

## Environmental stress tolerance, hydro-distilled essential oils characteristics and biological activities of *Eucalyptus torquata* Luehm.

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### Abstract

*Eucalyptus* has become one of the most widely planted genera in the world because of its tolerance to a wide range of soil types and climates, as well as for its many industrial, commercial and medicinal uses. *Eucalyptus torquata* Luehm. is a plantation species frequently planted in semi-arid and arid regions for its ecological, forestry, ornamental and melliferous interests. Based on literature, drought tolerance of this species was mostly directed to adaptation mechanisms. Physiological investigations reveal the importance of stomatal closure and increased solute contents suggesting that osmotic adjustment is one of the main responses to drought in *E. torquata*. On the other hand, it showed low sensitivity to salt stress. This paper also highlights the immense benefits of *E. torquata* which contains essential oils with variable chemical composition and rich essentially in 1,8-cineole, torquatone,  $\alpha$ -pinene, trans-myrtanol,  $\alpha$ -eudesmol,  $\beta$ -eudesmol, globulol, trans-pinocarveol and aromadendrene. These oils, as well as the methanol and aqueous extracts possess a wide variety of bioactivities of great importance which are particularly valuable as antibacterial and antifungal agents also have a strong toxicity against insects and mites in addition to antiproliferative and cytotoxic effects against different types of cancer cells.

**Keywords:** biological activities; chemical composition; coral gum; essential oil; *Eucalyptus*

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### Introduction

Eucalypts (*Eucalyptus* spp.) are endemic to Australia; however, its few species are indigenous to neighboring countries. The genus *Eucalyptus* comprises more than 800 species and hybrids, which includes

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shrubs and flowering trees. It is the most valuable genus and it is found in almost all parts of the world due to human introduction (Chemlali *et al.*, 2022). It has been broadly cultivated in many countries to utilize as wood for a diverse range of products and grow across a large range of climatic environments and soil types. Eucalypts are known for their adaptation to arid conditions and are considered drought, salt and heat tolerant compared to other trees (Teulières *et al.*, 2007). The mild climate is the most preferred for most species of *Eucalyptus*, they are highly distributed where there are warm summers, temperate winters, moderate rainfall, dry atmosphere and plenty of sunlight (Najum Rasheed *et al.*, 2005). The distribution of *Eucalyptus* all over the world also the dominance in Australia means ecological importance of this genus which provide food and habitat resources for a diverse range of fauna (Vuong *et al.*, 2015). Eucalypts are one of the most bio-economic plant species with high potential to grow in salt-affected soil with arid climatic condition also capable of providing an economic return in the future, it used for ornamental purposes, afforestation, providing feedstock for the pulp and paper industries or to obtain timber and gum, also a nectar resources for honey and known by cosmetic and medicinal values (Saadaoui *et al.*, 2022). In some countries dried *Eucalyptus* leaves are used as tobacco and smoked for asthma (Sefidkon *et al.*, 2008), aqueous extracts are used for aching joints, bacterial dysentery, ringworms, tuberculosis, etc. (Sefidkon *et al.*, 2010). Also, hot water extracts of dried leaves are traditionally used as analgesic, anti-inflammatory, and antipyretic remedies for the symptoms of respiratory infections, such as cold, flue and sinus (Silva *et al.*, 2003).

*Eucalyptus* has been prized as a rich source of essential oils that's more useful as it is easily extractable and has advantages as its superior quality and is regarded as safe and non-toxic by the United States Food and Drug Authority (FDA). *Eucalyptus* oils are volatile organic compounds found in fruits, flowers, bark, seeds, wood and roots, while, these compounds are mainly extracted from foliage (Boland *et al.*, 1991) because in the leaves that oils were most plentiful and more than 300 species of this genus contain volatile oils in their leaves (Pino *et al.*, 2002). *Eucalyptus* essential oils contain terpenoids, phenolic, flavonoids and alkaloids that possess many bioactivities that could be grouped into three classes viz: perfumery, industrial and medicinal (Abiri *et al.*, 2021). In fact, essential oils rich in 1,8-cineole are utilized as pharmaceuticals, whereas those rich in citronellal, citral and geranyl acetate are used in perfumery (Dhakad *et al.*, 2018). Under natural conditions, essential oils from the leaves of *Eucalyptus* known to provide allelopathic property to this plant (May and Ash, 1990) and defense to *Eucalyptus* leaves against attack by harmful insects, and thus acts as a natural pesticide (Batish *et al.*, 2008; Üstüner *et al.*, 2018; Gallon *et al.*, 2020; Sadraoui-Ajmi *et al.*, 2022). In fact, many researchers reviewed the biological properties of *Eucalyptus* essential oils including anti-microbial, fungicidal, antiviral, insecticidal/insect repellent, herbicidal, acaricidal and nematocidal also the anti-tumour and cytotoxic activities (Zhang *et al.*, 2010; Vuong *et al.*, 2015; Barbosa *et al.*, 2016; Dhakad *et al.*, 2018; Salehi *et al.*, 2019; Abiri *et al.*, 2021; Chandorkar *et al.*, 2021). The importance and the commercial uses of essential oils of *Eucalyptus* have increased the research on their extraction, exploring their chemical compositions and bioactivities.

In fact, *Eucalyptus torquata* Luehm. commonly known as coral gum or coolgardie gum is an attractive tree with a small to medium size growing to 6-8 m and a spread of some 5 m, presenting a single trunk, a greyish green foliage and the blade has a lanceolate shape. The length of leaves around 90-120 mm and wide of 15-20 mm, the profuse flowers are reddish-pink or coral colored and hang decoratively on reddish stems. Flowering is very conspicuous and occurs in spring to summer (Al-Snafi, 2017). It is a fast-growing tree known for tolerance to drought, often hybridising in cultivation with another commonly grown arid zone species, *E. woodwardii* to give a hybrid *E. torwood*. It recommends planting *E. torquata* in protected areas as an ornamental tree in the public and private gardens due to its beautiful flowers and its medium size (El-Juhany and Al Al-Shaikh, 2015). The researchers were also exploring the potency of *E. torquata* by valuing their extracts and essential oils which show different biological activities such as antibacterial, antifungal (Ashour, 2008), cytotoxic (Ashour, 2008; Bardaweel *et al.*, 2014; Lahmadi *et al.*, 2021) and pesticidal activity (Ebadollahi *et al.*, 2017; El Finti *et al.*, 2022; Ebadollahi *et al.*, 2022). Also, *E. torquata* considered an important sources of nectar

and pollen for honeybees as showing an abundant flowering of long period and good quality of pollen and nectar for the nutrition of bee; for these reasons, it is frequently planted in arid regions (Eisikowitch *et al.*, 2012; Saadaoui *et al.*, 2022).

The purpose of this study is to provide the readers with information concerning the tolerance and behavior of *E. torquata* under drought and salt stress, also, exploring the potency and diversity of extracts and essential oils of this species in terms of chemical composition and biological activities.

### **Evaluation of the tolerance of *E. torquata* to drought and salt stress**

Drought and the salinization of soil are a widespread environmental problems and an important factors determining plant productivity and distribution (Teulière, 2010). For landscape applications like reclamation of dry and arid saline lands, *Eucalyptus* is a good choice, it's a versatile woody species that develops an extensive deep root system and presents the challenge of finding a good compromise between adaptation to specific environmental conditions and productivity (Teulière *et al.*, 2007).

#### *Responses to drought*

Drought is the second productivity-limiting stress after cold to find subsequently biotic and abiotic stresses, it was suggested that the availability of water is the important determining factor for the distribution of *Eucalyptus* (Li and Wang, 2003). In fact, among 117 *Eucalyptus* species introduced in Tunisia, *E. torquata* is considered a drought-resistant species (Khouja *et al.*, 2001; Saadaoui *et al.*, 2017). Australian Native Plants Nursery (2015) mentioned that *E. torquata* is tolerant of extended dry periods (El-juhany *et al.*, 2008), also, in Saudi Arabia it was classified among the high tolerating species to drought (El-juhany and Al Al-Shaikh, 2015). In the Mediterranean arid regions, *E. torquata* showed a high tolerance level and flower abundance also in the southern provinces of North Africa (Chemlali *et al.*, 2022). Mechanisms employed for drought resilience of *E. torquata* were investigated by Souden *et al.* (2020) which reported physiological and biochemical responses of this species subjecting to a dehydration period followed by rehydration. It reported that *E. torquata* was less resilient to drought than *E. camaldulensis*. Nevertheless, common responses were shown during the dehydration phase including lowering cell water potential from -1MPa to -4.9MPa after 28 days and to -7.1MPa after 45 days of no irrigation which was restored with 88% after rewatering. In the face of water stress, lowering the water potential of the cells by the plant, help to maintain the water content of the cells and, consequently, the turgor (White *et al.*, 2000). Other physiological responses for *E. torquata* are observed including the early closure of stomata which starting from -3.5MPa to prevent water loss, the net photosynthesis was decreased to achieve less than  $2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The chlorophyll fluorescence parameters *Fv/Fm* (maximum photochemical efficiency of PSII) was decreased and after 30 days of re-watering, *E. torquata* restored the structural and functional integrity of its photosynthetic machinery. Changes in xylem conductivity under water deficit also showed which conducted to minimal xylem embolism for *E. torquata* and the value of  $\Psi$  xylem which induced 50% PLC ( $\Psi_{50}$ ) is -4.6MPa, obviously, the level of xylem cavitation decreased after rehydration (Souden *et al.*, 2020). It has been shown also that in case of drought and/or salinity, osmotic adjustment is the key to the adaptation of plants at the cellular level this by the accumulation of organic and inorganic solutes which helps to reduce the water potential without reducing the actual water content (Sanders and Arndt, 2012). For *E. torquata*, water stress induced accumulation of soluble sugars (glucose and fructose) and cyclitols (pinitol, myo-inositol) for its osmotic adjustment (Souden *et al.*, 2020). These adaptive traits are the key factor in the determination of *E. torquata* drought resistance.

*Responses to salinity*

Another major stress for plants is the salinity of soils. In fact, the exposure to salt stress triggers many common reactions in *Eucalyptus* species which have developed several strategies to cope with these challenges (Assareh, 2016). However, three strategies for achieving greater salt tolerance: damage prevention, homeostasis establishment and growth regulation (Zhu, 2001). How *E. torquata* deal with and respond to salinity stress has been reported by Balti *et al.* (2021) and the study showed that *E. torquata* was the salt-sensitive even at lower salt concentrations (80 mM NaCl) among other species such as *E. gomphocephala* and *E. loxophleba*. Salt stress induces certain biochemical and physiological changes in *E. torquata*, also visible symptoms mainly by the development of necrotic spots in leaves after exposure to 170 mM of NaCl for 30 days which indicates salt-induced damage at cellular level. Slower growth was not observed for *E. torquata* indicating the inability of growth modulating under salt stress. Changes in photosynthesis also observed, salt stress majorly affect optimal protein function in the photosynthetic electron transport chain (pETC). The chlorophyll fluorescence-based PSII-related parameters calculated showed lower values, in addition to that, a decline in chlorophyll and carotenoids contents in leaves has been observed. The  $K^+/Na^+$  ratio for *E. torquata* declined significantly than other species mainly for *E. loxophleba* which have the ability to selectively increase  $K^+$  amounts over  $Na^+$  (Balti *et al.*, 2021). Moreover, NaCl salinity causes a significant effect on  $Na^+$ ,  $K^+$  and  $Cl^-$  uptake and their distribution, in this, higher levels of external  $Na^+$  interfere with  $K^+$  acquisition limiting plant  $K^+$  uptake. Therefore, one of the important physiological mechanisms for salinity tolerance is the  $K^+$  selective absorbance (Nasim *et al.*, 2008). Germination also is strongly influenced by osmotic pressure caused by salts in the soil solution (Madsen and Mulligan, 2006). Mechergui *et al.* (2019) reported that seeds of *E. torquata* were not able to germinate at up to 9, 12 and 15  $g.L^{-1}$  NaCl that means that the salinity levels influenced significantly the percentage of germination. For the responses of *E. torquata* to salt stress in relation to growth, it reported that this species showed low survival percentages and volume growth to age 20 years under salt water irrigation (El-Juhany and Al Al-Shaikh, 2015). All these results suggest a good drought tolerance of this forest species; however, it shows a relative sensitivity to the presence of NaCl in the growing medium and soils.

**Yields of *E. torquata* on essential oils**

The essential oils of *E. torquata* may be obtained from different plant parts; however, as observed in Table1, the highest was found in the leaves whose production was much higher than that in the trunk bark. Hydro distilled leaves of *E. torquata* ranged 1.15-3% of essential oil. Similar essential oils yield (1.21-3.1%) has been reported for *E. globulus*, as the principal source of *Eucalyptus* oil in the world (Derwich *et al.*, 2009; Mossi *et al.*, 2011; Mulyaningsih *et al.*, 2011; Harkat-Madouri *et al.*, 2015). The geographical origin also highly affects this production; in this, good extraction yields were observed for plants from Tunisia. In fact, several studies reviewed the parameters that can influence the total essential oil content of plant including part of plant (Silva *et al.*, 2011), geographic origin (Gilles *et al.*, 2010; Almas *et al.*, 2018), the seasonal variations (Silva *et al.*, 2011), the phenological stage (Salem *et al.*, 2018), method of extraction (Ben Hassine *et al.*, 2010; Herzi *et al.*, 2013; Chamali *et al.*, 2021), rainfall and harvesting regime (Gilles *et al.*, 2010).

**Table 1.** Essential oil yields in *E. torquata* obtained by hydro distillation in Tunisia, Iran, Morocco and Cyprus

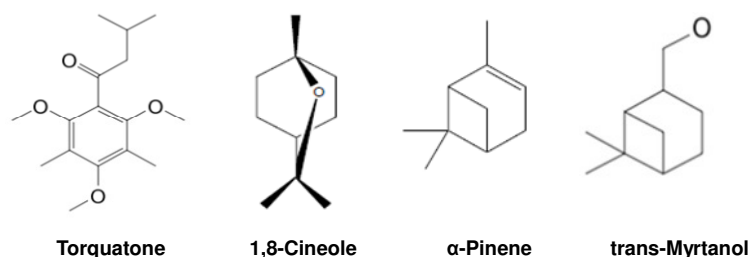
| Origin  | Part used  | Harvest period/sample crushed or not               | Essential oils yields (%) | References                           |
|---------|------------|--|---------------------------|--------------------------------------|
| Tunisia | Trunk bark | February 2019/Dry sample ground into a fine powder | 0.006                     | (Lahmadi <i>et al.</i> , 2021)       |
|         | Leaves     | January 2005/Dry leaves boorishly crushed          | 3.2                       | (Elaiissi <i>et al.</i> , 2010)      |
|         |            | January 2007/Dry leaves medially crushed           | 1.86                      | (Ben Hassine <i>et al.</i> , 2010)   |
| Iran    |            | Dry leaf powder                                    | 1.15                      | (Ebadollahi <i>et al.</i> , 2017)    |
| Morocco |            | April 1991   | 1.17                      | (Zrira <i>et al.</i> , 1994)         |
| Cyprus  |            | March 2021/ Dry leaves crushed into tiny pieces    | 1.6                       | (Yiğit Hanoğlu <i>et al.</i> , 2022) |

### Chemical profiling of *E. torquata* essential oils

Essential oils obtained from *Eucalyptus* are usually rich in monoterpenes and in some cases sesquiterpenes. Nevertheless, the chemical profile and main components of oils from *Eucalyptus* varied significantly between species. Mostly, the main components were the oxygenated monoterpenes 1,8-cineole and the monoterpene hydrocarbons  $\alpha$ -pinene with various percentages dependent on the specific species (Goldbeck *et al.*, 2014; Ishnava *et al.*, 2013). Other compounds also are detected as major component in *Eucalyptus* oils as example; limonene in *E. crebra* oils, citronellal in *E. citriodora* oils (Ghaffar *et al.*, 2015) and p-cymene in *E. oleosa* (Chamali *et al.*, 2019). In Tunisia, most of *Eucalyptus* species oils showed that the oxygenated monoterpenes constituted the major fraction as the 1,8-cineole was the major component (Elaiissi *et al.*, 2010; Elaiissi *et al.*, 2011a, 2011b, Elaiissi *et al.*, 2011; Elaiissi *et al.*, 2012, Elaiissi *et al.*, 2012; Sebei *et al.*, 2015; Limam *et al.*, 2020; Ameer *et al.*, 2021).

A wide number of terpenes have been identified in the leaves essential oil of *E. torquata*, using analyses by GC-FID, GC or GC-MS (Table 2). In spite of current variations of the origin of the analyzed plants, there is consistence that 1,8-cineole and  $\alpha$ -pinene are a characteristic compound of this species. 1,8-cineole was isolated in concentrations between 11 and 70% also the  $\alpha$ -pinene obtained with concentration between 10 and 20%. Other compounds detected such as trans-pinocarveol,  $\alpha$ -terpineol and borneol from chemical class of oxygenated monoterpenes. The oils also contain considerable amount of the monoterpene hydrocarbons p-cymene, also the aromadendrene and alloaromadendrene from chemical class of sesquiterpene hydrocarbons.  $\alpha$ -eudesmol,  $\beta$ -eudesmol,  $\gamma$ -eudesmol and globulol are the main oxygenated sesquiterpenes. A high percentage of torquatone also was detected. This last compound forms a member of acylphloroglucinols which was a class of specialized metabolites with relatively high content in *Eucalyptus* with diverse structures and bioactivities (Singh *et al.*, 2009; Yao *et al.*, 2021). Torquatone was first isolated from *E. torquata* and *E. caesia* Benth growing in Australia with 25 and 50% of the essential oil fraction respectively (Bowyer and Jefferies, 1959). Its derivative also from the essential oils of number of *Eucalyptus* species and absent in others and present with relative high concentration in *E. torquata* (Ghisalberti, 1996; Bignell *et al.*, 1997a, 1997b; Elaiissi *et al.*, 2010; Yiğit Hanoğlu *et al.*, 2022). The chemical formula of torquatone is  $C_{16}H_{24}O_4$ ; there is 4,6-trimethoxy-3,5-dimethyl-1-(3-methylbutyryl)-benzene (Menut *et al.*, 1999; Figure 1). When the composition of twelve *Eucalyptus* essential oils (*E. torquata* Luehm, *E. woodwardi* Maiden, *E. stricklandii* Maiden, *E. occidentalis* Endl, *E. brockwayi* C. A. Gardn, *E. salomonophloia* F. Muell, *E. gillii* Maiden, *E. oldfieldii* F. Muell, *E. largiflorens* F. Muell, *E. loxophleba* Benth, *E. sargentii* Maiden, *E. gracilis* F. Muell,) are compared, the high percentage of torquatone is recorded

for *E. torquata* with 42% also the low percentage of sesquiterpenes hydrocarbons and oxygenated monoterpene with a low quantity of 1,8-cineole (12%) and a relative high amounts of  $\alpha$ -pinene (10.5%),  $\alpha$ -eudesmol (2.9%),  $\beta$ -eudesmol (10.1%) and  $\gamma$ -eudesmol (1.3%). *Eucalyptus woodwardii* oil had resembling chemical characteristics to *E. torquata* oil essentially in major compounds detected (Elaissi *et al.*, 2010; Ben Amor, 2021).



**Figure 1.** Chemical structure of major elements of essential oil of *E. torquata*

Plants cultivated in different countries produce essential oils with variable composition as can be seen from Table 2. Torquatone is detected as a major component of *E. torquata* leaves essential oils from Tunisia (42%), Australia (42%) and Cyprus (29%) while totally absent in oils from Iran and Morocco. The same for  $\alpha$ -eudesmol,  $\beta$ -eudesmol and  $\gamma$ -eudesmol that are not detected in Iranian species. An intra-specific variation is also recorded and explained by geographical, environmental and climatic variations which affect the chemical composition of essential oils. Also, it was proven that essential oils of different plant parts have different chemical composition (Table 2). The trunk bark essential oil of *E. torquata* growing in Tunisia has a completely distinct chemical profile compared to the leaf essential oils. The 1,8-cineole was totally absent and the major constituents being the oxygenated monoterpenes (84.7%), with trans-myrtanol (73.4%) and myrtenol (4.7%) as the main components. The apocarotene cis- $\beta$ -ionone and the fatty acid nonanoic acid also identified in significant percentages of 3.9% and 2.4% respectively, the sesquiterpene hydrocarbons were represented with only 2% with  $\gamma$ -maaliene as the main component (1.3%) (Lahmadi *et al.*, 2021). Therefore, there are notable quantitative and qualitative differences in *E. torquata* essential oils compositions; it is mentioned in literature that these differences are attributed to several exogenous factors: harvest time, seasonal factors, soil composition, geographical position and the method of drying of plants. Endogenous factors are involved including genetic makeup and the ontogenetic development stage (Marzoug *et al.*, 2011; Zandi-Sohani and Ramezani, 2015).

Since the chemical composition of the *Eucalyptus* essential oils is directly associated with their biological activities, the following discussion will be focused on such activities of *E. torquata*. The specific and different composition in *E. torquata* can only act on these activities.

**Table 2.** Major components of *E. Torquata* essential oils obtained by hydro distillation from Tunisia, Iran, Australia, Morocco and Cyprus

| Origin  | Part used  | Major Components (%)   | Identification method | References                           |
|---------|------------|--|-----------------------|--------------------------------------|
| Tunisia | Trunk bark | trans-Myrtanol (73.4), myrtenol (4.7), (E)- $\beta$ -ionone (3.9), nonanoic acid (2.4), $\alpha$ -terpineol (1.9), decanoic acid (0.9), $\gamma$ -maaliene (1.3), cis-myrtanol (1.2), $\beta$ -cyclocitral (0.8), geranylacetone (0.8), (E)-ocimene (0.7)  | (GC-EI-MS)            | (Lahmadi <i>et al.</i> , 2021)       |
|         | Leaves     | Torquatone (42), 1,8-cineole (12), $\alpha$ -pinene (10.5), $\beta$ -eudesmol (10.1) trans-pinocarveol (5.1), $\alpha$ -eudesmol (2.9), p-cymene (2), globulol (2), $\gamma$ -eudesmol (1.3), aromadendrene (1.1), $\delta$ -cadinol (0.9)   | (GC (RI) and GC/MS)   | (Elaiissi <i>et al.</i> , 2010)      |
| Iran    | Leaves     | 1,8-Cineole (69.6), $\alpha$ -pinene (9.5), terpinen-4-ol (0.8), $\alpha$ -terpineol (1.1), alloaromadendrene (7.8), aromadendrene (4.5), limonene (1.5), p-cymene (0.7)   | GC-FID and GC-Mass    | (Nikbakht <i>et al.</i> , 2015)      |
|         |            | 1,8-Cineole (66.9), $\alpha$ -pinene (13.9), trans-pinocarveol (6.3), p-cymene (4.2)   | (GC and GC/MS)        | (Sefidkon <i>et al.</i> , 2010)      |
|         |            | 1,8-Cineole (28.57), $\alpha$ -pinene (15.74), globulol (13.11), alloaromadendrene (7.26) $\alpha$ -terpineol (2.64), epiglobulol (2.50), p-cymene (2.46), trans-pinocarveol (2.09), viridiflorol (1.86), endo-borneol (1.72), neoalloocimene (1.53), terpineol-4 (1.51), ledene (1.39), $\alpha$ -gurjunene (1.25), $\delta$ -selinene (1.04) | (GC-MS)               | (Ebadollahi <i>et al.</i> , 2017)    |
|         |            | 1,8-Cineole (24.2), $\alpha$ -pinene (20) globulol (8.4), aromadendrene (7.8), $\alpha$ -terpineol (2.5), cubeban-11-ol (2.4), trans-sabinol (2), alloaromadendrene (1.8)  | (GC-MS)               | (Ebadollahi <i>et al.</i> , 2022)    |
|         |            | Torquatone (42), 1,8-cineole (11.2), $\alpha$ -pinene (10.2), $\alpha$ -eudesmol (10.2), $\beta$ -eudesmol (11.1), $\gamma$ -eudesmol (4.8)  | (GC-FID and GC-MS)    | (Baranska <i>et al.</i> , 2005)      |
| Morocco | Leaves     | 1,8-cineole (46.9), $\alpha$ -pinene (16.7), bornéol (10.8), 4-terpinéol (3.2), globulol (1.6), p-cymene (1.3)   | (GC-FID)              | (Zrira <i>et al.</i> , 1994)         |
| Cyprus  | Leaves     | Torquatone (29.2), 1,8-cineole (18.8), $\alpha$ -pinène (18.6), $\beta$ -eudesmol (10.3), $\alpha$ -eudesmol (6.8)   | (GC and GC-MS)        | (Yiğit Hanoglu <i>et al.</i> , 2022) |

## Biological activities

### *Antimicrobial activity of E. torquata essential oils*

*Eucalyptus* essential oils endowed antimicrobial action against a large spectrum of bacteria and fungi which consist of its therapeutic properties as a promising alternative to drugs for several diseases and disorders (Zhang *et al.*, 2010; Barbosa *et al.*, 2016; Dhakad *et al.*, 2018). Additionally, the possible interactions of *Eucalyptus* essential oils with conventional antimicrobial agents was studied that could lead to new treatment strategies involving reduced antibiotic doses and for higher therapeutic efficacy (Knezevic *et al.*, 2016; Scazzocchio *et al.*, 2016; Al-Qaysi *et al.*, 2020). The bioactivity of *Eucalyptus* essential oils may be due to their monoterpene components; in fact, antimicrobial activity could be attributed to the presence of compounds such as 1,8-cineole,  $\alpha$ -pinene,  $\beta$ -pinene and limonene (Dhakad *et al.*, 2018). However, the interactions of different constituents may be responsible for the total bioactivity of *Eucalyptus* essential oils that can potentially lead to additive, synergistic, or antagonistic effects (Mulyaningsih *et al.*, 2010).

*Eucalyptus torquata* essential oils marked antimicrobial activities against a large spectrum of bacteria based on agar diffusion method and the microdilution method (Table 3) the bioassays confirm that Gram-positive bacteria are more sensitive compared to Gram-negative ones. Indeed, leaves, stems and flowers essential oils of *E. torquata* exhibited a moderate to high antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Bacillus subtilis* with inhibition zones in the range of 10 and 22 mm of diameter and against the two bacteria *Klebsiella pneumoniae* and *Proteus mirabilis* with inhibition zones in the range of 8 and 10 mm of diameter while not active against *salmonella typhi*. Also, *E. torquata* flowers essential oil demonstrated antibacterial action against *Pseudomonas aeruginosa* with inhibition zone of 11 mm (Ashour, 2008; Bardaweel *et al.*, 2014) this last bacteria was resistant to essential oils obtained from several *Eucalyptus* species and from other plants (Elaiissi *et al.*, 2011; Wilkinson and Cavanagh, 2005). In other hand, *Pseudomonas aeruginosa* with *Escherichia coli* are resistant to leaves essential oils of *E. torquata* grown in Egypt while susceptible to that grown in Jordan with inhibition zones of 11 and 9 mm respectively (Ashour, 2008; Bardaweel *et al.*, 2014). Minimum inhibitory concentration (MIC) of leaves essential oils of *E. torquata* from Jordan was calculated by the microdilution method, Norfloxacin 1 mg/ml was used as reference controls for antibacterial activity. The MIC values for *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* are 198, 201, 197, 204 and 217  $\mu$ g/ml respectively (Bardaweel *et al.*, 2014).

*E. torquata* essential oils also cause growth inhibition of some fungal species, oils from flowers, stems and leaves of *E. torquata* from Egypt and Jordan exhibited a moderate to high antifungal activities against mycelial fungi *Aspergillus flavus* and *Aspergillus niger* also against the yeast *Candida albicans*. Flowers essential oil from Egypt showed the maximum zone inhibition against *Aspergillus flavus* and *Candida albicans* with inhibition zones of 17 and 15 mm respectively, while the leaves essential oil is active with inhibition zones of 10 and 14 mm respectively. Nevertheless, essential oils from Jordan are active against *Aspergillus flavus* and *Candida albicans* with inhibition zones of 10 mm and with MIC values of 198 and 192  $\mu$ g/ml respectively (Ashour, 2008; Bardaweel *et al.*, 2014).

As a result, the difference in the chemical composition of *E. torquata* essential oils shown previously could be the cause of the difference in their biological and therapeutic activities.

### *Antimicrobial activities of E. torquata extracts*

Hence, there is an urgent need to find alternative antimicrobial agents for the treatment of resistant pathogenic microorganisms. The use of plant-based antimicrobials has several advantages over synthetic chemicals since the lower incidence of numerous side effects, low toxicity for mammals and high degradability



(Raja, 2014). *Eucalyptus* species are known to be a rich source of bioactive compounds, including phenolic, flavonoid, terpenoids, tannins, phloroglucinol and cardiac glycosides, which had potential antimicrobial activities (Luís *et al.*, 2016; Elansary *et al.*, 2017; Bhuyan *et al.*, 2017; Sabo and Knezevic, 2019). Indeed, phenolic compounds are those which contribute significantly to the antioxidant activities of plant extracts (Siramon and Ohtani, 2007; Ghaffar *et al.*, 2015). Two studies conducted in Morocco revealed that aqueous extracts of powdered waste from *E. torquata* (leaves, stems, twigs and other parts) contained total polyphenols amounts of 73.48 and 76.68 mg GAE/g DW and flavonoids content of approximately 58 mg RE/g DW in which significant antioxidant capacity has been investigated for these extracts (Bouhlali *et al.*, 2020; Bouhlali *et al.*, 2021). Additionally, methanol and aqueous extracts from leaves, stems and flowers of *E. torquata* showed antibacterial action against different medically bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus subtilis* and *Escherichia coli* with different inhibition zones in the range of 7 and 25 mm, also, it marked an antifungal activity against the yeast *Candida albicans* with inhibition zones in the range of 9 and 14 mm of diameter (Ashour, 2008).

In another way, the growing interest in the use of natural plant products in the biological control of plant disease through the use of biological methods has been a great challenge for agriculture for a long time. In fact, *Eucalyptus* species possess fungicidal properties against large spectrum of phytopathogenic fungi (Zhou *et al.*, 2016; Gakuubi *et al.*, 2017; Abdelkhalek *et al.*, 2020). Also, aqueous extracts from *E. torquata* waste (leaves, branches, twigs) showed an antifungal activity against two fungal pathogens *Fusarium oxysporum* f. sp. *albedinis* and *Mauginiella scaettae* in a dose dependent manner and more stronger than extracts from other plants such as *Acacia cyanophylla*, *Cupressus atlantica*, *Nerium oleander* and *Schinus molle* (Bouhlali *et al.*, 2020; Bouhlali *et al.*, 2021). These soil-borne fungal pathogens caused a serious threat to date palm (*Phoenix dactylifera* L.) in Morocco. "Bayoud" disease and inflorescence rot are the principal enemy of palm trees caused by these pathogens and researchers suggest the use of this plant to control these diseases. It reported that at every concentration tested, *E. torquata* extract showed the strongest inhibition activity on fungal mycelia growth of pathogens. At a dose of 4% of extract, the spore germination of *Fusarium oxysporum* inhibited with 79.21% after 7 days of incubation and strong sporulation reductions is shown with 44.97% of extract after 10 days of incubation (Bouhlali *et al.*, 2020). 100% inhibition of spore germination of *Mauginiella scaettae* at a low concentration of 1% of *E. torquata* extracts after 24h of incubation and a great reduction in sporulation by 88.05% at a dose of 4%. The inhibitory effect of these extracts is related to their composition; moreover, content in polyphenols and flavonoids in aqueous extracts of *E. torquata* are found to be correlated with this antifungal activity as well as their antioxidant properties (Bouhlali *et al.*, 2021).

**Table 3.** Biological properties of *E. torquata* essential oils and extracts

| <i>E. torquata</i> effects    | Plant part (extract or oil)                             | Tested organism and /or cell line  | Effects and/or related mechanisms <sup>a</sup>   | References                       |
|-------------------------------|---|--|--|----------------------------------|
| <b>Antimicrobial activity</b> | Leaves essential oil from Jordan                        | <i>B. subtilis</i> ,<br><i>S. aureus</i><br><i>S. epidermidis</i><br><i>P. aeuriginosa</i><br><i>E. coli</i><br><i>C. albicans</i> , <i>A. flavus</i>  | Growth inhibition<br>IZD      MIC<br>11      198<br>12      201<br>10      197<br>9      204<br>11      217<br>10      192,198           | (Bardaweel <i>et al.</i> , 2014) |
|                               | Leaves, stems and flowers essential oils from Egypt     | <i>B. subtilis</i><br><i>S. aureus</i><br><i>S. epidermidis</i><br><i>E. faecalis</i><br><i>K. pnemounia</i><br><i>P. mirabilis</i><br><i>C. albicans</i><br><i>A. flavus</i><br><i>A. niger</i> | 11-15<br>10-16<br>19-22<br>12-19<br>8-10<br>8-9<br>10-15<br>14-17<br>12-15   | (Ashour, 2008)                   |
|                               | Flowers essential oil from Egypt                        | <i>P. aeuriginosa</i>  | 11   |                                  |
|                               | Leaves, stems and flowers methanolic extract from Egypt | <i>S. aureus</i><br><i>S. epidermidis</i><br><i>E. faecalis</i><br><i>B. subtilis</i> , <i>E. coli</i><br><i>C. albicans</i>   | 16-17<br>10-16<br>13-19<br>13-15<br>10-11  |                                  |
|                               | Leaves, stems and flowers aqueous extract from Egypt    | <i>S. aureus</i><br><i>S. epidermidis</i><br><i>E. faecalis</i><br><i>B. subtilis</i><br><i>C. albicans</i>  | 10-13<br>9-13<br>18-24<br>11-14<br>9-10  |                                  |
|                               | Stems and leaves essential oils from Egypt              | MCF7 Human breast adenocarcinoma cells   | Notable cytotoxic effect with IC50 values of 1.34(stem oil) and 5.22 µg/mL (leaf oil)  |                                  |
| <b>Anticancer activity</b>    | Leaves essential oil from Jordan                        | Nine mammalian cell line (MCF-7, HeLa, Caco, T47D, BJAB, Raji, A498, PC3 and Caki)   | Cytotoxic effect with IC50 values ranging from 33 and 115 µg/mL after exposure time of 48 h<br>Probably involved cell death by apoptosis | (Bardaweel <i>et al.</i> , 2014) |
|                               | Trunk bark essential oil from Tunisia                   | Two human cell lines MDA-MB-231 and SW620  | Inhibitory effect on cell proliferation with IC50 values of  | (Lahmadi <i>et al.</i> , 202)    |

|                              |  |                    |  |                                  |
|------------------------------|--|--------------------|--|----------------------------------|
|                              |  |                    | 40.66 and 26.71<br>$\mu\text{g}/\text{mL}$ after 48h   |                                  |
| <b>Insecticidal activity</b> | Aqueous extract of leaves from Morocco | <i>D. opuntiae</i> | 65% mortality in the population of adults females and 50% of nymphal stage after three application of extract (60%)    | (El Finti <i>et al.</i> , 2022)  |
|                              | Leaves essential oil from Iran         | <i>R. dominica</i> | Fumigant toxicity after 72h with LC50 and LC90 values of 31.567 and 105.017 $\mu\text{L}/\text{L}$ of Air respectively | Ebadollahi <i>et al.</i> , 2022) |
| <b>Acaricidal activity</b>   | Leaves essential oil from Iran         | <i>T. urticae</i>  | Strong fumigant toxicity against female adults (LC50 = 3.59 ( $\mu\text{l}/\text{l}$ air after 24h)                    | (Ebadollahi <i>et al.</i> (2017) |

<sup>a</sup>**IZD** – Inhibitory zone diameter (mm), **MIC**- minimal inhibitory concentration ( $\mu\text{g}/\text{ml}$ ), **IC50**- 50% inhibitory concentration, **LC50**-50% lethal concentration, **LC90** - 90% lethal concentration.

#### *Anticancer activity*

The cytotoxic effect of extracts and components isolated from different species of *Eucalyptus* has been studied by several researchers. Anti-tumor properties of phenolics, terpenoids (monoterpenes, sesquiterpenes, diterpenoids and triterpenoids) derived from *Eucalyptus* plants have been discussed by Abiri *et al.* (2021) which explain the broad spectrum of toxicity, antitumor properties, and mechanisms against cancerous cell lines of *Eucalyptus*-derived essential oil which can be a promising green anti-cancer drugs. Also, Bardaweel *et al.* (2014) demonstrated the cytotoxic effect of essential oils from *E. torquata* grown in Jordan which reported that it was varied and highly cell line dependent; in fact, various cytotoxicity levels have been observed on the cancer cell lines treated with essential oil after exposure time of 48 h at 37 °C based on MTT assay. It reported that the EBV-negative Burkitt's lymphoma BJAB cell and the human Burkitt's lymphoma Raji cell line are the most sensitive cell lines with IC50 values of 33 and 39  $\mu\text{g}/\text{ml}$  respectively. Although, cytotoxic properties also observed against the human breast adenocarcinoma MCF7 cell line (IC50 values of 115  $\mu\text{g}/\text{mL}$ ), the human ductal breast epithelial tumor cell line T47D (IC50 values of 82  $\mu\text{g}/\text{mL}$ ), the human clear cell renal cell carcinoma Caki cell (IC50 values of 94  $\mu\text{g}/\text{mL}$ ), the human kidney carcinoma cell line A498 line (IC50 values of 87  $\mu\text{g}/\text{mL}$ ), the human prostate cancer PC3 cell line (IC50 values of 108  $\mu\text{g}/\text{mL}$ ), the human colon adenocarcinoma Caco-2 cell line (IC50 values of 108  $\mu\text{g}/\text{mL}$ ) and the human epithelial carcinoma HeLa cell line (IC50 values of 91  $\mu\text{g}/\text{mL}$ ). Also, the Lactate dehydrogenase (LDH) activity and the decrease in DNA content of cell line treated indicated that the cytotoxic activity of *E. torquata* essential oils probably mediated through induction of cell death by apoptosis (Bardaweel *et al.*, 2014).

The trunk bark essential oil of *E. torquata* grown in Tunisia displayed significant antiproliferative effect against two human cancer cell lines: breast carcinoma cell lines MDA-MB-231 and colorectal cancer cell lines SW620 which demonstrated inhibitory effect on the tested cell lines proliferation in a dose-dependent manner after 48 h of incubation using Crystal Violet Staining (CVS) assay, the highest cytotoxic activity of essential oil is observed at 100 $\mu\text{g}/\text{mL}$  and it's shown that colon carcinoma cells are more sensitive against essential oils (with IC50 values of 26.71  $\mu\text{g}/\text{mL}$ ) than breast MDA-MB-231 (with IC50 values of 40.66  $\mu\text{g}/\text{mL}$ ) (Lahmadi *et al.*, 2021). According to the protein-staining sulphorhodamine B (SRB) assay for cell growth, essential oils of *E. torquata* from Egypt (extracted from stem with IC50 value of 1.34  $\mu\text{g}/\text{mL}$  and leaves with IC50 value of 5.22

µg/mL) have a cytotoxic effect on the Human breast adenocarcinoma cell line (MCF7) and failed to exert a considerable effect on Human hepatocellular carcinoma cell line (HEPG2) (Ashour, 2008). These studies increase the attention in exploring this species and improving the therapeutic opportunities against cancer.

#### *E. torquata* as pesticide

Attacks and infection by pests (especially weeds, pathogens and animal pests) are the largest competitor of agricultural crops that severely reduce crop productivity (Oerke, 2006). However, the excessive use of synthetic pesticide residue in food, accumulated in the environment and increasing health hazards to humans in addition to the increasing risk of pesticide resistance (Pimentel *et al.*, 1992), is thus pertinent to explore the pesticidal activities of natural products. *Eucalyptus* species are known to be a rich source of bioactive compounds that allow it to act directly as natural pesticide (Radwan *et al.*, 2000; Shukla *et al.*, 2002; Batish *et al.* 2008; Anita *et al.*, 2012; Barbosa *et al.*, 2016; Adak *et al.*, 2020). Absolutely, *E. torquata* has been shown insecticidal properties against the Cochineal, *Dactylopius opuntiae*, an insect that highly damaging the cactus plants in Morocco, it found that three applications of aqueous extract (60%) of *E. torquata* leaves are needed to reduce mealybug populations also caused the death of 65% of females and 50% of nymphal stages of *Dactylopius* spp. after 72 h after spraying with *E. torquata* extract which could be an alternative for the control of wild cochineal (El Finti *et al.*, 2022). Also, *E. torquata* essential oils showed a great insecticidal potential on the adults of *Rhyzopertha dominica*, an insect pest of stored products, in which significant fumigant toxicity against insect which was augmented by increasing the concentration of *E. torquata* essential oils and the exposure time. The LC50 value decreased with increasing exposure time from 37.728 (µL/L of Air) after 24 h to 31.567 (µL/L of Air) after 72 h of exposure with essential oil. In fact, sublethal biochemical disruption has been shown in treated insects, including the reduction of energy content resulting from the significant decrease of the protein and glycogen contents. In other hand, an inhibition of digestive amylase and protease enzyme activities, also, a significant decreases in the relative growth rate of insects (Ebadollahi *et al.*, 2022) which confirm that *E. torquata* extract and essential oils can be used to control insect pests. Also, it found that possess acaricidal properties as reported by Ebadollahi *et al.* (2017) which demonstrated that *E. torquata* leaves essential oils have strong toxicity against the adult females of *Tetranychus urticae* Koch and the observed LC50 values in the fumigation test was 3.59 µL/L air after 24 h.

#### Conclusions

With many *Eucalyptus* species adapted to arid conditions, *E. torquata* is considered drought tolerant. The corresponding tolerance mechanisms developed by this species were demonstrated in this review. To assume, drought resistance of *E. torquata* was manifested by stomatal closure to prevent water loss. Osmotic adjustment was a coping strategy to water stress by increasing the accumulation of solutes, including soluble sugars (glucose and fructose) and cyclitols (pinitol and myo-inositol) in addition to that, the resilience to xylem embolism. However, salt stress may have a negative impact on *E. torquata* which appears sensitive to the presence of NaCl that acts in particular on photosynthesis. This paper showed also the large variability in yields and chemical composition that exists among *E. torquata* hydro distilled essential oils from several origins. The majority of oils produced are rich in 1,8-cineole,  $\alpha$ -pinene and torquatone. It can be concluded that *E. torquata* derived essential oils are a rich resource of active phytochemicals which can possess a wide range of biological activities; From the present review, it is clear that possess potent antimicrobial capacity and exhibit an advances anticancer and biocidal effects.

### Authors' Contributions

Writing-original draft: SBR; drafting guidance: KBY, SD, IC, SB, CBR; Supervision: ES, MR.  
All authors read and approved the final manuscript.

### Ethical approval (for researches involving animals or humans)

Not applicable.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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