T.R.N.C

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

ESSENTIAL OIL COMPOSITION AND ETHNOBOTANICAL PROFILE OF SOME SELECTED SPECIES OF *EUCALYPTUS* GROWING IN CYPRUS

SAMUEL BUKUNMI ADEDIRAN

MASTERS THESIS

DEPARTMENT OF PHARMACEUTICAL BOTANY

ADVISOR

Prof. Dr. Dudu ÖZKUM YAVUZ

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THESIS APPROVAL

The signed one should added here for prepared hard copy

STATEMENT (DECLARATION)

Hereby I declare that this thesis study is my own study, I had no unethical behaviour in all stages from planning of the thesis until writing thereof, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of the information and comments which could not be obtained by this thesis study and took the reference list and had no behaviour of breeching patent rights and copyright infringement during the study and writing of this thesis.

Samuel B. Adediran

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ABBREVIATION AND SYMBOLS

GC: Gas Chromatography

GC-MS: Gas Chromatography and Mass Spectrometry

WHO: World Health Organization

NEU: Near East University

TRNC: Turkish Republic of Northern Cyprus

US: United States

NEUN: Near East University Herbarium

LRI: Linear Rention Index

ÖZET

Öğrencinin Adı-Soyadı: Samuel Bukunmi Adediran

Danışman: Prof. Dr. Dudu Özkum Yavuz

Eş Danışman: Yrd. Doç. Dr. Duygu Yiğit Hanoğlu

Departman: Farmasötik Botanik

Ökaliptüs cinsi, derimsi yaprakları olan uzun, yaprak dökmeyen ağaçlardan oluşur ve Myrtaceae familyasına aittir. Avustralya'ya özgüdürler ve yaklaşık 900 tür ve alt tür içerirler. Bu cinse ait türlerden elde edilen uçucu yağlar, tıbbi ve farmasötik endüstrilerde değerlidirler. Kıbrıs'ta 47'si sınıflandırılmış ancak 15'i sınıflandırılmamış veya melez olmayan yaklaşık 62 Okaliptüs türü bulunmaktadır. Kıbrıs'ta bulunan Okaliptüs türlerinden bazıları; *E. camaldulensis*, *E. occidentalis*, *E. torquata*, *E. gomphocephala*, *E. leucoxylon*, *E.odorata*'dır. Bu çalışmanın amacı, Kıbrıs'ta yetişen Okaliptüs ağaçlarının geleneksel kullanımlarını araştırmak ve kozmetik ve ilaç endüstrisinde değerli olan fitokimyasal uçucu bileşenlerini analiz etmektir. Kıbrıs'ta yetişen Ökaliptüs (*E. camaldulensis* ve *E. torquata*) örnekleri hidrodistilasyon yöntemi ile izole edilmiş ve GC ve GC –MS ile eş zamanlı olarak analiz edilmiştir. *E. camaldulensis* yağının verimi %2.4'tür. Tanımlanan ana bileşikler sırasıyla α-fellandren (%10.3) ve β- fellandren (%30.6)'dir. Diğer önemli bileşikler ise *p*-simen (%8.2), bisiklogermakren (%6.1) ve spatulenol (%9.3) olarak belirlenmiş. Öte yandan, *E. torquata* yağı verimi %1.6'tür. Ana bileşikler α-pinen (%18.6), 1,8-sineol (%18.8), β-ödesmol (%10.3) ve torkuaton (29.2%) olarak belirlenmiştir.

Anahtar Kelimeler: *Eucalyptus*, Uçucu Yağ, *E. camaldulensis*, *E. torquata*, GC, GC-MS, Etnobotanik, Kıbrıs

ABSTRACT

Name of the Student: Samuel Bukunmi Adediran

Advisor: Prof. Dr. Dudu Özkum Yavuz

Co-Advisor: Assist. Prof. Dr. Duygu Yiğit Hanoğlu

Department: Pharmaceutical Botany

The Genus *Eucalyptus* consists of tall, evergreen trees with leathery leaves and belongs to the family Myrtaceae. They are indigenous to Australia and contain about 900 species and subspecies. One important use is in the production of essential oils, which are valuable in medicinal and pharmaceutical industries. There are about 62 species of *Eucalyptus* found in Cyprus, of which 47 are classified but 15 are not classified, or are hybrid. *E. Camaldulensis*, *E. occidentalis, E. torquata, E. gomphocephala, E. leucoxylon, E.odorata,* are some of the species of *Eucalyptus* found in Cyprus. The aim of this study is to investigate the traditional uses of *Eucalyptus* trees growing in Cyprus and to analyse essential oil constituents, which are valuable in cosmetic and pharmaceutical industries. The essential oil constituents of leaves of two species of *Eucalyptus* (*E. camaldulensis and E. torquata)* growing in Cyprus were isolated by hydro-distillation and analysed by GC and GC –MS, simultaneously. *E. camaldulensis* oil yield was higher (2.4%), and major compound identified are α-phellandrene (10.3%) and β-phellandrene (30.6%), respectively. These compounds were followed by *p*-cymene (8.2%), bicyclogermacrene (6.1%) and spathulenol (9.3%). On the other hand, *E. torquata* oil yield was relatively lower (1.6%), with major compounds being α-pinene (18.6%), 1,8-cineole (18.8%), β-eudesmol (10.3%) and torquatone (29.2%).

Keywords: *Eucalyptus*, Essential oil, *E. camaldulensis*, *E. torquata*, GC, GC-MS, Ethnobotany, Cyprus

1. INTRODUCTION

The genus *Eucalyptus* (commonly known as gum trees) is native to Australia and belongs to the family Myrtaceae (Myrtle family). The family Myrtaceae consists of over 140 genera and 3800 species (Bachir & Benali, 2012). It is also recognized in addition to families like, Lauraceae, Apiaceae, Lamiaceae, as an essential oil-bearing family. This genus consists of tall, evergreen trees, with leathery leaves. *Eucalyptus* is widespread throughout Australia water bodies and is an ecological, and economic symbol of the community (Ghasemian et al., 2019). The genus *Eucalyptus* occupies about 74% of the Australian forests and woodlands (Sørensen et al., 2020). The discovery of *Eucalyptus* is generally attributed to the voyages of Captain James Cook in the Endeavour of the 1700s. David Nelson, a botanist on Cook's third voyage, collected specimen on Bruny Island, and returned to England. This specimen was studied by a French botanist, Charles Louis L'Héritier de Brutelle, who was at the time, working at the British Museum, Natural History. Charles published the specimen in 1788 as the sole species of a newly discovered genus, which he called *Eucalyptus.* This name was coined from the Greek terms, *eu* and *calyptos* meaning well, and covered respectively**.** The name is a representation of the operculum, which covers the stamens in the bud before anthesis. Several species of *Eucalyptus* have since been discovered over the years (Coppen, 2002).

Figure 1.1. Operculum covering the stamens (Wikipedia, 2013)

Members of this family are usually trees or shrubs with oil glands in leaves and sometimes, other parts of the plants. More than three hundred (300) species of *Eucalyptus* have volatile essential oil in their leaves (Al-snafi, 2017). The essential oil of *Eucalyptus* is at the top of the world's most traded oil, with *E. citriodora* leading the line (Dhakad et al., 2018). The genus *Eucalyptus* is generally divided into 13 sub-genera (Brezáni & Šmejkal, 2013; Salehi et al., 2019). This group of plants, more than any other, gives the Australian landscape a peculiar character (Landrum et al., 1988). They also represent a major source of timber supply in Australia. Some species of *Eucalyptus* are main sources of nectar and pollen in honey production. Some are valuable as ornaments, fuels, and even for creating shelter. The ability of *Eucalyptus* trees to absorb large amount of water makes them suitable for drying swamps (Meriçli, 2019).

Recent records indicates that the genus *Eucalyptus* contains about 900 species and subspecies (Stankov et al., 2020). This number continues to increase with addition of newly described taxa. The genus *Eucalyptus* is widely planted in several parts of the world and is one of the world's most important genera (Salehi et al., 2019). The trees of *Eucalyptus* are fast growing, and well adapted to a wide range of climatic and edaphic conditions. These are some of the reasons for the successful cultivation of *Eucalyptus* in countries all over the world (Coppen, 2002). An example is seen in

Turkey, where over 20,000 ha of *Eucalyptus* are planted and thriving (Doğanlar & Doğanlar, 2008). Species of *Eucalyptus* play a vital role in the production of pollen. Majority of *Eucalyptus* species are fire-resistant and can start growing leaves, just after a fire (Ilseven & Baştaş, 2018). This may be attributed to numerous aerial perennating buds, protective bark, lignotubers, and woody, fire-resistant seeds. *Eucalyptus* trees are the most widely planted hardwood forest trees in the world (Myburg et al., 2014). *Eucalyptus* are mainly fast growing, tall trees in wetter forests e.g. *E. grandis*. In drier and less fertile soil regions, smaller trees of woodland habit are pronounced e.g. *E. kumarlensis* Brooker. There are also numerous mallee species, which are supported by extensive sand plains of low fertility e.g. *E. livida* (Coppen, 2002). The most represented species in international pharmacopeia, being the main supplier of *Eucalyptus* essential oil, is *E. globulus* (Bachir & Benali, 2012). *Eucalyptus* is cultivated all over the world for its timber, medicine, gum, pulp, oil, and aesthetic value.

Generally, *Eucalyptus* species are renowned for their pharmacological activity, including – but not limited to – antioxidative, antifungal, antimicrobial, antiviral, analgesic, antibacterial, and antidiabetic properties (Sonker et al., 2017). The essential oil of *E. camaldulensis* has spasmolytic activity, antioxidative activity, cytotoxic effects (Al-snafi, 2017). Likewise, *E. torquata* essential oil possesses antibacterial, cytotoxic, antifungal effects (Ashour, 2008). In particular, the species *E. torquata* is widely used in the pulp industry, as well as for the production of *eucalyptus* oil extracted on a commercial scale in many countries as raw materials in perfumery, cosmetics, food, beverages, aromatherapy and phytotherapy.

Studies on the essential oils of *E. camaldulensis* leaves have revealed major compounds such as 1,8-cineole, *p*-cymene, β-pinene, terpinen-4-ol, globulol (Pagula et al., 2000). 1,8-cineole, α-pinene, *trans*-pinocarveol are some of the major compounds recorded for the *Eucalyptus torquata* essential oil (Sefidkon et al., 2013).

There are about sixty-two (62) species of *Eucalyptus* found in Cyprus, of which forty-seven (47) are classified but fifteen (15) are not classified, or are hybrid (Ilseven & Baştaş, 2018). In Cyprus, the leaves of *E. camaldulensis* are used orally and topically for abortion, as after labor tonic, cold / flu symptoms, decongestant, urinary tract antiseptic and syphilis (Yöney et al., 2010). In another study, the ethnobotanical usage of the same species was recorded for respiratory tract disorders, musco-skeletal disorders and skin disorders (González-Tejero et al., 2008). However, no such ethnobotanical usage information of *E. torquata* exists in Cyprus.

The aim of this study is to analyse and record the essential oil constituents of the selected *Eucalyptus* species (*E. camaldulensis* and *E. torquata*) growing in Cyprus. In this research, the ethnobotanical usage, comparision of the essential oil compositions of *Eucalyptus* species growing in Cyprus were investigated. This will be the first report on the essential oil composition of the *E. torquata* growing in Cyprus.

1.1. Taxonomy

- Kingdom – Plantae
- Division- Tracheophyta
- Class – Magnoliopsida
- Order – Myrtales
- Family – Myrtaceae
- Genus – *Eucalyptus* L'Hér.

1.2. Morphology

Trees of *Eucalyptus* are relatively large and can grow to heights of 20m, but usually not higher than 50m (Ghasemian et al., 2019). Mature leaves are petiolate, pendulous and lanceolate. Leaf venation is tertiary and quaternary (Coppen, 2002). This venation is shown in figure 1.2 below. Adult leaves of *Eucalyptus* are glabrous, mostly alternate, and rarely erect, with a distinct midvein, penninerved or with parallel veins. Flowers are three (3) or more per umbel, rarely single, sessile, or pedicellate. Ovary is inferior or partly superior. The stamens and ovaries are numerous. The fruit is capsule, seeds are numerous and have different shapes and colours (Landrum et al., 1988). Most *Eucalyptus* species develop lignotuber, which is a vital organ in the regeneration of trees or mallees. The bark of *Eucalyptus* can be smooth, such as in *E. Camaldulensis*, or rough, as in *E. globulus.* Regular decortications/shedding results in smooth barks while the retention of the dead barks, results in rough barks. The seeds of most species are flattened-ellipsoidal with ventral helium. Some large groups however, have terminal hilum. Germination is epigeal and leaves usually form decussately.

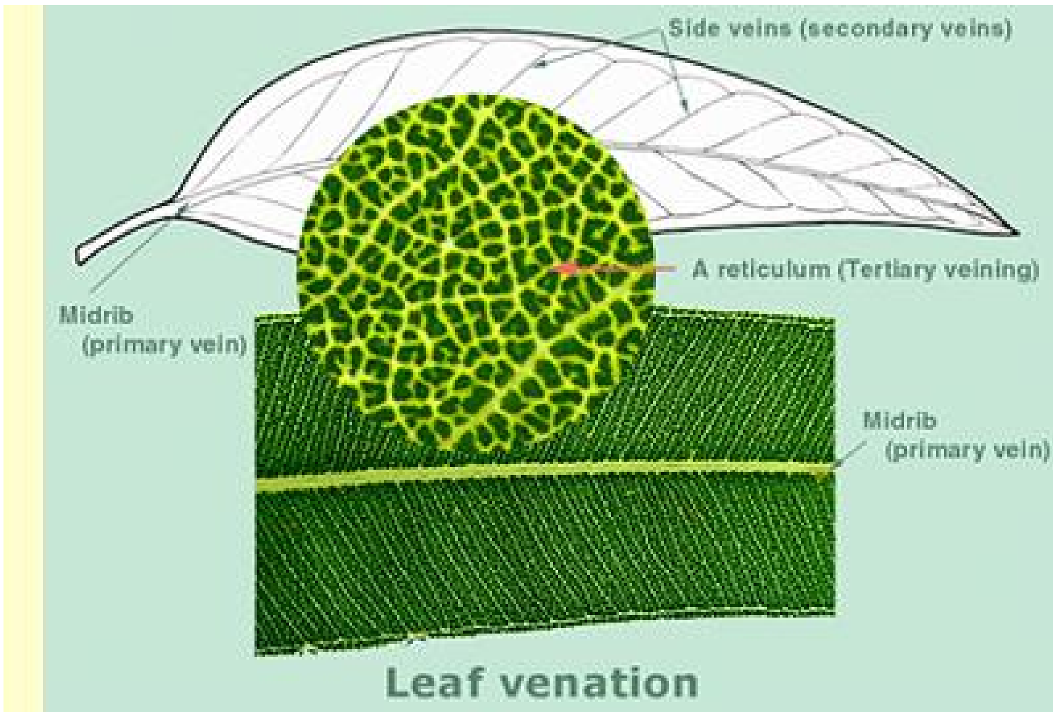

Figure 1.2. *Eucalyptus* leaf venation (Ladymaggic, 2011)

1.3. The Genus *Eucalyptus* L'Her. in Cyprus

Cyprus is an island on the eastern part of the Mediterranean Sea, surrounded by Africa, Asia, and Europe. The island is about 9251km^2 in size and has a high diversity. In terms of size, the island is only behind Sicily and Sardinia in this region (Christodoulou et al., 2018). The flora of Cyprus is diverse, comprising of about 2000 native or naturalized plant taxa (Karousou & Deirmentzoglou, 2011). Major vegetation types in Cyprus include oak forests, orchards, shrubs, pine forests (Fall, 2012).

Eucalyptus genus is not native to Cyprus; they were introduced as a measure to combat mosquitoes in the lowland, as they soak up the stagnant water. A similar method was done in Tunisia, where the trees of *Eucalyptus* were used to control erosion (Elaissi et al., 2012). *Eucalyptus* was first introduced to Cyprus by the French aborist, P.G Madon (Ilseven & Baştaş, 2018). There are about sixty-two (62) species of *Eucalyptus* found in Cyprus, of which forty-seven (47) are classified but fifteen (15) are not classified, or are hybrid. İn Cyprus, *Eucalyptus* is locally known as Efgalitto, Okaliptüs, Sıtma ağacı (Ozan, 2011). The absolute root depth of *Eucalyptus* in Cyprus is about 6 metre-deep roots. *Eucalyptus camaldulensis* has the deepest root system of the *Eucalyptus* species growing in Cyprus. Contrary to some opinions, trees of *Eucalyptus* in Cyprus do not extract water from the deep aquifers, but utilize water through hairy roots on or just below the surface. They grow best where the water level is high (Ilseven & Baştaş, 2018).

Eucalyptus can be seen growing in various areas of the Near East University (NEU) campus and many other regions in the Turkish Republic of Northern Cyprus (TRNC).

1.4. *Eucalyptus camaldulensis* Dehnh.

Eucalyptus camaldulensis: *Eucalyptus acuminata* Hook, *Eucalyptus canalouensis* Dehnh, *Eucalyptus rostrata* Schltdl, *Eucalyptus longirostris* F. Muell. ex Miq, *Eucalyptus subulata* A. Gray, *Eucalyptus camaldulensis* subsp. *camaldulensis*, *Eucalyptus camaldulensis* subsp. *obtusa* (Blakely) Brooker & M.W. McDonald, *Eucalyptus camaldulensis* var. *acuminata* (Hook.) Blakely, *Eucalyptus camaldulensis* var. *brevirostris* (F. Muell. ex Miq.) Blakely, *Eucalyptus camaldulensis* var. *camaldulensis*, *Eucalyptus camaldulensis* var. *obtusa* Blakely, *Eucalyptus camaldulensis* var. pendula Blakely & Jacobs, *Eucalyptus camaldulensis* var. *subcinerea* Blakely, *Eucalyptus longirostris* f. brevirostris F.Muell. ex Miq, *Eucalyptus longirostris* forma longirostris Miq; *Eucalyptus rostrata* var. *acuminata* (Hook.) Maiden, *Eucalyptus rostrata* var. *borealis* R. T. Baker & H. G. Sm; *Eucalyptus rostrata* var. *brevirostris* Maiden and *Eucalyptus tereticornis* var. *rostrata* (Schldl.) Ewart (Al-snafi, 2017).

Eucalyptus camaldulensis (commonly known as the red gum) typically grows on river edges and can last for up to a thousand year. This tree generally has a single stem, large trunk, and can grow as tall as 30-45m (Ghasemian et al., 2019). The trunk diameter of this species is usually between 1m-2m. Leaves are alternate, colour is grey-blue, length is between 8-22 cm long, width is 1-2 cm, often curved or sickle shaped, tapering, short pointed at base. Inflorescence is auxiliary, solitary, 7-11 flowered; flower buds white, globularrostrate or ovoid-conical; operculum hemispherical, rostrate or conical, 4-6 x 3-6 mm, obtuse. Flowering period is betweeen August-November (Ozan, 2011). Bark is smooth, can have colour variations of white, yellow, green, grey, and pink. The barks of *Eucalyptus* camaldulensis occasionally shreds in strips or irregular flakes. Fruits is capsule, small, and contain small seeds (Al-snafi, 2017).

Eucalyptus camaldulensis shows highly efficient water usage. It can also grow in soils that are low in nutrients. In Thailand, they serve as raw materials for shelter, oil, pulp. They are therefore, of great economic importance. This species has also been recorded to adapt to saline soils and varying climatic conditions (Klinsukon et al., 2021). *E. camaldulensis* is the most planted *Eucalyptus* species in Montenegro (Barbosa et al., 2016).

Figure 1.3. *E. camaldulensis* bark (Friday, 2008)

1.5. *Eucalyptus torquata* Luehm.

This species grow to heights of 4-10metres. Propagation is from seeds. The bark is rough and persistent, unlike that of *Eucalyptus camaldulenis*. Flowers are usually distinctly coral-pink, occasionally white or red, and can grow as large as 35mm in diameter. Flowering typically occurs in spring, often two years from seed (*Eucalyptus torquata*, 2008). Length and width of leaves are between 90-120mm and 15-20mm. Leaves are lanceolate and have greyish green colouration. They are also sometimes referred to as coral trees or Christmas trees (Al-snafi, 2017). *Eucalyptus* trees are suitable for ornamental purposes (*Eucalyptus torquata*, 2008). In Cyprus, this species mainly flowers in March-April (Ilseven & Baştaş, 2018).

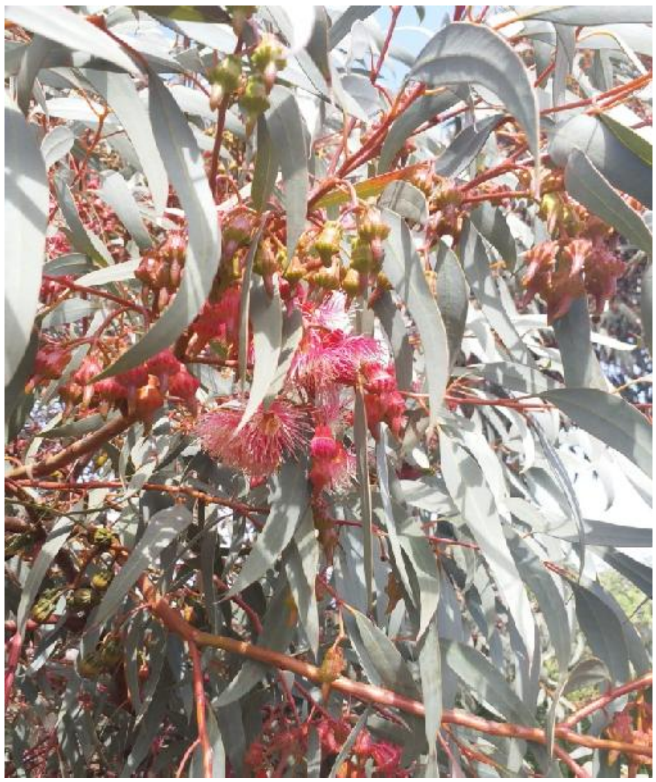

Figure 1.4. *E. torquata* flowers and leaves (Photo by: Azmi Hanoğlu)

1.6. Traditional and Medicinal Uses of *Eucalyptus*

The importance of traditional medicine, especially in Asia and Africa, cannot be overemphasized. A significant number of developing nations' population (about 80%) depend on traditional medicine for health care (Salehi et al., 2019). Ethnobotanical studies, is therefore, of utmost importance in these areas, and other regions all over the world. The knowledge of traditional applications of medicinal plants is mostly retained within families, and passed down from one generation to the next (Nguyen et al., 2019). This greatly limits the information readily available to the public, hence the need to take ethnobotanical records such as this. Ethnobotanical studies can be carried out via interviews, use of questionnaires, observation. According to the World Health Organization (WHO), 74% of the modern application of plant-derived medicine is similar to the their ethnobotanical usage (Yilmaz, 2020).

Traditionally, *Eucalyptus* species were consumed as uncooked “seed pastes” by deserts aboriginal people (Nangala et al., 2019). In countries such as Thailand and Burkina Faso, trees of *Eucalyptus* are simply grown as decorative plants (Chalchat et al., 2001). In Africa, powdered barks of *Eucalyptus* are used as insecticides, treatment of snakebites, and diarrhoea. In Chinese folk medicine, many species of *Eucalyptus* are used as anti-inflammatory, analgesic, and antipyretic remedies. Extracts from leaves of *Eucalyptus* have been approved as natural food additives and listed among the antioxidants in Japan (Brezáni & Šmejkal, 2013). The leaves of *E. globulus* are usually used traditionally in the treatment of asthma and bronchitis. They also serve usage in the production of herbal tea. In Thailand, *E. urophylla* is grown for its wood quality, *E. camaldulensis* for the high rate of growth, *E. citriodora* for the production of essentials oil for use in fragrances (González-Burgos et al., 2018). Extracts from leaves of *Eucalyptus* have been used traditionally by the Aboriginal people to treat wounds, as well as, fungal infections (Ashour, 2008). The leaves of *Eucalyptus* are used by the Aborigines to treat toothaches, cold, fevers. In Egypt, the wood of *E. camaldulensis* is valued in construction and furniture-making (Fadel et al., 1999).

Recently, Benkhaira and Koraichi were carried out ethnobotanical studies on medicinal plants in the city of Fez, Morocco. In this study, *Eucalyptus globulus* was listed as one of the most important species that showed promising signs in the treatment and prevention of COVID-19 (Benkhaira & Koraichi, 2021). Another study showed a frequent usage of *Eucalyptus globulus* in the treatment of respiratory infections by the Moroccan people. Extracts of the leaves of this species is made into syrups which is then administered in the treatment of cough, cold and chronic bronchitis (Belhaj et al., 2021). In Iran, several members of the *Eucalyptus* genus are used in the treatment of fever, and skin burns (Sefidkon et al., 2013). *Eucalyptus* is not only valuable for its medicinal purpose; in East Africa, for example, *Eucalyptus* was introduced, based on their fast growth, to be used in the railroad system expansion (Salehi et al., 2019).

In Cyprus, the leaves of *E. camaldulensis* orally and topically for abortion, after labor tonic, cold / flu symptoms, decongestant, urinary tract antiseptic and syphilis (Yöney et al., 2010). The Cypriot people treat flu, cold, and other upper respiratory tract infections by inhaling the steam of the boiled leaves of *Eucalyptus camaldulensis*. This is usually done once a day, preferably in the evening, until recovery. Additionally, the liquid obtained from this boiling process is used externally for antirheumatic effect (Kaya Yıldırım, 2010; Ozan, 2011). In another study, the ethnobotanical usage of the same species was recorded for respiratory tract disorders, musco-skeletal disorders and skin disorders (González-Tejero et al., 2008). However, no such ethnobotanical usage information of *E. torquata* exist in Cyprus.

1.7. Biological Activity

Eucalyptus oil has antibacterial and antifungal activity, this is however dependent on the cineole content (at least 70%) of the oil. *Eucalyptus* oil is generally safe for direct application on skin as ointment/cream (Ghasemian et al., 2019). The medicinal value of *Eucalyptus* essential oil is rated based on the amount of Eucalyptol (1,8-cineole) contained in the oil. China currently dominates the world market for essential oil of *Eucalyptus* species (Barbosa et al., 2016). Bardaweel et al. reported significant anticancer of essential oil extracted from the leaves of *Eucalyptus*, especially against lymphoma tumours (Bardaweel et al., 2015). Studies show that chewing gum made of *Eucalyptus* extracts can have significant effects on inflammation of the gums (gingivitis) in healthy humans (Al-snafi, 2017). Bachir et al. carried out experiment to show that the leaves essential oil of *E. globulus* has antimicrobial activity (on both gram-negative and gram-positive bacteria) and thus, can be used as natural antibiotic for disease treatment. This activity is linked to the presence of constituents such as eucalyptol, eucamalol, linanool, limonene, citronellal, *p*-cymene, citronellol, aromadendrene, α-terpinol, β-pinene etc (Bachir & Benali, 2012). Essential oils of some plants can act as bioherbicide, thereby promoting a green environment (Mancianti & Ebani, 2020).

Leaves extracts of *Eucalyptus camaldulensis* has been reported to show interesting antioxidative activities. The leaves essential oils of this species have also been demonstrated to show cytotoxic potential on human breast cancer cell lines (Al-snafi, 2017). The essential oil of this species has also been recorded to be active phyto-pathogenic fungi (Elaissi et al., 2012). The essential oil of *E. camaldulensis* can be used to control mosquito larva (Grbović et al., 2010). This species has also been recorded to have allelopathic properties (Muller et al., 2015).

1.8. Phytochemical Content

Phytochemical content is interesting for a couple of reasons. While a compound within the constituent can help deter herbivores from the plant, another compound (mostly floral volatiles) can attract herbivores to the plant (Müller et al., 2020). Essential oils are main sources of secondary metabolites and play important roles in the ecosystem, such as, contributing to the defence mechanisms of the tree (Özgenç et al., 2017).

1.8.1. Essential Oil

The chemical composition of *Eucalyptus* oil may be dependent on the *Eucalyptus* species (Chahomchuen et al., 2020). It can also vary depending on the extraction technique employed (Benabdesslem et al., 2020; Fadel et al., 1999). One important use of species in the *Eucalyptus* genus is in the production of essential oils, which are valuable in medicinal and pharmaceutical industries (Akin et al., 2010). Some of the most important plant species include *E. camaldulensis, E. grandis, E. globulus, E. torquata,* and their hybrids. Species such as *E. camaldulensis* that thrive under local conditions and responds positively to coppicing can be grown specifically for oil production (Pagula et al., 2000).

Essential oil can be produced from plant parts such as flowers, leaves, stems, gums, seeds, fruits, roots, barks etc. The essential oils of *Eucalyptus* are usually stored in the sub-dermal secretory cavities of the mature leaves (Naidoo et al., 2014). The major compound found in *Eucalyptus* oil is the monoterpene ether 1,8-cineole (eucalyptol). Eucalyptol accounts for over 70% of the oil mass and is responsible for the campor-like smell, it is valuable and has been widely applied in the pharmacopeias of countries like Germany, USA, Japan, and France (Vuong et al., 2015). It is also largely responsible for the medicinal value of *Eucalyptus* EOs (Salehi et al., 2019). Other main constituents are limonene and α-terpineol. The most detected constituents of EO of *Eucalyptus* are 1,8-cineole (=Eucalyptol), β-pinene, *p*-cymene, γ-terpinene. Eucalyptol is a monotropic substance containing carbon, hydrogen and oxygen atoms (Ghasemian et al., 2019). It is particularly important because it is neither carcinogenic nor genotoxic (Salehi et al., 2019).

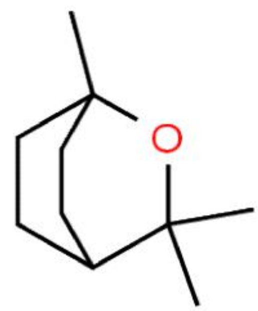

Figure 1.5. The molecular structure of Eucalyptol (*(±)-Eucalyptol | C10H18O | ChemSpider*, 2021).

The chemical compositions of essential oils can vary with respect to seasons (Tsiri et al., 2003). It is also possible for plants of the same species to produce similar EOs but with different chemical composition and therapeutic effects due to genetic and epigenetic factors. Different plant parts have different chemical composition of EOs. Factors that can affect EO composition include plant development stage, maturity of plant used for extraction, plant culture condition, soil salinity etc (Aleksic Sabo & Knezevic, 2019). *Eucalyptus* oils differ depending on factors such as geographical location, species, age of leaf, date of harvesting, season and the method of extraction (Chahomchuen et al., 2020) (Ashour, 2008).

EOs are found in the leaves of over 300 species of *Eucalyptus*, however less than 20 of these species have been explored commercially. The leaves majorly contain terpenes and terpenoids. The *Eucalyptus* plants rich in 1,8-cineole are usually useful in pharmaceutical industries while those rich in citral, citronellal, geranyl acetate are used in perfumery. The EOs produced in *Eucalyptus* are usually rich in monoterpenes and sometimes sesquiterpenes (Barbosa et al., 2016). Essential oil of

Eucalyptus can be extracted from the barks and leaves by steam distillation. Other methods include solvent extraction, Soxhlet extraction, hydrodistillation. In steam distillation, the plant sample can be recovered after the extraction process is conmpleted. However, if hydrodistillation technique is employed, the sample is not easily recoverable. The GC/MS analysis is a convenient and helpful analytical tool in profiling the individual compounds present in the *Eucalyptus* oil. Just as in the extraction of oils from other plants, the extraction yield of EOs of Eucalyptus and volatile organic compounds are affected by factors such as extraction temperature, solvent-to-sample ratios, agitation rates, time, and the physiochemical properties of the extraction solvent (Vuong et al., 2015).

Systemic toxicity can occur if EO of *Eucalyptus* is ingested without following proper recommendation, or if a substandard alternative is consumed. Although it is rare, it is an important condition that has been recorded and should therefore, be considered (Ittyachen et al., 2019). Symptoms of toxicity can include – but not limited to – vomiting, coughing (Al-snafi, 2017). The antimicrobial activity of *Eucalyptus* EO can be traced to the presence of compounds such as 1,8-cineole, α-pinene β-pinene, limonene. The leaf essential oil content of *Eucalyptus* is also used as natural pain reliever. *Eucalyptus* oil is mixed with coconut oil, olive oil to produce hair cream (Dhakad et al., 2018). The use of *Eucalyptus* oils have been approved for use by the European Commission, as well as, the US Food and Drug Administration. However, when consumed in excess, Eucalyptus oil can have adverse effect on humans (Ghasemian et al., 2019). The continued interest of pharmaceutical companies, food, and cosmetic industries in essential oil will ensure that plants essential oils will continue to be of great value, and hence, propel more studies that are scientific.

Essential oils from species of *Eucalyptus* have been reported to be included in the list of the world's most traded oil. The essential oil of *Eucalyptus* is considered to have allelopathic property to the tree. *E. citriodora* is at the top of the world's major oils in relation to trade volume. *Eucalyptus* oil has multipurpose uses and ranks higher in quality than oil of other tree crops. *E. citriodora* (Lemon-scented *Eucalyptus*), *E. globulus* (Tasmanian blue gum), *E. camaldulensis* (River red gum), *E. polybactea* (blue mallee) are some of the common oil-yielding species of *Eucalyptus* (Batish et al., 2008).

The leaves extracts of *Eucalyptus torquata* contains compounds such as jensenone and torquatone (Schliebs & Foley, 1999).

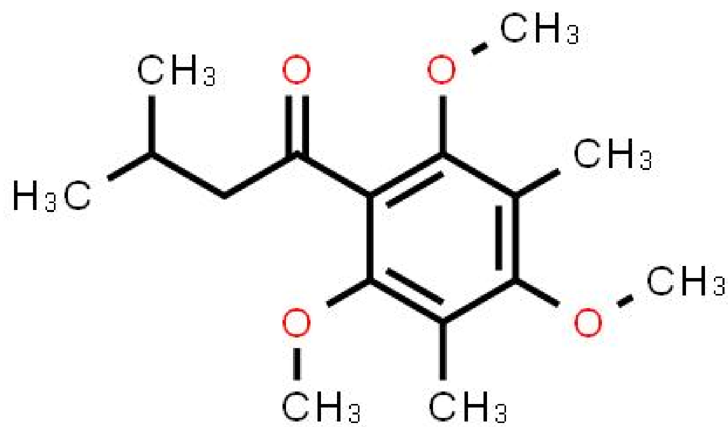

Figure 1.6. The molecular structure of Torquatone

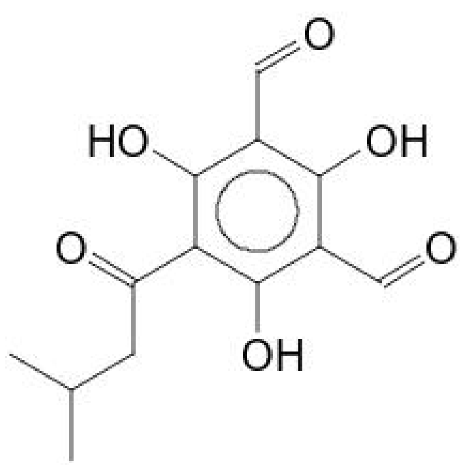

Figure 1.7. The molecular structure of jensenone

1.8.2 Non-volatile Compounds

The non-volatile compounds extracted from different *Eucalyptus* species are more than twenty. Phenolic compounds are reported as the major non-volatile compounds found in *Eucalyptus* and are the major contributors to the antioxidant activities of extracts of *Eucalyptus*. Therefore, the extracts of *Eucalyptus* are potentially important source of phenolics, for value adding to food and pharmaceutical products. Health benefits associated to some of these compounds include reduction of diabetes, cardiovascular diseases and strengthening of the immune system (Vuong et al., 2015). Recent research has shown pharmacologically active non-volatile compounds (such as euglobals and macrocarpals) specific to *Eucalyptus* with potentials for use in treating cancer and AIDS. This, and more, strongly indicates that the genus will continue to be researched for many years to come (Coppen, 2002)

The essential oil of *Eucalyptus* is a complex mixture of monoterpenes, sesquiterpenes, aromatic phenols, esters etc. The exact composition and proportion however differs with species.

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2. MATERIALS AND METHOD

2.1. Collection of Plant Samples

Leaves of *E. camaldulensis* and *E. torquata* were collected on 21.03.2021, *E. torquata* was from Cengizköy-Lefke and *E. camaldulensis* was from Girne kapı-Lefkoşa. Identification of the collected *Eucalyptus* species was done by Prof. Dr. Dudu Özkum Yavuz and Assist. Prof. Dr. Duygu Yiğit Hanoğlu. The samples are dried, prepared into herbarium samples, and kept at the Near East University herbarium (NEUN).

Figure 2.1. *E. camaldulensis* sample (Photo by: Samuel Adediran)

Figure 2.2. *E. torquata* sample (Photo by: Duygu Yiğit Hanoğlu)

2.2. Preparation of Plant Samples

Dried leaves of *E. camaldulenis* sample were gently crushed into tiny particles, 50g of the sample was weighed and put into the flask. *E. torquata* leaves was cut (using a scissors) into small sections and 50g of the sample was weighed and transferred into a different flask.

2.3. Isolation of the Essential Oil

This was carried out at the Pharmacognosy laboratory of the Faculty of Pharmacy, Near East University (NEU). The air-dried leaves samples were hydro-distilled for three (3) hours, using a Clevenger type apparatus. The distillation process for both *Eucalyptus* samples was done separately, on the 25th of May 2021. The resulting oils were collected in a coloured bottle and stored at 4℃ until the analysis.

2.4. Gas Chromatography (GC) and Gas Chromatography Mass Spectrometry (GC-MS) Analysis

2.4.1 Gas Chromatography

GC analysis is carried out using an Agilent 7890B GC system. FID detector temperature is 300^{o}C. To obtain the elution order with GC-MS, simultaneous auto-injection is done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatographs.

2.4.2. Gas Chromatography Mass Spectrometry (GC-MS)

The GC-MS analysis is carried out using an Agilent 5977B GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

2.5. Identification of the Constituents

This was carried out by comparing the relative retention times of the essential oil constituents with those of authentic samples or by comparisons of their linear retention index (LRI) to series of *n*-alkenes. Computer matching against commercial (Wiley GC/MS Library, NIST Chemistry WebBook) (McLafferty and Stauffer, 1989; Linstrom and Mallard, 2001) and in-house “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data was used for the identification (Joulain and Koenig, 1998; ESO, 1999).

3. RESULTS AND DISCUSSION

The chemical compositions of essential oil of the two studied species of *Eucalyptus* (*E. camaldulensis and E. torquata)* growing in Cyprus can be seen in Table 3.1 below.

The EOs obtained from both *Eucalyptus* species studied showed a pale yellow characteristic colour.

Oil yield of 2.4% was recorded for *E. camaldulensis.* 29 compounds were identified representing 91.5% of total essential oil of *E. camaldulensis*. The GC chromatogram and some of the major peaks detected compound names can be seen in Figure 3.1.

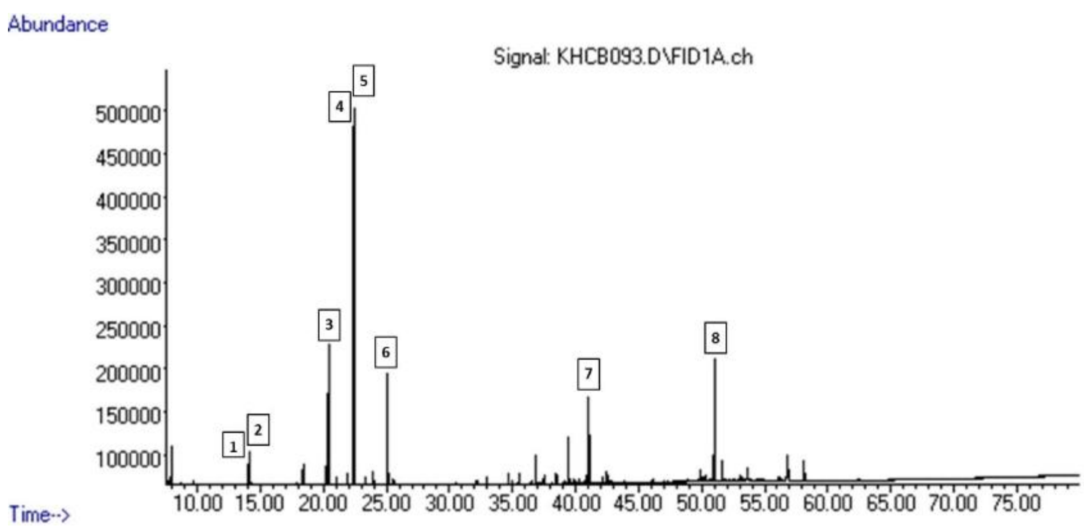


Figure 3.1. The essential oil chromatogram of *E. camaldulensis* (**1:** α-pinene, **2:** α-thujene, **3:** α-phellandrene, **4:** Limonene, **5:** β-phellandrene, **6:** *p*-cymene, **7:** Bicyclogermacrene, **8:** Spathulenol)

The major compounds of *E. camaldulensis* essential oil were identified as α-phellandrene (10.3%) and β-phellandrene (30.6%), respectively. These compounds were followed by *p*-cymene (8.2%), bicyclogermacrene (6.1%) and spathulenol (9.3%).

Oil yield of 1.6% was recorded for *E. torquata.* 20 compounds were identified representing 96.8% of total essential oil of *E. torquata*. The GC chromatogram and some of the major peaks detected compound names can be seen in Figure 3.2.

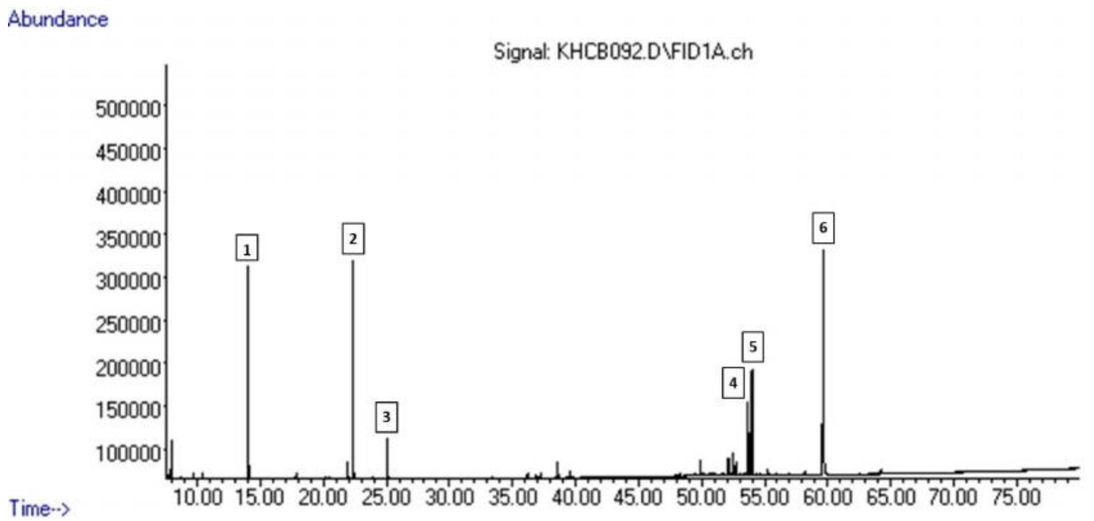


Figure 3.2. The essential oil chromatogram of *E. torquata* (**1:** α-pinene, **2:** 1,8-cineole, **3:** *p*-cymene, **4:** α-eudesmol, **5:** β-eudesmol, **6:** Torquatone)

The major compounds of *E. torquata* essential oil were identified as α-pinene (18.6%), 1,8-cineole (18.8%), β-eudesmol (10.3%) and torquatone (29.2%).

Table 3.1. The essential oil compositions of two *Eucalyptus* (*E. camaldulensis* and *E. torquata*) species growing in Northern Cyprus

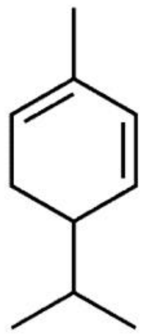
LRI	Compound Name	Relative Percentage amount (%)	
		A	B
1015	α-pinene	1.7	**18.6**
1019	α-thujene	2.6	-
1114	β-pinene	-	0.4
1127	Sabinene	1.6	
1167	Myrcene	1.2	0.1
1172	α-phellandrene	**10.3**	0.1
1187	α-terpinene	0.5	-
1206	Limonene	0.9	1.5
1216	1,8-cineole	-	**18.8**
1218	β-phellandrene	**30.6**	-
1240	(w)-β-ocimene	0.5	-
1255	γ-terpinene	0.9	0.1
1282	*p*-cymene	**8.2**	3.3
1294	Terpinolene	0.4	-
1472	*trans*-sabinene hydrate	0.3	-
1494	Bicycloelemene	0.5	-
1549	γ-humulene	0.8	-
1597	Pinocarvone	-	0.5

1618	α-bergamotene	-	0.3
1619	Terpinen-4-ol	2.0	-
1632	Aromadendrene	-	0.4
1640	4-terpinenyl acetate	0.6	-
1672	Alloaromadendrene	0.7	-
1676	*trans*-pinocarvyl formate	-	1.3
1694	(w)-ocimenyl acetate	0.2	-
1704	Cryptone	3.7	-
1710	γ-terpinyl acetate	-	0.6
1720	Ledene	0.4	-
1721	Isoborneol	-	0.2
1755	Phellandral	0.9	-
1759	α-terpinyl acetate	0.3	-
1762	Bicyclogermacrene	**6.1**	-
1802	Aromadendra-1(10),4(15)-diene	0.5	-
1815	Cuminaldehyde	1.0	-
2151	Spathulenol	**9.3**	-
2197	γ-eudesmol	-	1.6
2209	Hinesol	-	2.1
2252	α-eudesmol	-	6.8
2264	β-eudesmol	-	**10.3**
2307	Apodophllane	-	0.6
2355	Eremophilone	2.6	-
2394	Marsupellone	2.2	-
2433	Torquatone	-	**29.2**
	Total	91.5	96.8

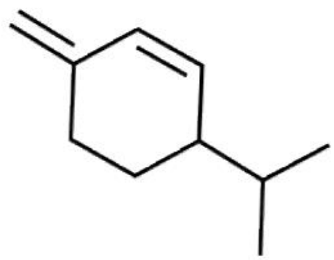
LRI: Linear retention indices calculated against *n*-alkanes, %: calculated from FID data
A: *Eucalyptus camaldulensis* essential oil; B: *Eucalyptus torquata* essential oil

3.1. Molecular structures of the major compounds detected

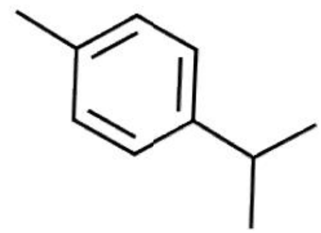
3.1.1. *E. camaldulensis* Major Compounds



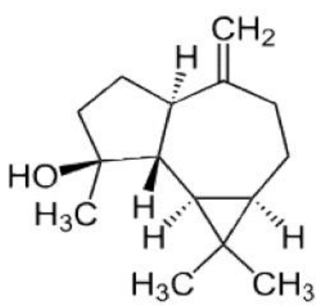
α-phellandrene



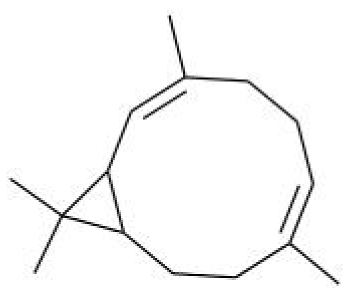
β-phellandrene



p-cymene

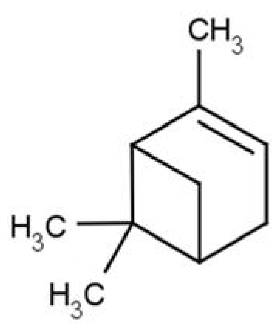


Spathulenol

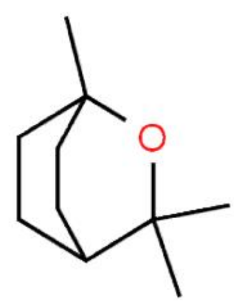


Bicyclogermacrene

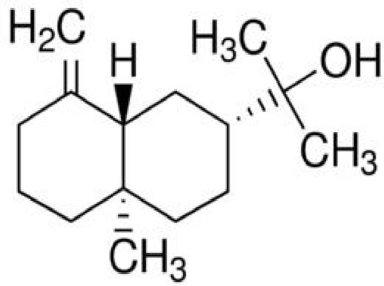
3.1.2. *E. torquata* Major Compounds

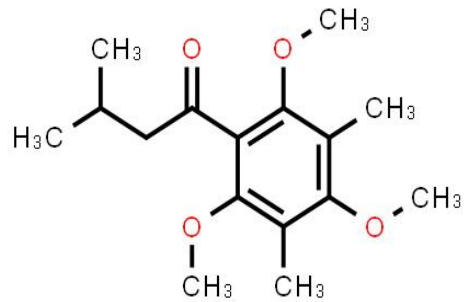


α-pinene



Eucalyptol





β-eudesmol Torquatone

According to the plant originated clone and the height and/or age of the tree, the major compounds and some of the other minor compounds, which are also important, of the essential oil of *Eucalyptus* species can be varied (Barra et al., 2010; Chalchat et al., 2001). In general, concerning the essential oil composition of *E. camaldulensis*, two chemotypes can be distinguished in literature, one of which is dominated by 1,8-cineole (=Eucalyptol) (Zrira et al., 1992; Barra et al., 2010; Moudachirou et al., 1999) and the other by β-phellandrene, *p*-cymene and cryptone as main compounds, some of which contain also spathulenol as main compound, and small quantities or absence of 1,8-cineole (Chalchat et al., 2001; Grbović et al., 2010; Barra et al., 2010; Dagne et al., 2000).

In recent study, we assume that the essential oil of *E. camaldulensis* is one of the chemotype rich in phellandrene (α- and β-), *p*-cymene, spathulenol and absence of 1,8-cineole. It is different compared with the reported chemotypes due to the α-phellandrene ratio (10.3%) in addition with the absence of 1,8-cineole. Moreover bicyclogermacrene ratio is also a distinct difference to the literature, due to the fact that there is only one literature having approximately same ratio (3.60-3.88%) of bicyclogermacrene (Grbović et al., 2010). Dominant compounds obtained in that study which was carried out in different locations in the Montenegro coastline include *p*-cymene, β-phellandrene, β-pinene, and spathulenol (Grbović et al., 2010).

The report of essential oil composition of the leaves of same species collected from Famagusta region of Northern Cyprus was very different from the obtained results of our investigation. It was reported as ethanone (25.36%), eucalyptol (13.73%), βcaryophyllene (11.55%) and carvacrol (9.05%) as the major compounds and pulegone (0.14%), thujone (0.16%), γ-terpinene (0.17%), nerolidol (0.19%) as the minor components (Akin et al., 2010).

The essential oil compositions of leaves and twigs of *E. camaldulensis* growing in Tarsus, Mersin reported as 21.1-55.7% depending on the season (Azcan et al., 1995).

The essential oil composition of the leaves of *E. camaldulensis* collected from Mersin, Turkey were reported as *p*-cymene (42.1%), eucalyptol (1,8-cineole) (14.1%), α-pinene (12.7%) and α-terpinol (10.7%) of which different to the results of our study (Dogan et al., 2017).

Major components identified here is closely similar to investigation of the EOs of same species by Dagne et al. (Dagne et al., 2000), except for the absence of bicyclogermacrene and low quantity of spathulenol.

A study carried out in Burkina Faso by Samaté et al reported the major constituents of *Eucalyptus camaldulensis* to be α-phellandrene, 1,8-cineole, α-pinene, γ-terpinene (Samaté et al., 1998). This is in significantly different to the result obtained in our study, with only α-phellandrene being the common main compound.

Tsiri et al., (2002) reported the EOs composition obtained from the fruits of *Eucalyptus camaldulensis* collected in Greece. Spathulenol and *p*-cymene are the only main compounds common with our study. Although these findings does not agree with the EOs composition record of Tsiri, it confirms that the EOs composition of members of the genus *Eucalyptus* is tissue-specific (Tsiri et al., 2003).

Lucia et al., (2008) investigated the bioactivity of the EOs of several *Eucalyptus* species in Argentina. The main compounds in the EOs constituents recorded for *E. camaldulensis* was very similar to ours, except for the presence of 1,8-cineole in Lucia's study.

Fadel et al., (1999) studied the effects of two extraction techniques on the chemical composition of *E. camaldulensis.* The results from the hydrodistillation method is consistent with our studies; exceptions being cryptone as a chief constituent, and decreased amounts in phellandrene and bicyclogermacrene (Fadel et al., 1999).

The major compounds of the essential oil *E. torquata* α-pinene (10.5%), 1,8-cineole (12%), β-eudesmol (10.1%) and torquatone (42.0%) harvested from Tunisia (Elaissi et al., 2010). The results of this study agrees with our findings. However, the other study results disagreed with our findings. The fresh leaves of *E. torquata* cultivated from Australia orginated seed in Iran were analysed and the major compounds were found as 1,8-cineole (66.9%) α-pinene (13.9%) and *trans*-pinocarveol (6.3%) (Sefidkon et al., 2010). Ebadollahi et al., (2017) was reported that the major compounds of the *E. torquata* essential oil as 1,8-cineole (28.57%), α-pinene (15.74%) and globulol (13.11%). In addition, it is stated that the essential oil of *E. torquata* had a potential acaricidal activity (Ebadollahi et al., 2017). In another research, the main compounds of the essential oil of *E. torquata* leaves collected from Iran was reported as 1,8-cineole (69.6 %), α-pinene (9.5 %), aromadendrene (4.5 %), and alloaromadendrene (7.8 %) (Nikbakht et al., 2015).

4. CONCLUSION

In conclusion, the essential oil composition of *E. torquata* was reported for the first time in addition with the *E. camaldulensis* essential oil composition. The research showed that there are variations in the chemical composition of the essential oil of *E. camaldulensis* growing in Northern Cyprus. We assume that the essential oil of *E. camaldulensis* is one of the chemotype rich in phellandrene (α- and β-), *p*-cymene, spathulenol, while 1,8-cineole is absent. Considering the findings of Lucia et al., (2008), the high concentration of *p*-cymene found in *E. camaldulensis* in Cyprus may confer insecticidal activity on the plant. (Lucia et al., 2008). On the other hand, the essential oil composition of *E. torquata* growing in Cyprus compared with the literature data of the same species showed variations. Further research is in progress in order to reveal the infra- and inter- specific chemical variations and among the species, including the biological activities of the essential oil compositions of *Eucalyptus sp.* growing in Cyprus.

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		7						

	Math	Equally weighted	Non-math
ALES Grade			
(Other) Grade			

Computer Knowledge

Program	Use proficiency