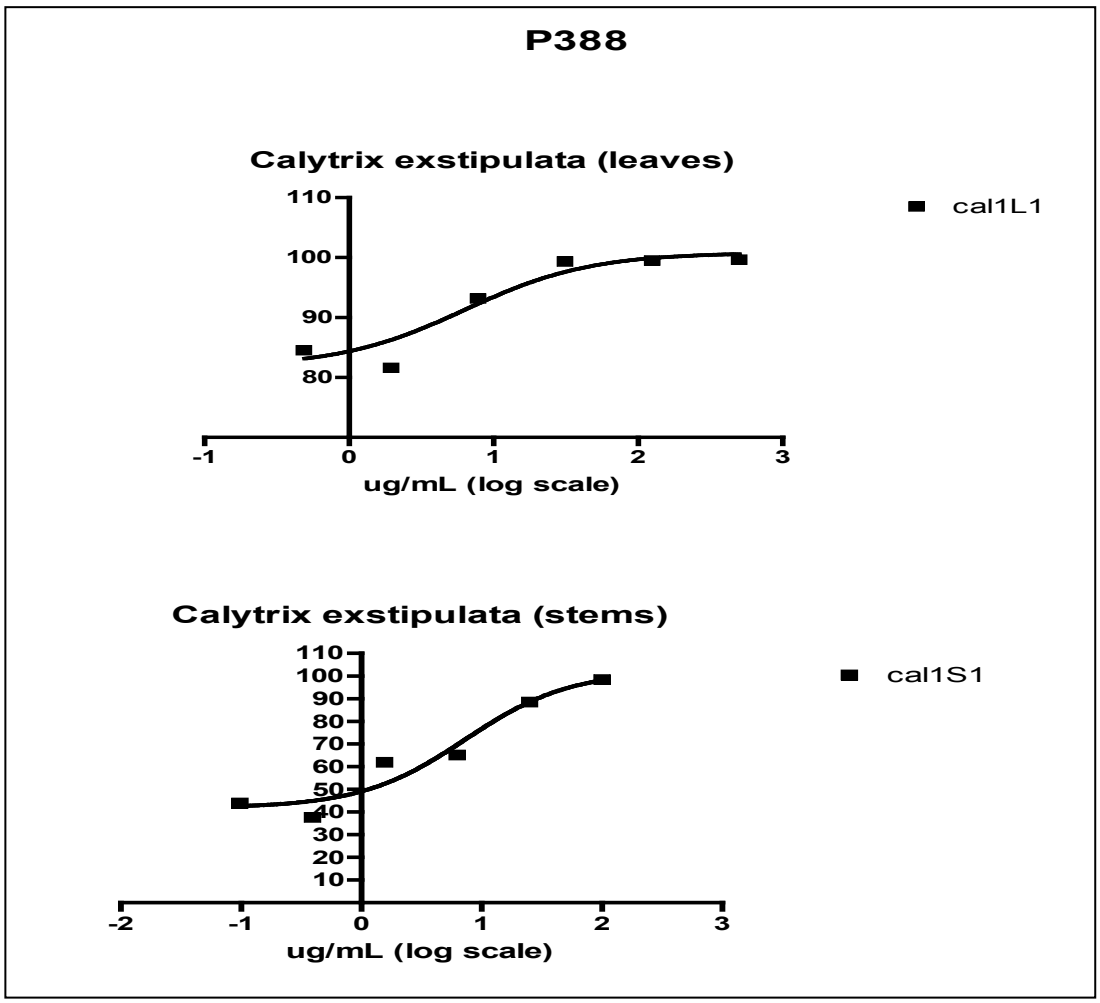
(Detailed cytotoxic results)

Appendix Table 1. Cytotoxic results on P388 cells of *Calytrix exstipulata* leaf oil (Cal1L1).

Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Cal1L1	500.000	9.001	99.6
	125.000		99.5 ± 0.1
	31.250		99.4
	7.813		93.2
	1.953		81.6
	0.488		84.5 ± 1.1

Appendix Table 2. Cytotoxic results on P388 cells of *Calytrix exstipulata* stem oil (Cal1S1).

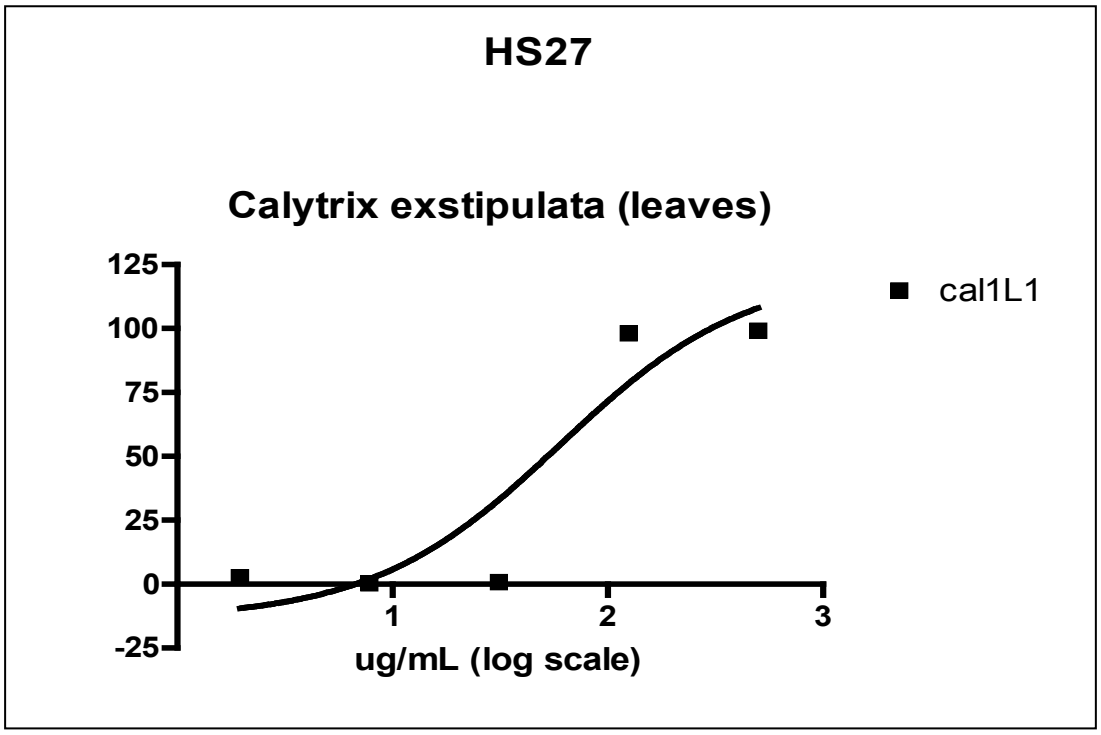
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
cal1S1	100.000	25.35	98.4 ± 1.4
	25.000		88.5
	6.250		65.2
	1.563		61.9
	0.391		37.6
	0.098		44.0



Appendix Figure 1. Cytotoxic results on P388 cells of *Calytrix exstipulata* leaf oil (Cal1L1).

Appendix Table 3. Cytotoxic results on HS27 cells of *Calytrix exstipulata* leaf oil (Cal1L1).

Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
cal1L1	500.000	59.78	99.2
	125.000		98.2
	31.250		0.8
	7.813		0.3
	1.953		2.7
	0.488		-



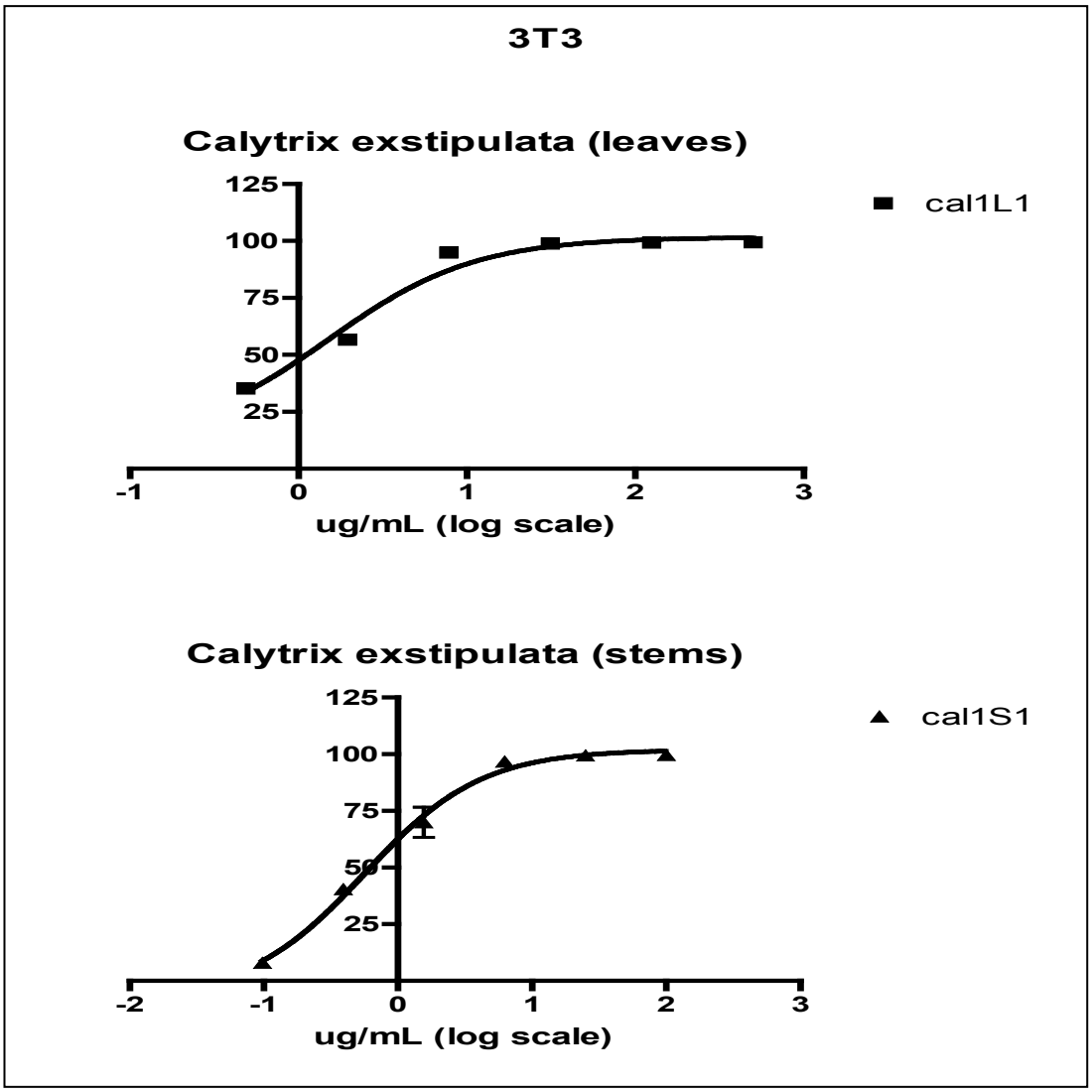
Appendix Figure 2. Cytotoxic results on HS27 cells of *Calytrix exstipulata* leaf oil (Cal1L1).

Appendix Table 4. Cytotoxic results on 3T3 cells of *Calytrix exstipulata* leaf oil (Cal1L1).

Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
cal1L1	500.000	1.5	99.4 ± 0.2
	125.000		99.4 ± 0.3
	31.250		98.9 ± 0.1
	7.813		94.9
	1.953		56.7
	0.488		-

Appendix Table 5. Cytotoxic results on 3T3 cells of *Calytrix exstipulata* stem oil (Cal1S1).

Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
cal1S1	100.000	0.6	99.6
	25.000		99.4
	6.250		96.8 ± 2.5
	1.563		69.9 ± 9.4
	0.391		40.4
	0.098		7.9



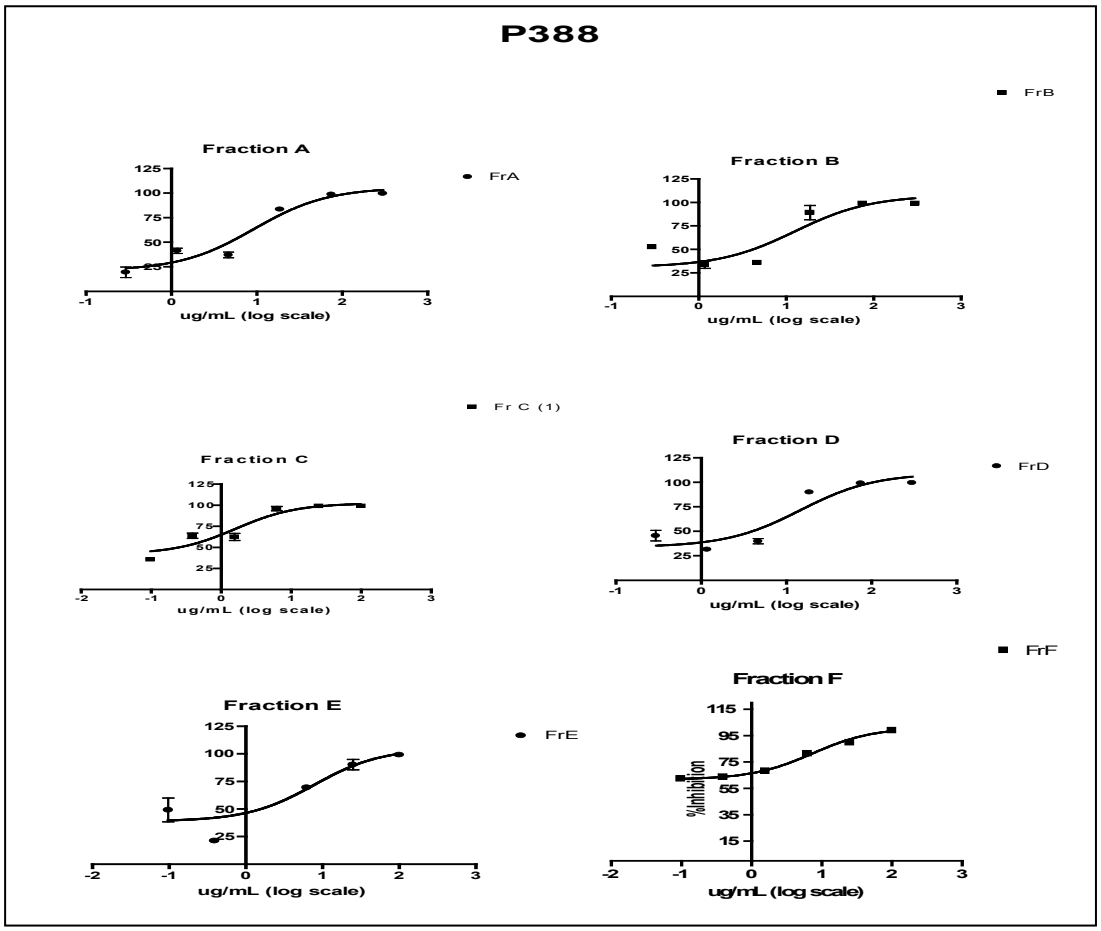
Appendix Figure 3. Cytotoxic results on 3T3 cells of *Calytrix exstipulata* leaf and stem oil (Cal1L1; Cal1S1).

Appendix Table 6. Cytotoxic results on P388 cells of Fraction A, B, C.

Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
FrA	300.000	9.23	99.6 ± 0.1
	75.000		98.7 ± 1.8
	18.750		83.5 ± 3.7
	4.688		37.1 ± 5.0
	1.172		41.3 ± 4.6
	0.293		19.5 ± 9.3
FrB	300.000	12.75	99.0
	75.000		99.0 ± 0.2
	18.750		89.2 ± 10.7
	4.688		36.0 ± 3.2
	1.172		33.8 ± 5.8
	0.293		52.6
FrC	100.000	1.62	99.0 ± 0.1
	25.000		98.9 ± 0.1
	6.250		96.0 ± 3.6
	1.563		62.4 ± 6.0
	0.391		63.7 ± 4.4
	0.098		35.4

Appendix Table 7. Cytotoxic results on P388 cells of Fraction D, E, F.

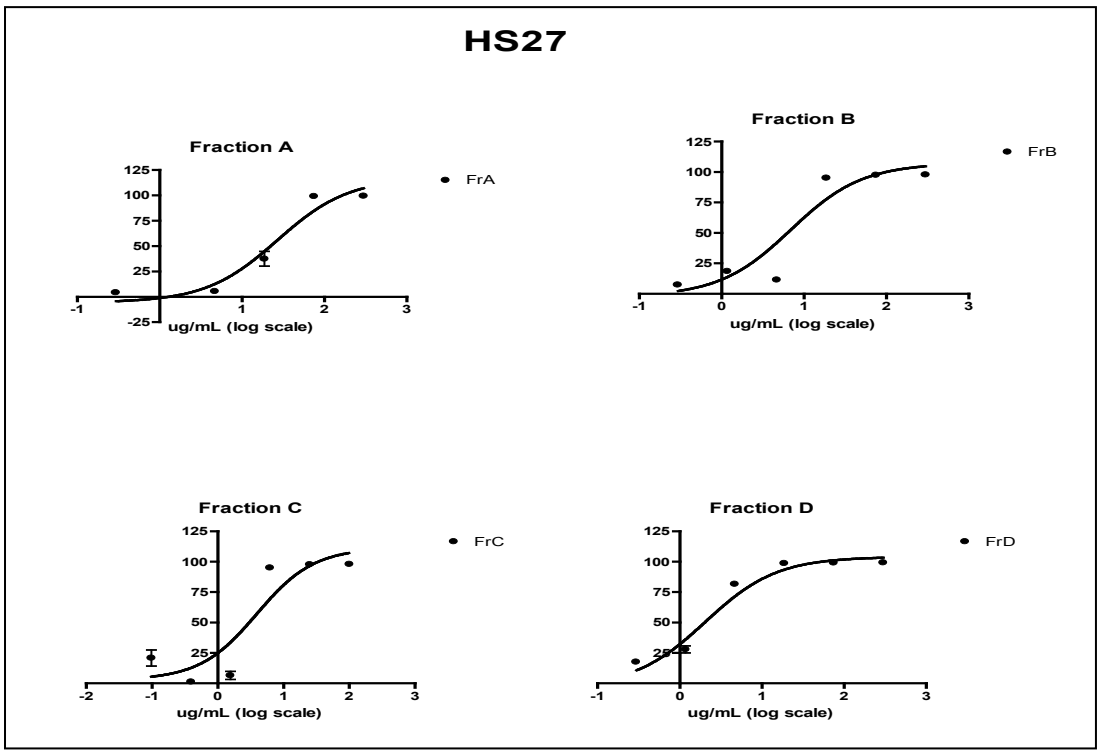
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
FrD	300.000	14.6	99.6 ± 0.1
	75.000		99.2 ± 0.2
	18.750		89.9 ± 0.5
	4.688		39.9 ± 4.6
	1.172		31.5 ± 1.3
	0.293		45.5 ± 9.3
FrE	100.000	7.78	99.2 ± 0.3
	25.000		90.2 ± 8.2
	6.250		69.4
	1.563		-
	0.391		21.2
	0.098		49.2 ± 15.3
FrF	100.000	7.37	99.1 ± 0.5
	25.000		89.9
	6.250		81.5
	1.563		68.2
	0.391		63.7 ± 1.4
	0.098		62.4



Appendix Figure 4. Cytotoxic results on P388 cells of Fraction A, B, C, D, E, F.

Appendix Table 8. Cytotoxic results on HS27 cells of Fraction A, B, C, D.

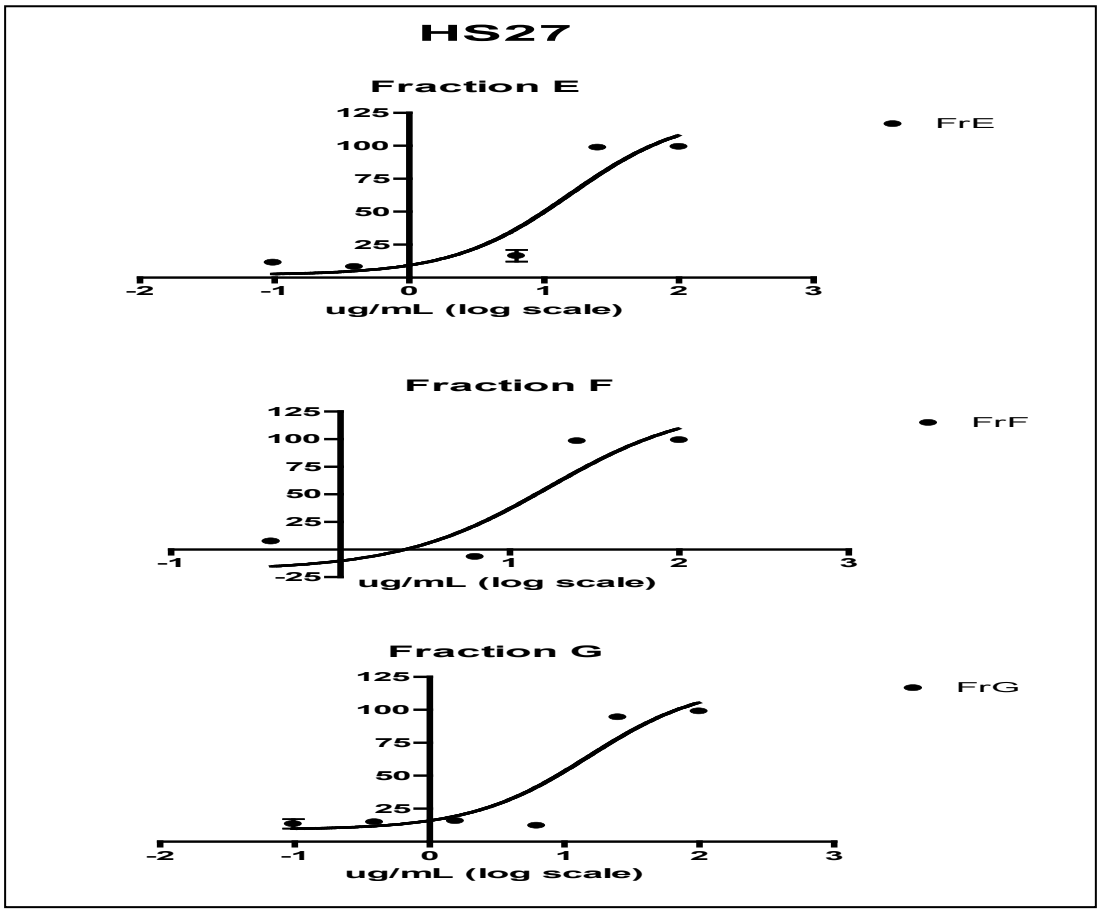
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
FrA	300.000	26.84	99.4
	75.000		99.0
	18.750		37.5 ± 10.3
	4.688		5.5
	1.172		-
	0.293		4.3
FrB	300.000	6.9	97.8 ± 0.7
	75.000		97.6 ± 0.6
	18.750		95.2 ± 2.2
	4.688		11.5
	1.172		18.6
	0.293		7.5
FrC	100.000	3.92	98.1 ± 0.8
	25.000		97.8 ± 0.7
	6.250		95.1 ± 2.7
	1.563		6.4 ± 4.6
	0.391		1.2
	0.098		20.8 ± 9.4
FrD	300.000	2.05	99.3
	75.000		99.2
	18.750		98.7 ± 0.1
	4.688		81.7
	1.172		27.9 ± 4.1
	0.293		17.5



Appendix Figure 5. Cytotoxic results on HS27 cells of Fraction A, B, C, D.

Appendix Table 9. Cytotoxic results on HS27 cells of Fraction E, F, G.

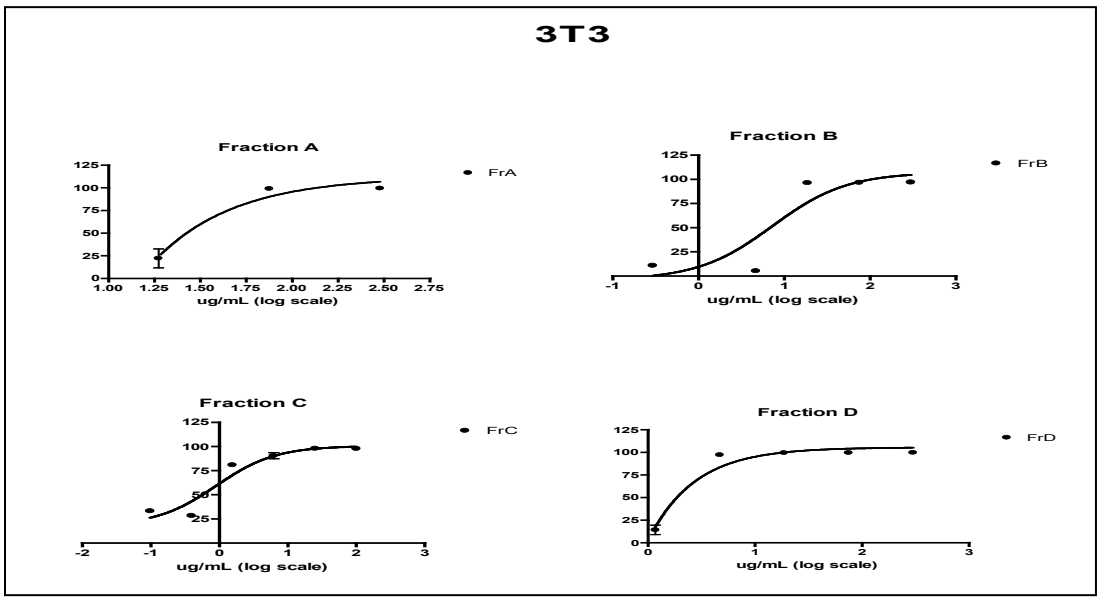
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
FrE	100.000	15.59	99.5
	25.000		98.8
	6.250		16.6 ± 6.3
	1.563		-
	0.391		8.3 ± 3.2
	0.098		11.5
FrF	100.000	16.97	99.3
	25.000		98.4 ± 0.1
	6.250		6.5 ± 0.3
	1.563		-
	0.391		7.5
	0.098		-
FrG	100.000	15.09	99.0 ± 0.4
	25.000		94.6 ± 0.2
	6.250		12.3
	1.563		15.8
	0.391		15.0 ± 2.7
	0.098		13.4 ± 5.1



Appendix Figure 6. Cytotoxic results on HS27 cells of Fraction E, F, G.

Appendix Table 10. Cytotoxic results on 3T3 cell of Fraction A, B, C, D.

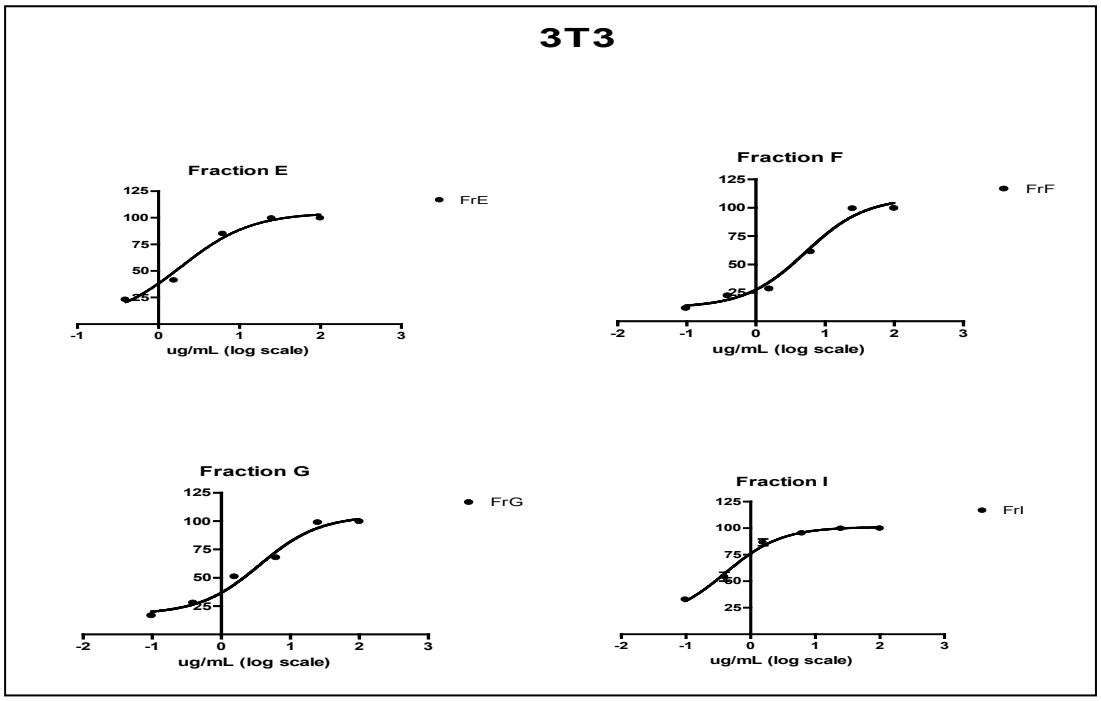
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
FrA	300.000	0.07	99.5 ± 0.2
	75.000		99.2 ± 0.8
	18.750		22.3 ± 14.8
	4.688		-
	1.172		-
	0.293		-
FrB	300.000	7.27	97.1 ± 1.2
	75.000		96.6 ± 1.7
	18.750		96.4 ± 2.3
	4.688		5.2
	1.172		-
	0.293		10.9
FrC	100.000	0.9	97.9 ± 1.7
	25.000		98.0 ± 1.4
	6.250		90.5 ± 4.5
	1.563		80.9
	0.391		28.4
	0.098		33.3
FrD	300.000	0.003	99.8
	75.000		99.6 ± 0.1
	18.750		99.5 ± 0.2
	4.688		97.3 ± 1.4
	1.172		14.3 ± 7.3
	0.293		-



Appendix Figure 7. Cytotoxic results on 3T3 cell of Fraction A, B, C, D.

Appendix Table 11. Cytotoxic results on 3T3 cell of Fraction E, F, G, I.

Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
FrE	100.000	1.9	99.7
	25.000		99.6 ± 0.1
	6.250		84.9 ± 0.5
	1.563		41.3
	0.391		23.0
	0.098		
FrF	100.000	5.18	99.7
	25.000		99.3 ± 0.1
	6.250		61.3
	1.563		28.6
	0.391		22.5 ± 0.3
	0.098		11.5
FrG	100.000	3.6	99.7
	25.000		98.9 ± 0.7
	6.250		67.9 ± 1.9
	1.563		51.0
	0.391		27.9
	0.098		16.7
FrI	100.000	0.41	99.8
	25.000		99.6
	6.250		95.3 ± 0.3
	1.563		86.7 ± 4.5
	0.391		54.2 ± 6.0
	0.098		32.8



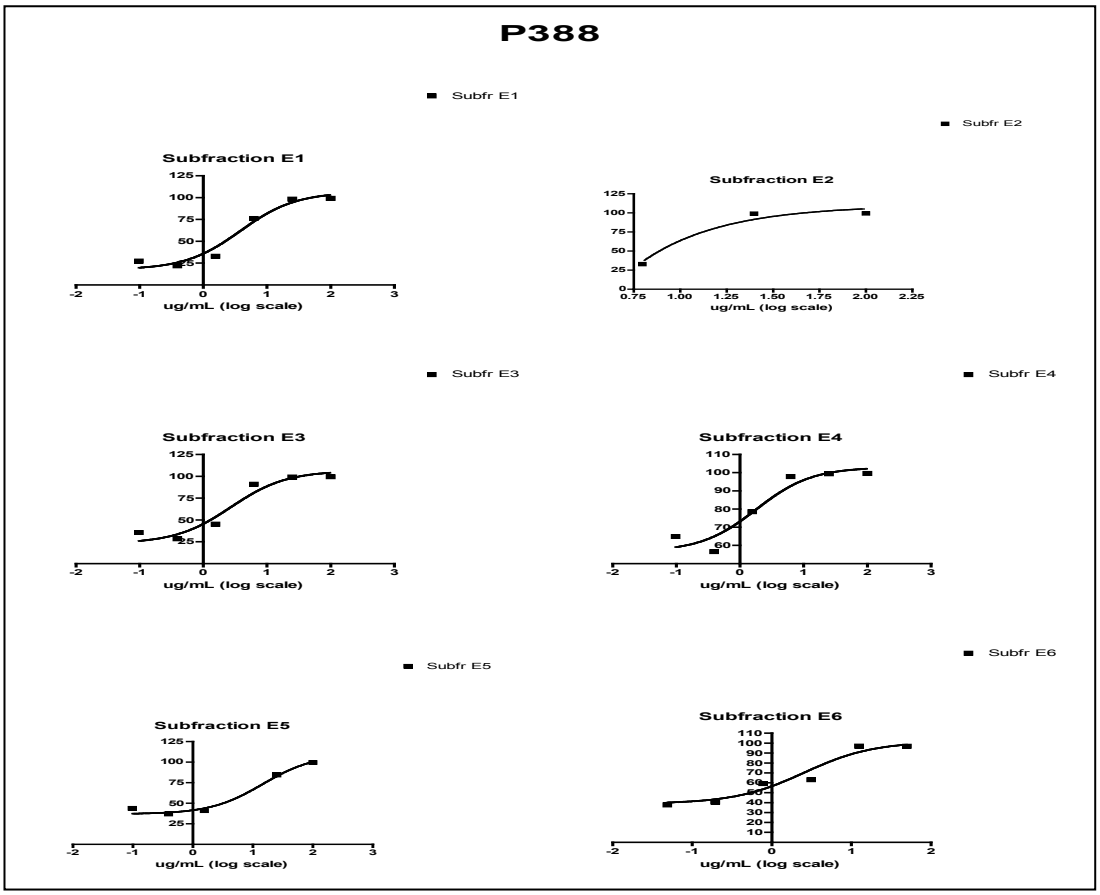
Appendix Figure 8. Cytotoxic results on 3T3 cell of Fraction E, F, G and I

Appendix Table 12. Cytotoxic results on P388 cells of subfraction E_1, E_2 and E_3.

Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Fr E_1	100.000	3.84	99.1 ± 0.107
	25.000		98.3 ± 0.6
	6.250		76.1 ± 1.9
	1.563		32.9
	0.391		21.8
	0.098		27.3
Fr E_2	100.000	0.01	99.4 ± 0.1
	25.000		99.0 ± 0.2
	6.250		33.0
	1.563		-
	0.391		-
	0.098		-
Fr E_3	100.000	2.72	99.5
	25.000		99.2 ± 0.1
	6.250		91.0 ± 0.7
	1.563		45.1 ± 12.0
	0.391		28.3
	0.098		35.5

Appendix Table 13. Cytotoxic results on P388 cells of subfraction E_4, E_5 and E_6.

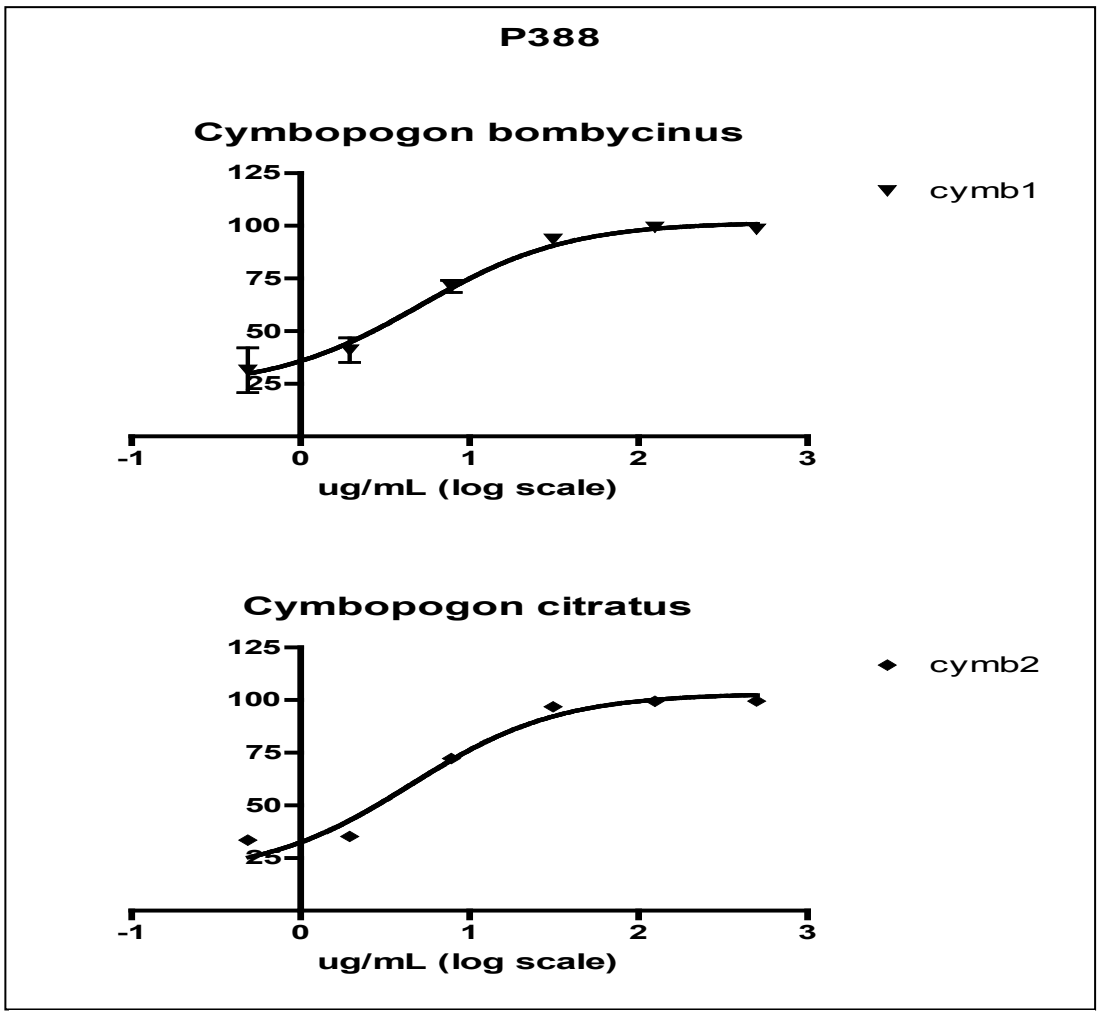
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Fr E_4	100.000	1.81	99.6 ± 0.1
	25.000		99.4 ± 0.2
	6.250		97.9 ± 1.3
	1.563		78.5 ± 2.5
	0.391		56.6 ± 3.5
	0.098		64.9 ± 4.3
Fr E_5	100.000	14.5	99.5
	25.000		84.3 ± 11.6
	6.250		-
	1.563		40.9 ± 4.4
	0.391		36.9
	0.098		43.8
Fr E_6	50.000	2.55	96.8
	12.500		97.0
	3.125		63.1
	0.781		59.3 ± 1.2
	0.195		40.7
	0.049		37.7



Appendix Figure 9. Cytotoxic results on P388 cells of all subfractions (Subfr. E_1 –

Appendix Table 14. Cytotoxic results on P388 cells of *Cymbopogon bombycinus* and *citratus* (Cymb1; Cymb2).

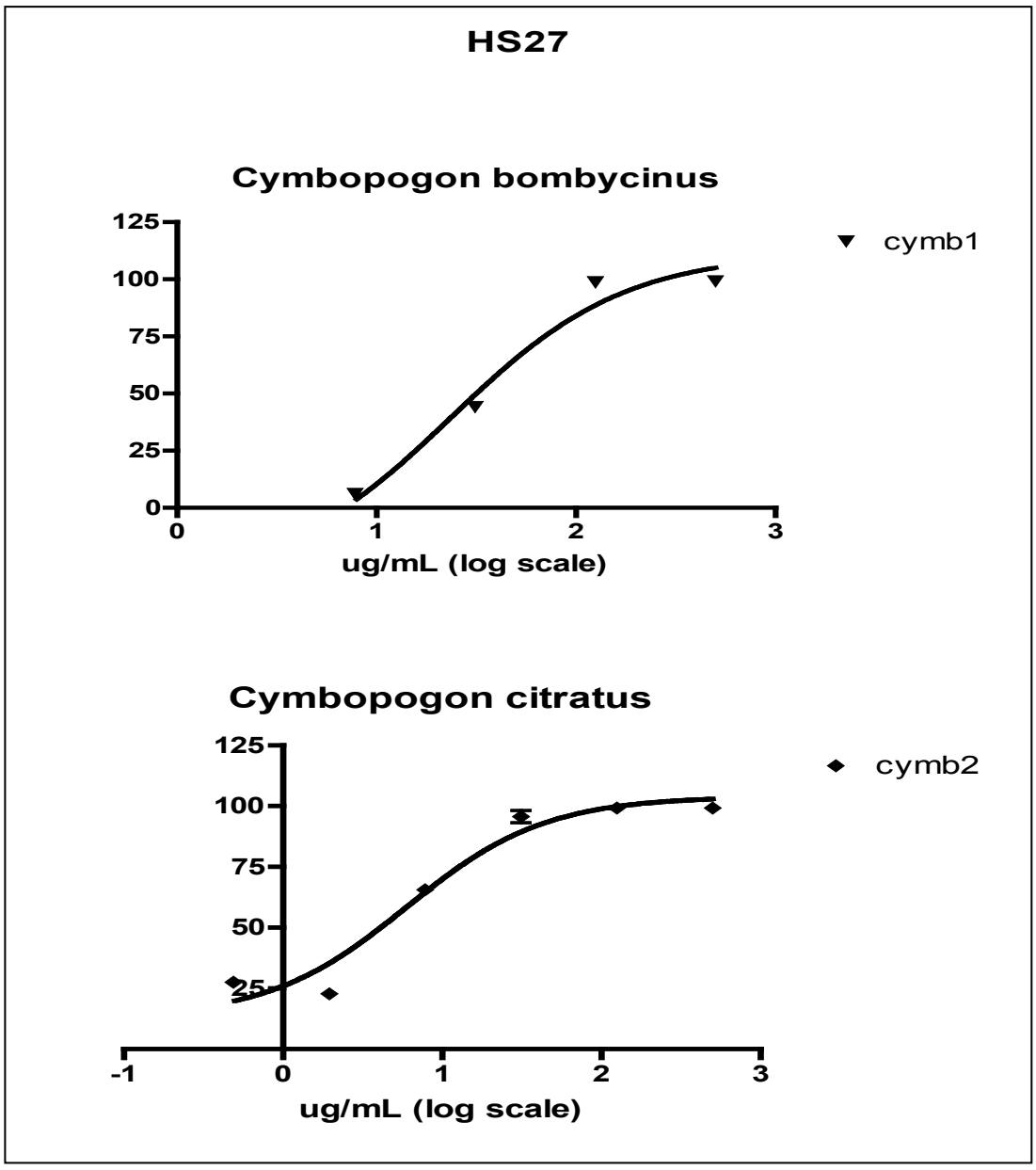
Sample	**Conc. µg/mL**	**IC_{50} (µg/mL)**	**% Inhibition**
Cymb1	500.000	5.14	98.4 ± 2.2
	125.000		99.4 ± 0.2
	31.250		93.6 ± 4.2
	7.813		71.2 ± 5.0
	1.953		41.0 ± 10.1
	0.488		31.4 ± 21.2
Cymb2	500.000	4.52	99.6 ± 0.1
	125.000		99.4 ± 0.1
	31.250		96.8 ± 3.0
	7.813		72.2 ± 4.0
	1.953		35.3 ± 0.1
	0.488		33.5



Appendix Figure 10. Cytotoxic results on P388 cells of *Cymbopogon bombycinus* and *citratus* (Cymb1; Cymb2).

Appendix Table 15. Cytotoxic results on HS27 cells of *Cymbopogon bombycinus* and *citratus* (Cymb1; Cymb2).

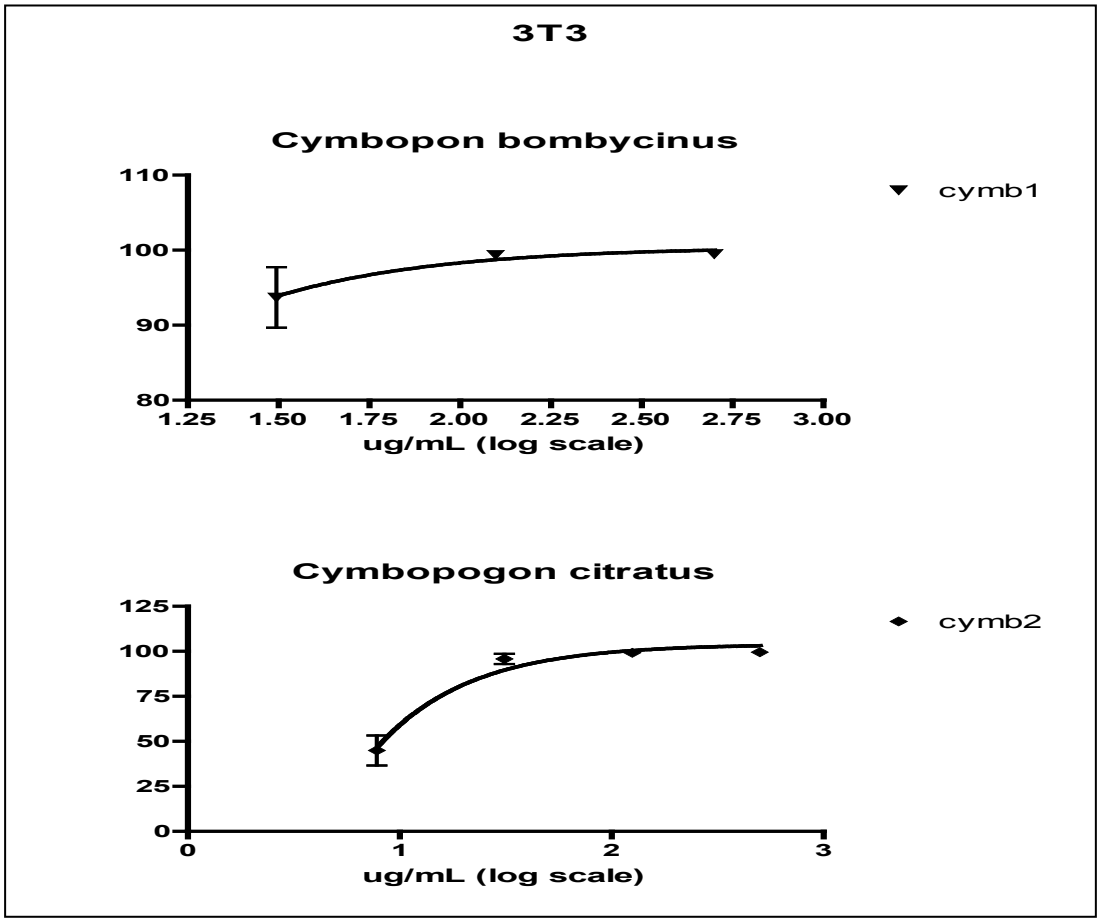
Sample	**Conc. µg/mL**	**IC_{50} (µg/mL)**	**% Inhibition**
Cymb1	500.000	23.6	99.1 ± 0.2
	125.000		98.7 ± 0.4
	31.250		44.1 ± 3.3
	7.813		6.1
	1.953		-
	0.488		-
Cymb2	500.000	5.6	99.3
	125.000		99.2 ± 0.1
	31.250		95.7 ± 3.5
	7.813		65.6
	1.953		22.8
	0.488		27.6



Appendix Figure 11. Cytotoxic results on HS27 cells of *Cymbopogon bombycinus* and *citratus* (Cymb1; Cymb2).

Appendix Table 16. Cytotoxic results on 3T3 cells of *Cymbopogon bombycinus* and *citratus* (Cymb1; Cymb2).

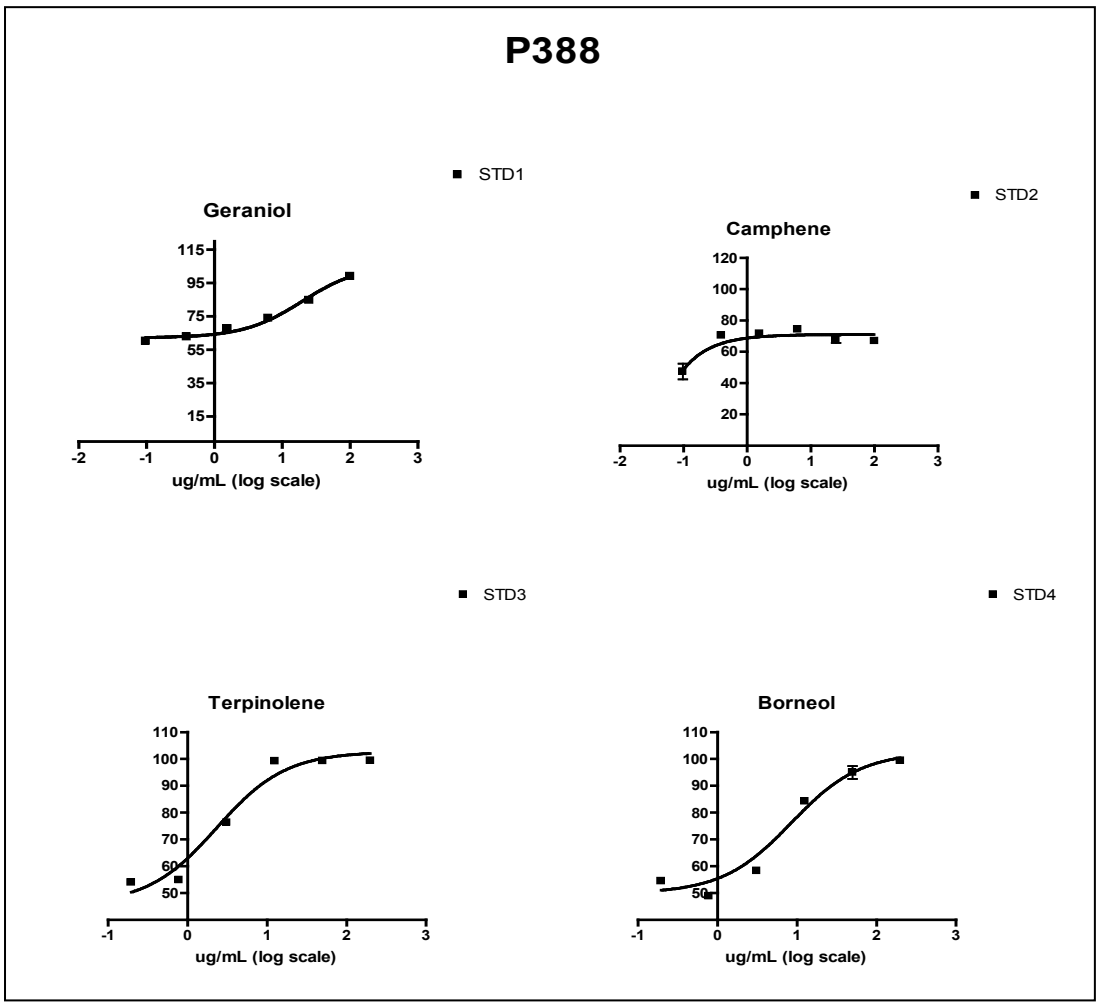
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Cymb1	500.000	1.4	99.5
	125.000		99.4
	31.250		93.7 ± 5.7
	7.813		-
	1.953		-
	0.488		-
Cymb2	500.000	0.03	99.5
	125.000		99.3
	31.250		95.8 ± 4.0
	7,813		45.0 ± 11.8
	1953		-
	0488		-



Appendix Figure 12. Cytotoxic results on 3T3 cells of *Cymbopogon bombycinus* and *citratus* (Cymb1; Cymb2).

Appendix Table 17. Cytotoxic results on P388 cells of geraniol, camphene and terpinolene.

Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Geraniol	100.000	19.89	99.1 ± 0.7
	25.000		84.6
	6.250		74.0
	1.563		67.6
	0.391		62.9
	0.098		60.2
Camphene	100.000	0.001	66.9
	25.000		68.0 ± 3.4
	6.250		74.5
	1.563		71.7
	0.391		70.6
	0.098		47.3 ± 7.1
Terpinolene	100.000	2.32	99.4 ± 0.1
	25.000		99.3
	6.250		99.2 ± 0.2
	1.563		76.2
	0.391		54.8
	0.098		54.0



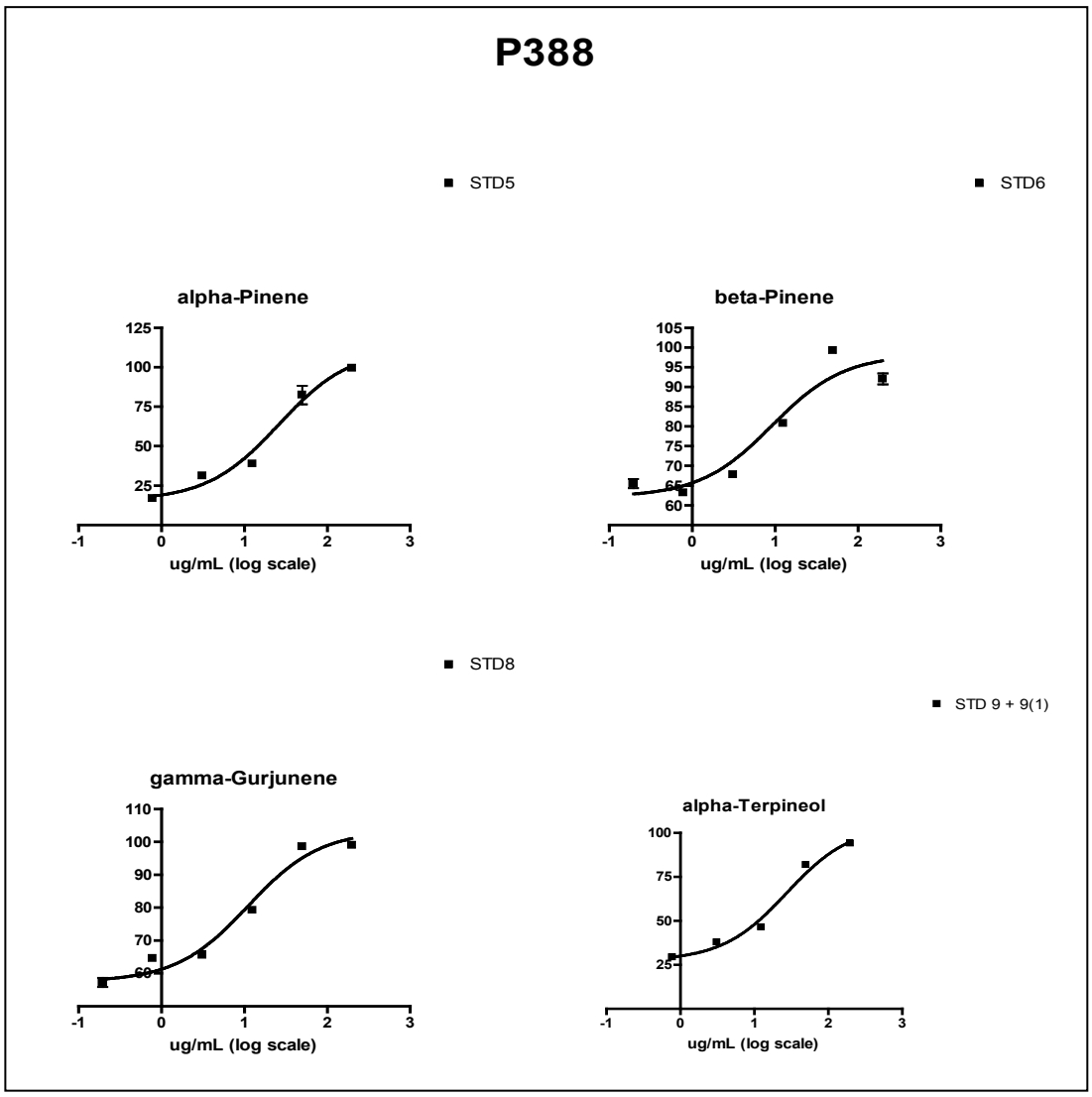
Appendix Figure 13. Cytotoxic results on P388 cells of geraniol, camphene, terpinolene and borneol.

Appendix Table 18. Cytotoxic results on P388 cells of borneol, α–pinene and β–pinene.

Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Borneol	100.000	8.47	99.4 ± 0.1
	25.000		95.0 ± 3.4
	6.250		84.2 ± 1.5
	1.563		58.3
	0.391		48.8
	0.098		54.5 ± 0.5
α-Pinene	100.000	26.03	99.5
	25.000		82.5 ± 8.3
	6.250		38.8
	1.563		31.2
	0.391		16.8
	0.098		-
β-Pinene	100.000	9.13	92.0 ± 2.0
	25.000		99.3 ± 0.1
	6.250		80.8
	1.563		67.8
	0.391		63.2 ± 0.7
	0.098		65.5 ± 1.6

Appendix Table 19. Cytotoxic results on P388 cells of γ-gurjunene and α–terpineol.

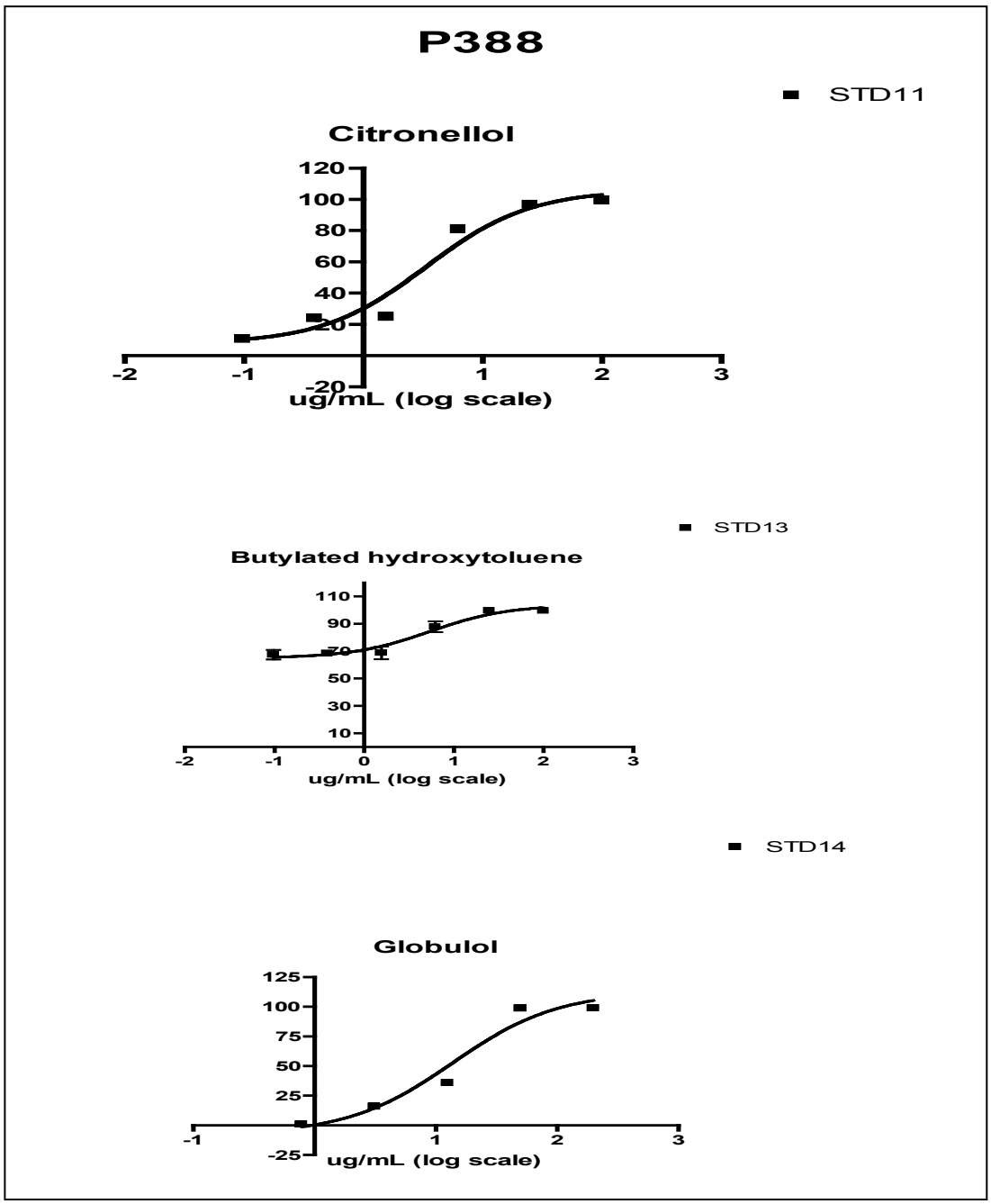
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
γ–Gurjunene	100.000	11.03	99.0
	25.000		98.6 ± 0.6
	6.250		79.2
	1.563		65.6
	0.391		64.6
	0.098		57.2 ± 1.9
α-Terpineol	100.000	27.27	94.0 ± 0.4
	25.000		81.8
	6.250		46.4
	1.563		37.7
	0.391		29.3
	0.098		-



Appendix Figure 14. Cytotoxic results on P388 cells of α–pinene, β–pinene, γ–gurjunene and α–terpineol.

Appendix Table 20. Cytotoxic results on P388 cells of citronellol, butylated hydroxytoluene and globulol.

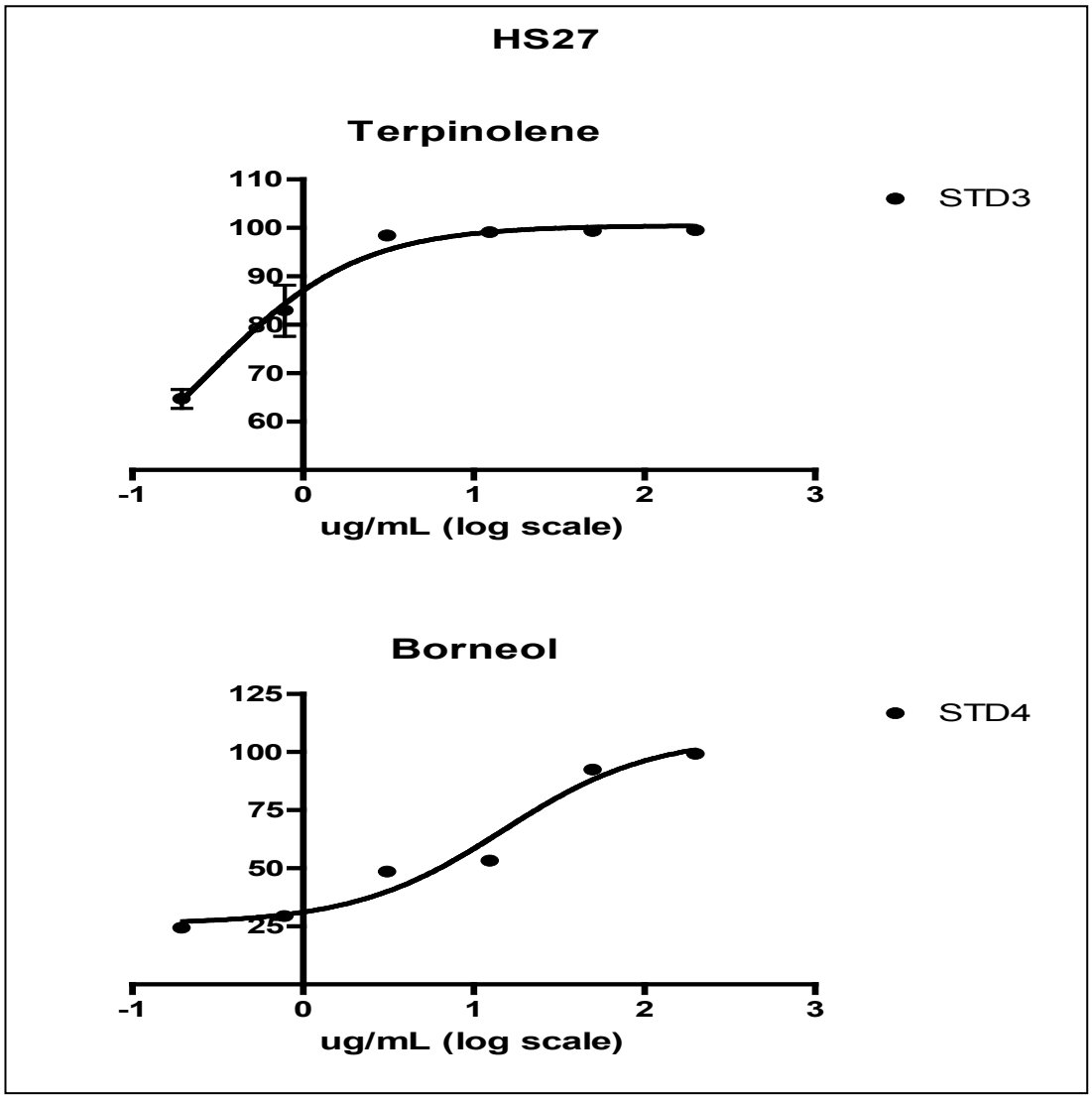
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Citronellol	100.000	3.36	99.5
	25.000		96.8 ± 3.4
	6.250		81.1
	1.563		24.9
	0.391		24.0
	0.098		10.7
Butylated Hydroxytoluene	100.000	4.42	99.4 ± 0.3
	25.000		99.5 ± 0.2
	6.250		83.9
	1.563		73.5
	0.391		66.5
	0.098		63.9
Globulol	100.000	13.75	98.9
	25.000		98.6 ± 0.4
	6.250		35.8
	1.563		16.0
	0.391		0.8
	0.098		-



Appendix Figure 15. Cytotoxic results on P388 cells of citronellol, butylated hydroxytoluene and globulol.

Appendix Table 21. Cytotoxic results on HS 27 cells of terpinolene, borneol, α–pinene and β–pinene.

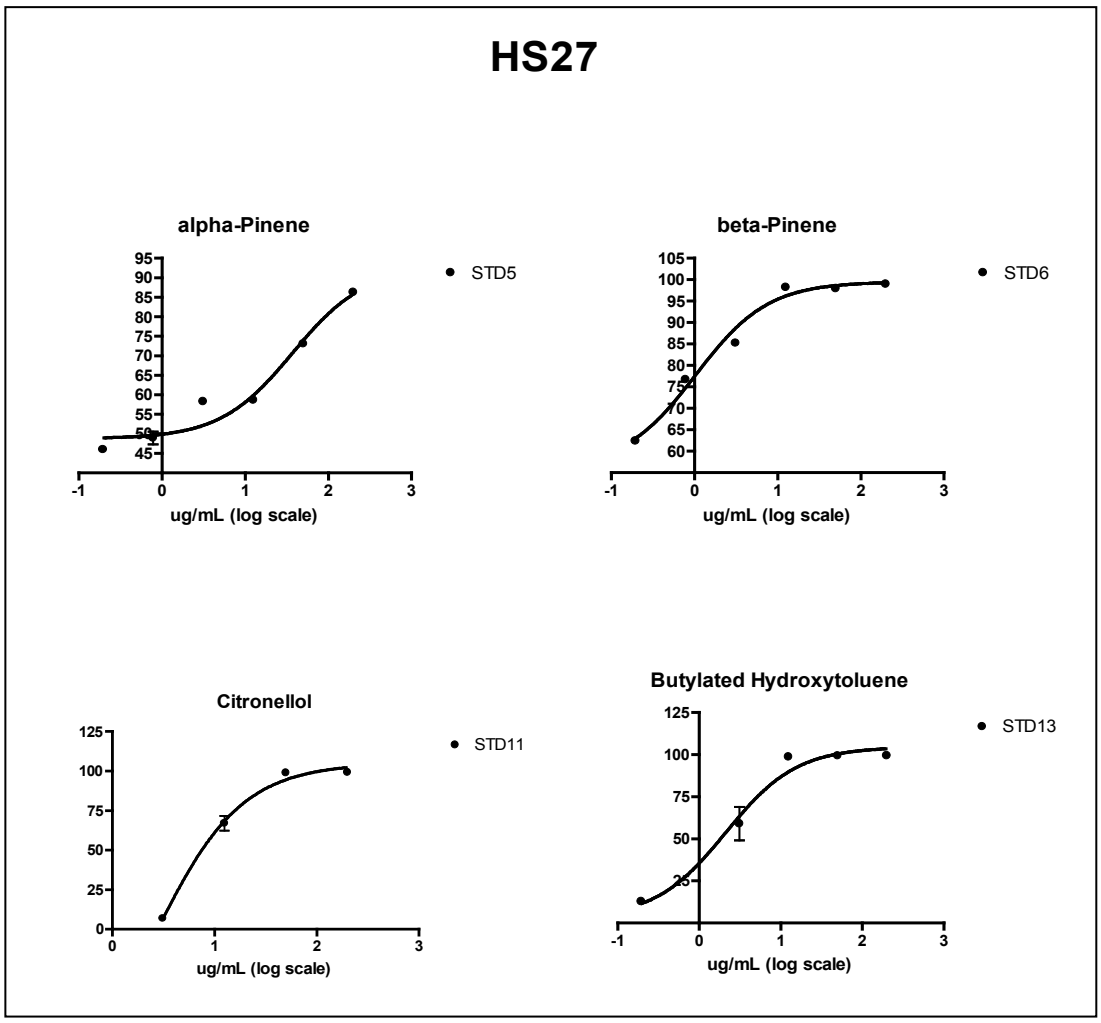
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Terpinolene	100.000	0.28	99.5
	25.000		99.3 ± 0.2
	6.250		99.1
	1.563		98.4
	0.391		82.9
	0.098		64.7
Borneol	100.000	14.89	99.1 ± 0.2
	25.000		92.3 ± 1.5
	6.250		53.2
	1.563		48.5
	0.391		29.3
	0.098		24.4
α–Pinene	100.000	36.93	86.3
	25.000		73.0
	6.250		58.6
	1.563		58.3
	0.391		48.9 ± 2.3
	0.098		45.9
β-Pinene	100.000	1.04	98.9
	25.000		97.9 ± 0.3
	6.250		98.2 ± 0.6
	1.563		85.2
	0.391		76.7
	0.098		62.4



Appendix Figure 16. Cytotoxic results on HS 27 cells of terpinolene and borneol.

Appendix Table 22. Cytotoxic results on HS 27 cells of citronellol and butylated hydroxytoluene.

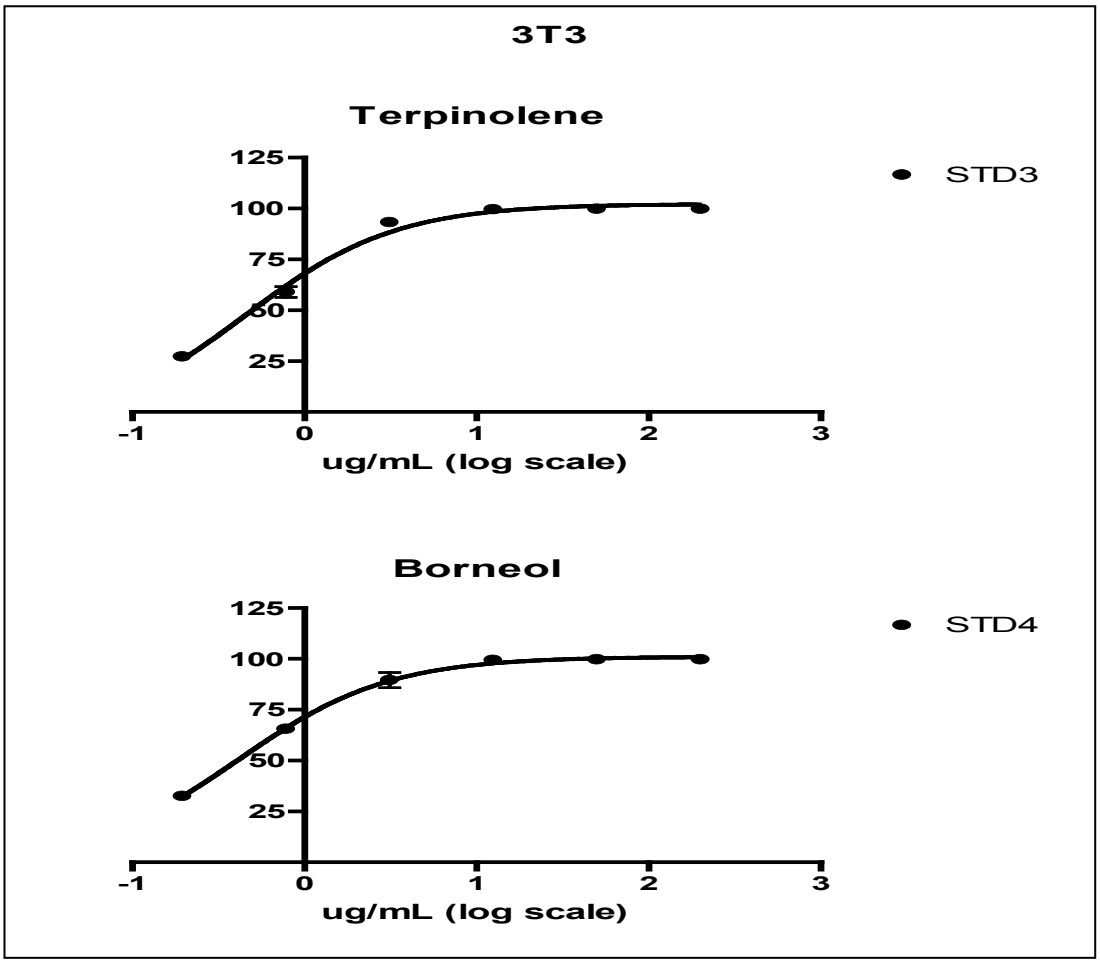
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Citronellol	100.000	2.42	99.2
	25.000		98.9 ± 0.3
	6.250		67.0 ± 6.6
	1.563		6.8
	0.391		-
	0.098		-
Butylated Hydroxytoluene	100.000	2.12	99.5
	25.000		99.3
	6.250		98.8 ± 0.1
	1.563		59.0
	0.391		-
	0.098		12.8



Appendix Figure 17. Cytotoxic results on HS 27 cells of α–pinene, β–pinene, cironellol and butylated hydroxytoluene.

Appendix Table 23. Cytotoxic results on 3T3 cells of terpinolene, borneol, α–pinene and β–pinene.

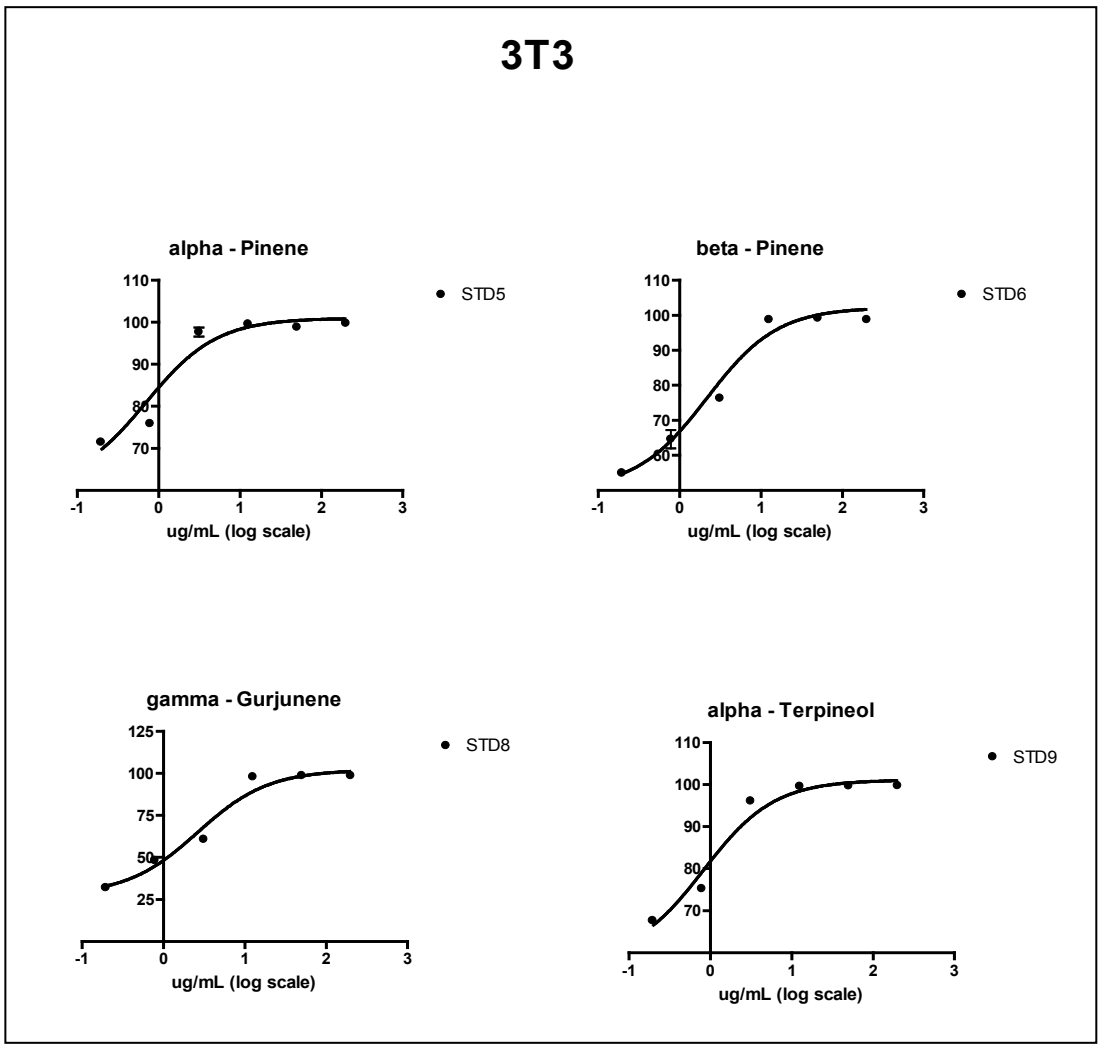
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Borneol	100.000	0.42	99.8 ± 0.1
	25.000		99.8
	6.250		99.5 ± 0.1
	1.563		89.6 ± 5.3
	0.391		65.6 ± 1.3
	0.098		32.6 ± 3.0
α–Pinene	100,000	0.71	99.8 ± 0.1
	25,000		98.9 ± 1.3
	6,250		99.6 ± 0.1
	1,563		97.7 ± 1.5
	0,391		75.9
	0,098		71.5
β-Pinene	100.000	2.17	98.8
	25.000		99.2 ± 0.7
	6.250		98.8 ± 0.7
	1.563		76.4
	0.391		64.6 ± 3.7
	0.098		55.0
Terpinolene	100.000	0.46	99.9
	25.000		99.8
	6.250		99.6 ± 0.1
	1.563		93.3 ± 0.2
	0.391		59.0 ± 3.7
	0.098		27.2



Appendix Figure 19. Cytotoxic results on 3T3 cells of terpinolene and borneol.

Appendix Table 24. Cytotoxic results on 3T3 cells of γ–gurjunene and α–terpineol.

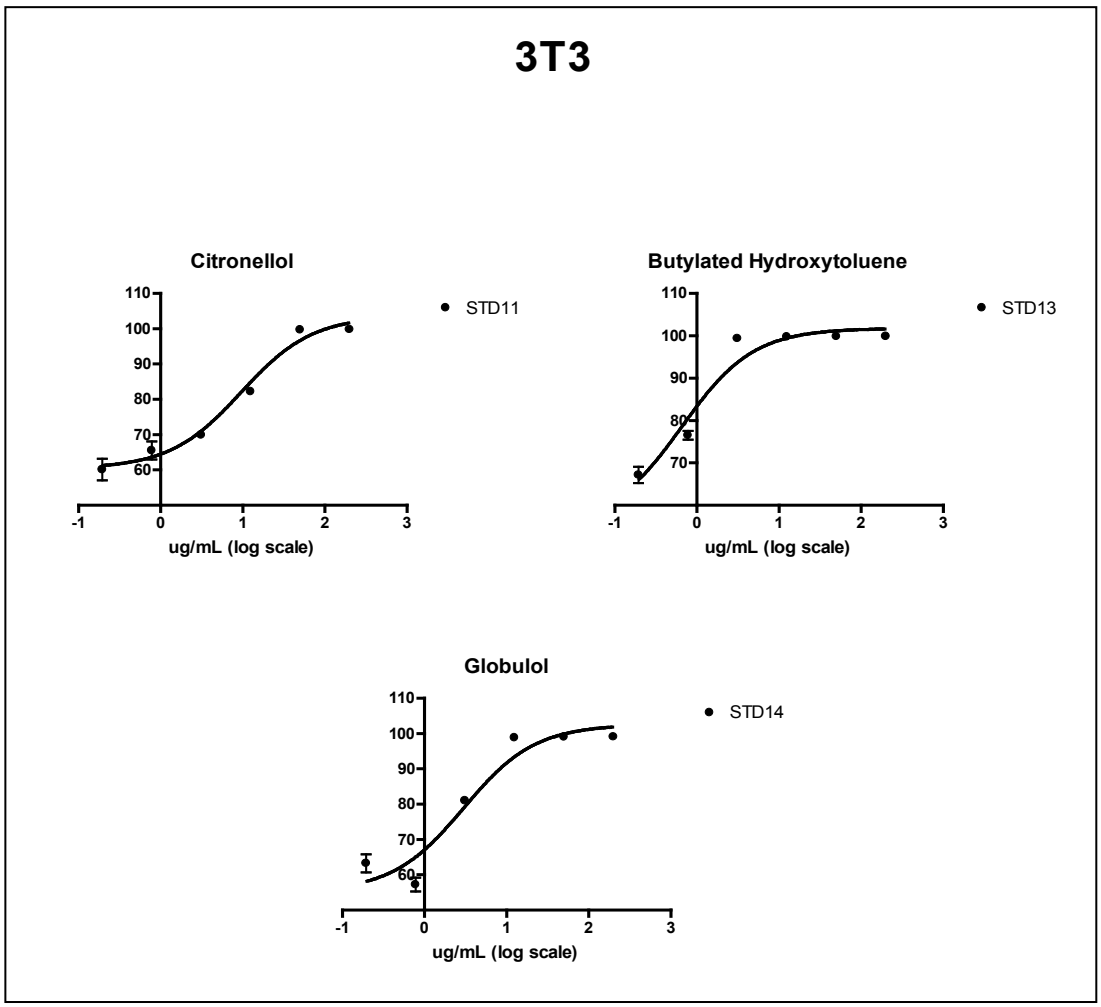
Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
γ-Gurjunene	100.000	2.64	98.8 ± 0.7
	25.000		98.7 ± 0.5
	6.250		98.1
	1.563		60.9
	0.391		48.3
	0.098		32.0
α-Terpineol	100.000	0.82	99.8
	25.000		99.7
	6.250		99.6
	1.563		96.2
	0.391		75.3
	0.098		67.7



Appendix Figure 19. Cytotoxic results on 3T3 cells of α–pinene, β–pinene, γ–gurjunene and α–terpineol.

Appendix Table 25. Cytotoxic results on 3T3 cells of citronellol, butylated hydroxytoluene and globulol.

Sample	Conc. µg/mL	IC_{50} (µg/mL)	% Inhibition
Citronellol	100.000	5.63	99.8 ± 0.1
	25.000		99.7 ± 0.1
	6.250		82.2
	1.563		69.9
	0.391		65.5 ± 3.6
	0.098		60.1 ± 4.3
Butylated Hydroxytoluene	100.000	0.66	99.9
	25.000		99.9
	6.250		99.8
	1.563		99.4 ± 0.2
	0.391		76.5 ± 1.5
	0.098		67.2 ± 2.7
Globulol	100.000	3.00	99.2 ± 0.4
	25.000		99.1 ± 0.3
	6.250		98.9 ± 0.2
	1.563		81,0
	0.391		57.2 ± 2.7
	0.098		63.3 ± 3.6



Appendix Figure 20. Cytotoxic results on 3T3 cells of citronellol, butylated hydroxytoluene and globulol.

Appendix 2

(Antioxidant activity results)

Appendix Table 26. Antioxidant activity results summarized.

Sample	ORAC value µmol TE/g
Calytrix exstipulata leaves	66 ± 4
Calytrix exstipulata stems	n.a.
Cymbopogon bombycinus	356 ± 35
Cymbopogon citratus	298 ± 42
Fraction A	n.a.
Fraction B	n.a.
Fraction C	59 ± 32
Fraction D	209 ± 30
Fraction E	756 ± 24
Fraction F	865 ± 124
Fraction G	416 ± 93
Fraction H	n.a.
Fraction I	n.a.
Subfraction E_1	1053 ± 20
Subfraction E_2	578 ± 128
Subfraction E_3	589 ± 14
Subfraction E_4	1185 ± 240
Subfraction E_5	597 ± 62
Subfraction E_6	n.a.
Geraniol	969 ± 220
Borneol	n.a.
Terpinolene	n.a.
Camphene	n.a.
α–Pinene	n.a.
β–Ppinene	n.a.
α–Terpineol	1343 ± 588
Citronellol	586 ± 58
Globulol	574 ± 366
Butylated Hydroxytoluene	n.a.
γ–Gurjunene	n.a.
γ–Terpinene	n.a.

Curriculum Vitae

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Personal Details

Nationality:	Austria
Date of birth:	15.05.1985
Place of birth:	Kotor - Varos (Bosnia- Herzegovina)
Marital status:	Single

Academic studies

Mar 2006 – Feb 2012	Master study Pharmacy at University of Vienna
Oct 2008 ~	Bachelor study International Business Studies at Vienna University of Economics and Business
Mar 2011 – Jul 2011	Visiting student at Southern Cross University in Lismore Master thesis: *„Phytochemistry and pharmacology of volatile components of Calytrix exstipulata & Cymbopogon bombycinus“*

Education

Sept 1993 – Jun 1996	Elementary School (Austria)
Sept 1996 – Jun 2005	Secondary School (Wels, Austria)

Language skilss

Bosnian/ Croatian/ Serbian	native language
German	native language niveau
English	fluently
Spanish	very good written and spoken knowledge

Work experience

Feb 2001 – Jul 2003	Studentjob Mc Donald's (Wels, Upper Austria)
Aug 2003 – Dec 2003	Studentjob at Burger King (Wels, Upper Austria)
Sept 2003 – Sept 2005	Studentjob at Orsay; apparel store (Wels, Upper Austria)
Jun 2006 – Jun 2008	Studentjob at ITH; catering company (Wels, Upper Austria)
Aug 2007	Internship at Schutzengel Apotheke (Wels, Upper Austria)
Jul 2008	Internship at Stern Apotheke (Wels, Upper Austria)
Aug 2008	Internship at Linden Apotheke (Wels, Upper Austria)
Jun 2008 – Nov 2009	Studentjob at Siemens; promotion company (Vienna, Austria)
Jun 2010 – Feb 2011	Studentjob at Pol 1; promotion company (Vienna, Austria)
Oct 2011 – Feb 2012	Private tutor at Lernexpress (Vienna, Austria)