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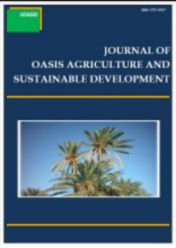

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Essential oils, chemical composition, and biological activities of *Eucalyptus oleosa* F. Muell. : A review

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Abstract

Many *Eucalyptus* species are growing in the border of oasis areas. *Eucalyptus* sp. are generally known for their richness in essential oils and their virtues and economic interests. However, the great taxonomic diversity affects the quantity and quality of these oils. This study is designed to summarize the chemical composition of *Eucalyptus oleosa* and their biological activities. The yield of essential oils in the leaves of this species varies from 0.45% to 6.7%. These oils contain many chemical compounds of which 1,8-cineole is the main component (15.31% – 89.4%) followed by α-pinene (1%– 24.7%). *Eucalyptus oleosa* essential oils exhibited antioxidant, antibacterial, anti-fungal and insecticidal activities with high variability. This variability is associated to many factors such as subspecific diversity, geographical location, part of plant and essential oil's extraction method.

1. INTRODUCTION

Eucalyptus is a large genus of the Myrtaceae family (Grattapaglia et al., 2012) that includes 900 species and subspecies of which more than 300 species contain volatile essential oil in their leaves (Al-Snafi, 2017), and about twenty of these species are known for their richness in 1,8-cineole, used in pharmaceutical and cosmetic industries (Pino et al., 2002; Dhakad et al., 2018). Although *Eucalyptus* is widely grown in many countries all over the world (Booth, 2013), Australia is probably the only one where such a single group of plants dominate most of the landscape (Crisp et al., 2011). Worldwide production of *Eucalyptus* essentials oils is around 3000 tons, and the major producers are China, Spain, Portugal, South Africa, and Chile (Ghaffar et al., 2015). It has a long history of use in traditional medicine in the treatment of cold, fever, flu, and general sickness (Salehi et al., 2019). In addition to its richness in essential oils, *Eucalyptus* is used for ornamentation, afforestation, and to obtain timber, gum, pulp and paper and it's known by its cosmetic and medicinal values (Saadaoui et al., 2017). Furthermore, Green synthesis of silver nanoparticles is possible to use the aqueous extract of *E. oleosa* as a green synthesis procedure without any catalyst, template, or surfactant. Colloidal gold and silver nanoparticles are synthesized with *Eucalyptus* leaf extract at non-photomediated conditions (Pourmortazavi et al., 2015; Pourmortazavi et al., 2017). In addition to these biological activities, *Eucalyptus* oils have ecological roles; they act as herbivore deterrents, alleviate ozone toxicity, and mitigate the temperature throughout the time of fires (Sawalha et al., 2021). In food, the efficiency of *Eucalyptus* essential oils as a natural beverage preservative is verified; an effective and potent inhibitor of spoilage fungi and their *in vitro* antimicrobial effect is assessed against 17 food spoilage microorganisms (Boukhatem et al., 2020).

This study focused on *Eucalyptus oleosa*, a xeric species, resistant to environmental stresses including salinity and drought; and grows in very low rainfall (Merchant et al., 2006; Hobbs et al., 2009). The choice was made essentially for its high content of 1,8-cineole more than 80% (Jaymand et al., 2009) and its diversified biological activities. In terms of chemical composition, essential oils of *E. oleosa* were complex mixtures of substances generally terpenes and terpenoids (Marzoug et al., 2011). Considering the versatility of *Eucalyptus* essential oils in terms of bioactivities as well as their industrial importance, the purpose of this study is to provide the readers with the latest information concerning the essential oils yields, the chemical composition, and biological activities of *E. oleosa*.

2. YIELD OF *E. OLEOSA* ESSENTIAL OILS

The *Eucalyptus* essential oils were extracted ~~by~~ using different methods such as supercritical CO_2, microwave and by solvents. Hydrodistillation is typically the most used method to obtain volatile compounds produced by plants (Richter and Schellenberg, 2007). A considerable variation in the yields of essential oils extracted from *E. oleosa* leaf has been detected and the values were rang~~ing~~d from 0.06 to 7 % (Elaissi et al., 2007). Previous studies have reported that the essential oils yield from plants collected in Iran was 6.7% (Ebadollahi et al., 2013; Rahimi-Nasrabadi et al., 2013), whereas others reported that the yield of *E. oleosa* essential oils varied from 2.31% to 3.2% collected from the same country (Jaymand et al., 2009; Ebadollahi et al., 2017). Relatively essential oils yield extracted from *E. oleosa* harvested in Tunisia was 4.90% (Marzoug et al., 2011) and similar results was detected with *E. oleosa* volatile oils from Australia that contained 4.60% of essential oils (Bignell et al., 1995). The detected values were more important than that reported in other species in Tunisia (*E. maideni, E. astrengens*, *E. cinerea, E. leucoxylon*, *E. lehmani*, *E. sideroxylon* and *E. bicostata*) in which the yields were ranging from 1.2% to 3% (Sebei et al., 2015). According to the literature, the yields of essential oils varies significantly between species of the genus *Eucalyptus* such as 0.29% in *E. microtheca* (Hashemi-Moghaddam et al., 2013), 0.5% in *E. camaldulensis* (Ndiaye et al., 2018), 3.9% in *E. sargentii* (Fathi and Sefidkon, 2012), and 1.8% in *E. globulus* (Damjanović-Vratnica et al., 2011). Moreover, other investigation reported variable oil content (0.45%–1.12%) from different aerial parts of *E. oleosa* originating from Tunisia (Marzoug et al., 2011). Other *Eucalyptus* species analyzed from Marocco (*E. cinerea, E. baueriana, E. smithii, E. bridgesiana, E. microtheca, E. foecunda, E. propinqua* and *E. erythrocorys*) have similar oil yields ranging from 0.2% to 1.15% (Zrira et al., 2004). As previously stated, the observed variability not only might have been derived from harvest time, local, climatic, and seasonal factors but also it could be greatly depending upon the different parts of the plants extracted. Other study reported the effect of the extraction methods on the yield (Chamali et al., 2019).

3. CHEMICAL COMPOSITION

The *E. oleosa* essential oils were analyzed using GC-MS to identify their components. Generally, monoterpenes are the major components of *Eucalyptus* essential oils (Ohara et al., 2010). The essential oils composition of *E. oleosa* leaves showed by some studies that all of them contained 1,8-cineole, the highest content is (89.4%) (Jaymand et al., 2009) followed by (57.89%) (Safaei-Ghomi et al., 2009), (52.04%) (Bignell et al., 1995), (45.1%) (Rahimi-Nasrabadi et al., 2013) and (31.96%) (Marzoug et al., 2011), while some studies ~~gave~~ presented the lowest rate (15.31% and 22.94%) (Chamali et al., 2019; Ben Hassine et al., 2012). These data supported previous published results and confirmed that 1,8-cineole is the major ~~one~~ compound in leaf essential oils of *E. oleosa* and the most important volatile component in most of *Eucalyptus* species (Fadel et al., 1999; Vilela et al., 2009; Maghsoodlou et al., 2015; Vivekanandhan et al., 2020). Besides, other investigation confirmed that the main component of the essential oils of all parts of the *E. oleosa* (stems, adult leaves, immature flowers, and fruits) is 1,8-cineole (31.5%, 8.7%, 47.0% and 29.1%, respectively). For the other molecules there is a high diversity between plant tissues (Marzoug et al., 2011). The other species, especially *E. camaldulensis*, had relatively low monoterpene contents. In the case of *E. camaldulensis*, α-pinene (22.52%) and 1,8-cineole (9.48%) were the predominant compounds, as indicated by Sebei et al. (2015).

Other studies reported on *E. microtheca* showed the following composition: α-pinene (6.752 %) and β-pinene (5.006 %) as a major compound (Maghsoodlou et al., 2015). Thus, for many *Eucalyptus* species, several factors may influence monoterpenes synthesis, especially seasonal and diurnal emission activity cycles (He et al., 2000). The second major class in *E. oleosa* identified also with high rates is represented essentially by α-pinene (1.0%- 24.7%), β-pinene (1.2%- 11.36%), α-thujene (0.1%- 11.42%) and p-cymene (0.38%- 10.91%).

The third class in *E. oleosa* essential oils is the oxygenated sesquiterpenes constituted by spathulenol, thymol and borneol. The chemical composition of the essential oils of *E. oleosa* has been evaluated in many studies and they were presented in Table 1.

Tableau 1. Major components present in essential oils of *Eucalyptus oleosa* samples.

compounds	RI	Chamali et al. 2019	Ben Hassine et al. 2010	Rhimi et al. 2013	Jiamand et al. 2009	Marzoug et al. 2011	Safaei-Ghomi et al. 2009	Bignell et al. 1995	Ebadol-lahi et al. 2017
α thujene	**931**	--	11.42	0.1	--	--	--	--	2.42
α-pinene	**936**	12.28	10.91	14.5	1.0	21.80	11.19	24.7	15.25
β-pinene	**982**	11.36	--	1.5	1.2	--	1.62	2.59	2.22
p-cymene	**1026**	--	10.91	--	--	3.30	5.86	3.39	0.38
m-cymene	**1028**	17.02	--	--	--	--	--	--	--
1,8-Cineole	**1036**	15.31	22.94	45.1	89.4	41.20	57.89	52.04	31.96
γ –Terpinene	**1060**	--	--	0.7	0.70	3.00	--	--	--
soamyl valerianate	**1106**	--	--	1.9	--	0.30	--	--	--
α campholenal	**1126**	--	3.66	0.2	--	--	--	--	--
Trans-pinocarveol	**1139**	--	0.86	3.9	0.6	5.70	7.79	0.63	3.59
(E)-verbenol	**1155**	8.86	--	--	--	--	--	--	--
Pinocarvacrone	**1163**	--	1.58	--	--	--	3.18	--	--
Pinocarvone	**1165**	--	--	1.1	--	1.80	--	0.31	1.19
Borneol	**1169**	--	--	0.9	--	0.90	--	--	--
4-Terpineol	**1177**	--	--	1.9	--	--	--	--	--
p-cymén-8-ol	**1185**	--	--	--	--	1.70	--	--	--
α-Terpineol	**1189**	--	--	4.3	--	--	1.17	--	5.33
verbenone	**1211**	13.70	--	0.1	--	0.20	--	--	
Isodihydrocarveol	**1222**	4.44	--	--	--	--	--	--	--
cuminaldéhyde	**1237**	--	--	--	--	0.90	--	--	--
Thymol	**1310**	--	2.04	0.1	--	--	--	--	0.73
Spathulenol	**1578**	--	--	0.4	--	--	0.34	0.83	0.36
Viridiflorol	**1593**	--	--	0.3	--	1.10	--	--	--
γ-eudesmol	**1635**	0.34	--	--	--	5.30	--	--	0.57
β-Eudesmol	**1651**	--	--	2.2	--	--	0.44	0.25	1.36
Total		99.97	99.10	98.00	93.50	97.60	99.64	97,44	99.87
Monoterpene hydrocarbons (%)		41.47	26.3	18.70	2.2	28.20	18.67	38.04	23.05
Oxygenated monoterpenes (%)		46.89	33.95	68.60	91.3	50.70	78.67	54.17	66.08
Sesquiterpene hydrocarbons (%)		2.54	38.88	0.50	0	4.10	0.34	2.27	0.77
Oxygenated sesquiterpenes (%)		6.01	0	6.50	0	7.70	1.05	2.54	3.69
Others (%)		3.44	0	3.70	0	6.50	0.91	0.42	6.28

(--) no identified, RI: Retention Index

All these variations might be due to the influence of geographical differences, environmental and growing conditions, physiological and biochemical states of plants, different extraction and analytical procedures, and genetic factors (Kokkini et al., 2004; Hassanpouraghdam et al., 2011). Furthermore, such variation can be attributed to several factors including plant age, climate, vegetative cycle stage, harvest time, geographical location, part plant used and genetic variation (Barra, 2009; Ben Hassine et al., 2012; Barbosa et al., 2016; Dorsaf et al., 2016; Almas *et al*., 2018). Several clinical studies indicate that Eucalyptol, the monoterpene, due to different medicinal properties including antioxidant (Ciftci et al., 2011), antimicrobial (Schürmann et al., 2019), anti-inflammatory (Zhao et al., 2014) and respiratory disorder treatments (Sudhoff et al., 2015). On other hand, the richness in 1,8-cineole revealed several potential applications; as an insect repellent (Aldoghaim et al., 2018). Furthermore, it is often used as a flavoring agent for food products (Santos and Rao, 2001).

4. BIOLOGICAL ACTIVITIES

Traditionally, the *Eucalyptus* oils are used to treat fever, bronchitis, asthma, and pulmonary diseases via inhalation (Horváth and Ács, 2015). Previous research found that *Eucalyptus* essential oils have numerous biological properties such as antioxidant, anti-bacterial, fungicide, anti-allergicantic, anti-cancer, anti-inflammatory, insecticidal and herbicidal effects

(Silva et al., 2003; Batish et al., 2006; Gilles et al., 2010; Salem *et al*., 2015; Vuong *et al*., 2015; Nakamura et al., 2020; Sharma, 2020; Pinto et al., 2021). Indeed, *Eucalyptus* essential oils play a central role in these biological functions by their active chemical substances (Barbosa et al., 2016; Migacz et al., 2018). Similarly, many essential oils produced by *E. oleosa* have been reported for their antioxidant, antimicrobial and insecticidal activities (Ebadollahi et al., 2013; Rahimi-Nasrabadi et al., 2013; Marzoug et al., 2015). Moreover, *E. oleosa* terpenes such as *α*-Terpineol, p-cymene, α- and β-pinene show much wider therapeutic uses antimicrobial, antiviral, antihyperglycemic, anti-inflammatory, antioxidant, antiparasitic and immune-modulatory (Upadhyay, 2022).

4.1. Antioxidant activities

Antioxidants play an important role in food preservation by inhibiting oxidation processes and contributing to the health promotion provided by many dietary supplements, nutraceuticals, and functional food ingredients (Shahidi and Zhong, 2015). Furthermore, natural antioxidants are in demand for pharmaceuticals products (Brewer, 2011). Therefore, in recent years, considerable attention has been detected towards the identification of plants with antioxidant activity (Moon and Shibamoto, 2009). Previous studies revealed that *E. oleosa* leaf essential oils from Tunisia exhibited high antioxidant potential in both assays (DPPH and ABTS), the IC_{50} were 52.8 ± 0.7 mg/mL and 176.5 ± 3.1mg/mL respectively (Ben Marzoug et al., 2010). In addition, the antioxidant capacity of four parts of *E. oleosa* essential oils (stems, adult leaves, fruits, and immature flowers) showed moderate antioxidant activities in which the best IC_{50} is found for the adult leaves essential oil (0.013 ± 0.0006 mg/mL) in the ABTS assay (Marzoug et al., 2011). Whereas another study showed that the leaf essential oils of *E. oleosa* from Iran did not show any antioxidant activity (Rahimi-Nasrabadi et al., 2013). This moderate antioxidant activity is probably due to the low content of phenolic compounds presents that is related to the extraction technical used and origin of plant.

4.2. Antimicrobial activities

The antimicrobial activity of the *E. oleosa* essential oils has been studied by several researchers and discussed in the text; the essential oils exhibit toxicity against a wide range of microbes, including bacteria, fungi, yeast but the bioactivity against virus has not investigated (Table 2).

4.2.1. Antibacterial activities

It has been reported that *E. oleosa* is active against Gram+ strains *Enterococcus feacalis* and *Staphylococcus aureus* with minimal inhibitory concentration which was situated between 0.028-0.056 mg/ml. In addition, the bactericidal dose against all organisms tested was ranged between 28-56 µg/ml (Ben Hassine et al., 2012). These results agreed with the study showed that the *E. oleosa* essential oils of different parts (stems, adult leaves, fruits, and immature flowers) appeared more active against the tested Gram+ such as *Staphylococcus aureus* and *Listeria monocytogenes* than Gram negative bacteria, although the immature flowers presented a larger prevalence of activity 0.39-3.72 mg/mL (Srinivasan et al., 2001). But another study revealed that the essential oil of *E. oleosa* exhibited high antibacterial activity against Gram negative ones, with highest inhibition zone 19.0 mm diameter and lowest MIC value 0.062 mg/ml against *E. coli* which shows that this microorganism is sensitive to *E. oleosa* essential oil (Rahimi-Nasrabadi et al., 2013). Hence, the activity against both types of bacteria Gram+ and Gram-, may be indicative of presence of broad spectrum antibiotic compounds or simply general metabolic toxins (Srinivasan et al., 2001).

4.2.2. Antifungal activities

An antifungal activity of the different part (stems, adult leaves, fruits, and immature flowers) of *E. oleosa* essential oils were tested against three pathogenic fungi *Aspergillus ochraceus*, *Mucor ramamnianus* and *Fusarium culmorum* and demonstrated that immature flowers and stems had strongest antifungal activity with minimal inhibition concentration value between 2.79-3.88 mg/ml (Srinivasan et al., 2001). Additionally, these findings were consistent with another study performed by Kouki et al. (2023) who reported that *E. oleosa* EOs exhibited a significant antifungal activity against five *Fusarium ssp.*

Generally, *E. oleosa* essential oils showed variable antimicrobial activity against the different test organisms. This variability could be related to several factors such as chemical composition of essential oils and geographic location of the plant material, also the sensitivity of the bacterial strains and its nature (Sabo and Knezevic, 2019).

Table 2. Antimicrobial effects of *Eucalyptus oleosa* EOs investigated on the pathogenic microorganisms.

***Eucalyptus oleosa* EOs origin**	**Part used**	**Inhibited microorganisms**	**References**
Gabes (Tunisia)	Arial parts	*Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella enterica, Escherichia coli, Klebsiella pneumoniae, Saccharomyces cerevisiae, Candida albicans, Aspergillus ochraceus, Mucor ramamnianus, Fusarium culmorum*	Marzoug et al., 2011
Gabes (Tunisia)	leaves	*Listeria monocytogenes, Klebsiella. pneumoniae, Saccharomyces cerevisiae, Candida albicans, Mucor ramamnianus, Aspergillus ochraceus*	Ben Marzoug et al., 2010
Kashan (Iran)	Leaves	*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella. pneumoniae, Bacillus subtilis, Bacillus cereus, Salmonella typhimurium*	Rahimi-Nasrabadi et al., 2013
Monastir (Tunisia)	Leaves	*Escherichia coli, Enterococcus feacalis, Staphylococcus aureus, Listeria monocytogens, Salmonella anatum, Salmonella enteritidis*	Ben Hassine et al., 2012

4.2.3. Insecticidal/Acaricidal activities

The intense application of insecticides leads to the development of insecticide resistance in insect pest populations worldwide (Pittendrigh et al., 2008) and resulted in an increased risk of pesticides resistance, toxicological implications for human health and environmental pollution (Batish et al., 2006; Mahmood et al., 2016; Alengebawy et al., 2021). Thus, there has been a growing interest in research concerning the possible use of plant extracts as alternatives to synthetic pesticides (Ghosh et al., 2012). Recently, the insecticidal activity elicited by certain essential oils indicates that these botanical compounds could be used as alternative tools (Isman, 2000). Furthermore, essential oils are applied similarly to other pesticides and their biological activity is manifested both by exposure to their vapors and by topical application (Isman, 2000; Tarelli et al., 2009). Further, it has been reported that *Eucalyptus* species essential oils have significant pesticidal potentials (Maciel et al., 2010; Alzogaray et al., 2011; Pant et al., 2014; Filomeno et al., 2017; Ainane et al., 2019). Insecticidal activity of *E. oleosa* oils from Iran are assessed against American white moth, *Hyphantria cunea* Drury 1773 (Lepidoptera: Arctiidae) at different concentrations (0.1, 0.21, 0.45, 0.95 and 2%) used at three times (24h, 48h and 72h) in which found the LC_{50} (Lethal Concentration to kill 50% of insects) values were estimated as 0.36% at a shorter duration (24h) (Ebadollahi et al., 2013). In addition, the acaricidal effects of *E. oleosa* essential oils against *Tetranychus urticae* Koch (Acarina: Tetranychidae) have been reported (Ebadollahi et al., 2017). Hence, volatile oils from *E. oleosa* show strong phytotoxicity towards *H. cunea* and *T. urticae.* Indeed, it could provide opportunities for new biodegradable products for pest control considering their noticeable effects at low applied concentrations and short times of exposure. Indeed, the toxicity of the essential oils tested varies widely depending on the nature of the essential oil, the concentration used and the duration of the treatment. Studies have revealed that monoterpenes have insecticidal activities against the stored–product insects (Rajendran and Sriranjini, 2008; Papachristos et al., 2004).

5. CONCLUSION

Eucalyptus oleosa shows high subspecific variability and resistance to arid conditions. It showed a great richness in essential oils and their chemical composition indicated that the most abundant component is 1,8-cineole, followed by α-pinene. These characteristics represent advantages for the use of the species in afforestation and the valorization of these oils. Furthermore, *E. oleosa* essential oils possess a broad spectrum of biological effects, such as antioxidant, antibacterial, anti-fungal and anti-insecticidal activities. *E. oleosa* deserve to be deepened by studies on the relation subspecies, essential oils, and activities for a selection of promising subspecies for their aridity tolerance and their oils quantity, quality, and activities.

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