Chemical composition, antibacterial and antioxidant activities of essential oil of Eucalyptus globulus from Algeria

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Chemical composition, antibacterial and antioxidant activities of essential oil of *Eucalyptus globulus* from Algeria

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ABSTRACT

Essential oils are known for their use in various fields such as cosmetic, pharmaceutical and food industries. The aim of this work is to investigate the chemical composition of essential oils from *Eucalyptus globulus* leaves (*E. globulus*) by gas-chromatography coupled with mass spectrometry (GC/MS) method, and to evaluate their antioxidant capacity (DPPH radical scavenging effect, reducing power, and inhibition of lipid peroxidation activity) as well as their antibacterial activity, against periodontopathogenic and cariogenic bacterial species, using microdilution method in 96-well microplates. In total, 26 compounds were identified with the predominance of oxygenated monoterpenes (78.58%); 1,8-Cineole (55.29%), Spathulenol (7.44%) and α-Terpineol (5.46%) being the main components. The analyzed oils exhibited a weak antioxidant capacity, but a marked antibacterial activity against Gram negative bacteria, mainly for *F. nucleatum* ATCC 25586 (MIC = 1.14 mg/mL) and *P. gingivalis* ATCC33277 (MIC = 0.28 mg/mL). Therefore, *E. globulus* essential oils may have a potential therapeutic application for the treatment of periodontal diseases.

1. Introduction

Eucalyptus globulus belongs to the family of Myrtaceae which is indigenous to Australia. It was introduced to Algeria in 1854 by Ramel (Boulekbache-Makhlouf et al., 2010), where it is now widely distributed. Essential oils of *E. globulus* contain more than 20 compounds with a prevalence of 1,8-Cineole (Batish et al., 2008; Boukhatem et al., 2014; Goldbeck et al., 2014; Maciel et al., 2010).

The limitation on the use of synthetic antioxidants and the increase interest for natural non-toxic antioxidants has spawned numerous studies on the antioxidant potential of essential oils. Essential oils of plants are a mixture of various components such as monoterpenes, sesqui-terpenes, alcohols, esters, aldehydes and ketones, which are involved in the defense of the plant against pests, herbivores, fungi, and bacteria (Batish et al., 2008). Furthermore, essential oils and aromatic plants are known for their multiple uses in flavor and fragrance, as preservatives, and as antimicrobials (Bakkali et al., 2008). Due to the toxicological effect of the synthetic products, renewed efforts were provided in respect of the use of essential oils as natural antioxidants and preservatives in the food processing, food supplement production and pharmaceutical industry (Wei and Shibamoto, 2007).

The essential oils of *Eucalyptus* species are widely used in the world, the United States Food and Drug Authority considered them as safe and non-toxic, even the Council of Europe has approved the use of eucalyptus oils as flavoring agent in foods (Batish et al., 2008). Consequently, a growing interest has been given to their use in the scientific research field and industry as a natural food additive, drugs and cosmetics. (Goldbeck et al., 2014; Ishnava et al., 2013). Several studies investigated the antioxidant potential of essential oils from various *Eucalyptus* species such as *E. polyanthemos*, *E. perriniana*, and *E. camaldulensis* (Barra et al., 2010; Lee and Shibamoto, 2001; Singh et al., 2012). Singh et al. (2012) have reported a strong antioxidant activity of decaying and fresh leaves of *E. tereticornis* against DPPH, $OH^{\bullet}$ and O2 radicals. However, Barra et al. (2010) have reported a moderate DPPH scavenging activity for oils extracted from aerial parts of *E. camaldulensis* and *E. radiate*. *Eucalyptus* leaves are rich sources of essential oils, flavonoids

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or tanins, which are responsible for their antibacterial, larvicidal, fumigant, antioxidant activities and anthelmintic properties. The major compounds of *E. globulus* essential oils are 1,8-cineole (eucalyptol), aromadendrene, globulol, D-limonene and pinene, their content depends on environmental, agronomic factors, plant parts and the age (Topiar et al., 2015; Armando et al., 1997).

Major oral infectious diseases (dental caries and periodontal diseases) are caused by bacteria colonizing the oral surfaces. Despite the advances concerning its prevention and control, dental caries, which is the result of the degradation of the enamel by the acid produced by bacteria, is still considered a public health problem worldwide affecting a large proportion of the young population (Ishnava et al., 2013). The major causative bacteria of dental caries are mutans group streptococci, such as *Streptococcus mutans* and *Streptococcus sobrinus* (Berezow and Darveau, 2011; Selwitz et al., 2007). Periodontal diseases are inflammatory disorders that lead to tooth loss. They are caused by Gram-negative anaerobic bacteria (*Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*) that destroy the periodontal tissue by interacting with the mucosal immune cells (Allakerand Douglas, 2009; Madianos et al., 2005). Natural products have been recently investigated as promising agents for the prevention of oral diseases, and herbal products are increasingly used as alternatives to traditional chemical drugs (Harzallah et al., 2011).

Few studies have reported the antioxidant activity of essential oils from *E. globulus* leaves (Mishra et al., 2010; Noumi et al., 2011) and only one work has been conducted on their activity against *S. mutans* (Goldbeck et al., 2014). In order to contribute to the evaluation of the biological activities of *E. globulus* plant, it should be important to test the antioxidant and the antibacterial capacities of its leaves, with a view of their pharmaceutical and industrial applications. This paper is a part of a study on the evaluation of the volatile components of *E. globulus* cultivated in Algeria, so the composition and the determination of two biological activities (antioxidant and antibacterial) of the essential oils of its leaves is the subject of this report. Therefore, in this work a GC/MS method was developed to characterize the volatile compounds from hydrodistillated extract of *E. globulus* leaves. Their antioxidant effect was tested by the reducing capacity, the inhibition of lipid peroxidation and the scavenging effect on DPPH$^{\bullet}$ free radical. Concerning their antibacterial activity, it was determined against Gram-negative periodontopathogenic and Gram-positive cariogenic bacterial species.

2. Materials and methods

2.1. Plant materials and chemicals

Plant samples were collected from the arboretum of Derguinah (36°31′13.56″ N, 5°17′ 18.43″ E), Bejaia, in the north east of Algeria, in February 2013. All solvents and reagents were of analytical grade. Samples were cleaned and dried in the drying oven at 30 °C. A sample of 150 g boorishly crushed leaves was subjected to extraction by hydrodistillation for 3 h/500 mL distilled water using a Clevenger type apparatus. The obtained oil was recovered and stored at 4 °C. The oil yield was calculated as the ratio of the weight of oil to the weight of leaves.

2.2. Determination of refractive index

The refractive index is used to confirm the purity of essential oils. It was determined as previously described by Boukhatem et al. (2014) and calculated using the Eq. (1).

$$n = \frac{\text{Speed of light in a vacuum}}{\text{Speed of light in medium}} \quad (1)$$

2.3. Determination of specific gravity

The specific gravity of *E. globulus* oils was determined as previously described in AOAC (2000) standard method. Briefly, a gravity bottle was weighted (W_0), then filled with water and stopper was inserted. The water of the bottle was wiped off and weighed again (W_1). The same process was repeated by using oil sample and reweighted (W_2). The specific gravity of the oils was calculated using the Eq. (2).

$$\text{Specific gravity of oil} = \frac{W_2 - W_0}{W_1 - W_0} \quad (2)$$

Where W_0 = weight of the empty gravity bottle, W_1 = weight of water + gravity bottle, W_2 = weight of oil + gravity bottle.

2.4. Analysis of the essential oils

Analysis of the essential oils was carried out with a TRACE Ultra Gas Chromatograph coupled to an ISQ Mass Spectrometer (ThermoScientific, Austin, Texas, USA), connected to a computer running Xcalibur 2.0 software (ThermoScientific, Austin, Texas, USA). A DB-5ms capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent J&W, Santa Clara, CA, USA) was used. The analysis was performed using helium (purity >99.99 vol.%) as a carrier gas at 1.2 mL/min with the following temperature program: 40 °C for 2 min, increased to 250 °C at 5 °C/min and to 300 °C at 30 °C/min and maintained at this temperature for 10 min. One μL of sample was injected at a constant temperature of 250 °C with a split ratio of 1:20 during 1 min. Masses were scanned between 40 and 650 uma. The essential components were identified by comparing their mass spectra with those stored in the NIST/EPA/NIH library.

2.5. Antioxidant activity

The antioxidant activity of the essential oils from *E. globulus* leaves was estimated by DPPH, reducing power, and inhibition of lipid peroxidation tests. The DPPH assay was estimated as described by Noumi et al. (2011) method. Different concentrations of the sample were prepared in pure methanol, then 1 mL of each of them was added to 0.25 mL of a 0.2 mmol/L DPPH methanolic solution (v/v). The obtained solutions were shaken vigorously and left at room temperature for 30 min, and their absorbance was measured at 517 nm after 30 min. The scavenging activity was calculated using the Eq. (3).

$$\text{DPHH scavenging activity}\,(\%) = \frac{[(A_0 - A_t) \times 100]}{A_0} \quad (3)$$

Where A_0 is the absorbance of the control after 30 min, and A_t is the absorbance of the sample after 30 min. Results were expressed as *IC*50 (mg/mL), it corresponds to the dose required to cause a 50% inhibition. A lower *IC*50 value corresponds to a higher antioxidant activity.

The reducing capacity of the tested oils was evaluated by the procedure of Singh et al. (2012). One mL of different concentrations (10, 20, 30, 40 and 50 mg/mL) was mixed with 1 mL of phosphate buffer (0.2 M 'w/v', pH 6.6) and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$], 1% 'w/v'. The obtained solutions were incubated at 50 °C for 20 min. Then 1 mL of Trichloroacetic acid (TCA) (10%'w/v') was added to the solution that was then centrifuged for 10 min at 3000 × *g*. The supernatant was recovered and mixed with 1.5 mL of distilled water and 150 μL of $FeCl_3$ (0.1% 'w/v'). The absorbance was measured at 700 nm and the Butylated hydroxyanisole (BHA) was used as standard. The result was expressed as *IC*50 (mg/mL).

The lipid peroxidation activity was determined by the β-carotene bleaching method (Tepe et al., 2006), which is based on the inhibition of the products of linoleic acid oxidation (volatile

organic compounds and the conjugated dienehydroperoxides). An emulsion of β-carotene/linoleic acid was prepared by mixing 25 μL of linoleic acid, 200 mg of Tween 40, 0.5 mg of β-carotene and 1 mL of chloroform. After evaporation of the solvent, under low pressure at 40 °C, 100 mL of distilled water were added. To 2.5 mL of the obtained solution, were added 350 μL of sample (2 mg/mL), after shaking, the mixture was incubated for 48 h at room temperature. Two controls were prepared, one with the standard BHA (positive control) and the other without BHA or extract (blank). Absorbance at 490 nm of each sample was immediately measured at 0 h, 2 h, 4 h, 13 h, and 48 h. Relative antioxidant activity was calculated according to the Eq. (4).

$$\text{Antioxidant activity}\,(\%) = \frac{A_t}{A_0} \times 100 \quad (4)$$

Where A_t is the absorbance of the sample after 48 h, and A_0 is the essential oil absorbance at the beginning of incubation. Results were expressed as *IC*50 (mg/mL).

2.6. Antibacterial activity

The antibacterial activity of essential oils from *E. globulus* leaves was tested against 12 bacterial strains. Six Gram-negative periodontopathogenic bacteria (*F. nucleatum* ATCC 25586, *A. actinomycetemcomitans* ATCC 29522, *P. gingivalis* ATCC 33277, ATCC 49417, HW24D1, and W83) and six Gram-positive cariogenic bacteria (*S. mutans* ATCC 35668, ATCC 33535, ATCC 25175, *S. sobrinus* ATCC 33478, ATCC 27607, ATCC 27352).

The growth of bacteria was carried out in Todd Hewitt broth (BBL Microbiology Systems, Cockeysville, MA, USA) in the presence of 0.001% hemin and 0.0001% vitamin K (THB-HK). After 24 h of incubation at 37 °C in an anaerobic chamber (N_2:H_2:CO_2;75:10:15), minimum inhibitory concentration (MIC) values were determined using a broth microdilution method in 96-well microplates (Azelmat et al., 2015). A 24 h culture bacterium was prepared in fresh THB-HK to obtain an optical density of 0.2 at 660 nm. Then 100 μL of both bacterium solution and serial dilutions of the essential oils, in culture medium containing 0.5% Tween 80, were mixed into wells. Two controls were prepared (with no bacteria or no essential oil), after that the microplates were incubated under anaerobic conditions for 24 h at 37 °C.

2.7. Statistical analysis

All tests were conducted in triplicate and results are expressed as mean ± standard error. *IC*50 value was calculated using the linear regression equation obtained from the curve, i.e. absorbance = *f* (extract concentrations). XLSTAT Release 10 (Addinsoft, Paris, France) was used to the analysis of variance (ANOVA). To compare means of each parameter, Tukey's multiple range test (HSD) was used. Differences were considered to be significant at $P < 0.05$.

3. Results and discussion

3.1. Physical parameters analysis

Two physical parameters were determined to assess the quality of oils of *E. globulus* leaves (refractive index and specific gravity). The refractive index was 1.4657 ± 0.0070, which was comparable to that previously reported in the literature (1.4602–1.46933) (Boukhatem et al., 2014; Subramanian et al., 2012; Zrira et al., 2004). Whereas, the relative density of the studied essential oils was 0.9135 ± 0.0036. The farmer result was closer to those reported in a previous work about the essential oils from Algerian *E. globulus* plant (0.919) (Boukhatem et al., 2014) and those reported for the Moroccan Eucalyptus species (0.918–0.919) (Zrira et al., 2004). Furthermore, the values of these two physical properties were in agreement with the AFNOR standards for *E. globulus* spices (1.4590–1.4670 for the refractive index, and 0.906–0.923 for the relative density) (AFNOR, 2000).

Table 1
Chemical composition of *E. globulus* essential oil obtained by hydrodistillation (HD) compared to the results of Topiar et al. (2015) * obtained by hydrodistillation (HD[a]) and supercritical fluid extraction (SFE).

Compounds	Type	KI	Composition (%)		
			HD	HD[a*]	SFE[*]
α-pinene	M	920	4.61	–	–
β-pinene	M	1122	0.07	9.25	3.50
o-Ocymene	M	1026	1.83	–	–
Total M	M		6.51	–	–
Isovaleraldehyde	OM	660	10.04	–	–
2-pentanone-4-hydroxy-4- methyl	OM	837	1.69	–	–
1,8-Cineole	OM	1033	55.29	36.68	21.01
Linalool	OM	1096	0.10	–	–
2-pinen-4-ol	OM	1145	0.07	–	–
4-Terpineol	OM	1181	0.70	–	–
L-Pinocarvone	OM	1162	0.10	–	–
α-Terpineol	OM	1196	5.46	–	–
Crypton	OM	1189	3.10	–	–
Cuminal	OM	1243	0.42	–	–
E-Neral	OM	1268	0.63	–	–
Phelandral	OM	1279	0.10	–	–
Piperitone	OM	1255	0.25	–	–
p-cymenol	OM	1297	0.45	–	–
Total OM			78.58	–	–
TOTAL (M + OM)			85.09	–	–
Aromadendrene	S	1462	0.02	6.33	5.30
Allo-Aromadendrene	S	1440	0.04	1.45	1.06
Ledene	S	1490	0.28	–	–
Total S			0.34	–	–
β Caryophyllene-oxide	OS	1551	0.14	–	–
Epiglobulol	OS	1563	0.21	1.00	0.32
Spathulenol	OS	1577	7.44	–	–
Caryophyllene-oxide	OS	1586	1.66	–	–
Globulol	OS	1589	2.96	5.11	1.23
Eudesmol	OS	1626	0.98	0.92	0.47
Total OS			13.39	8.77	2.75
TOTAL (S + OS)			98.82	–	–

Concentration (%): the percentage of concentrations based on peak area integration.
HD: hydrodistillation with 500 mL of water for 3 h.
HD[a]: hydrodistillation of Topiar et al. (2015) with 300 mL of water for 5 h.
SFE: CO_2 supercritical fluid extraction from Topiar et al. (2015) at best operation conditions of 12 MPa and 40 °C and flow rate of 1.8 g/min.
KI: compounds were tentatively identified by comparison with mass spectra data (MS) obtained from NIST/EPA/NIH library and confirmed by comparison with Kovat's index on DB5MS column.
M: monoterpenes.
S: sesquiterpenes.
OM: oxygenated monoterpenes.
OS: oxygenated sesquiterpenes.
[*] Topiar et al. (2015).

3.2. Extraction yield of essential oils

The volatile oils extracted from of *E. globulus* leaves were pale colored, having camphor like smell and pleasant odor, similar to the finding of Boukhatem et al. (2014) and Iqbal et al. (2003). The extraction yield of essential oils was 2.53 ± 0.1%, this value is considerably higher than those reported in previous studies, ranging from 0.77% to 1.29% (da Silva et al., 2006; Joshi, 2012; Selvakumar et al., 2012). Furthermore, it was higher than that obtained from other species considered as economically important for essential oils production, such as *E. uniflora* with 0.4–1.1% (Melo et al., 2007), *Psidium guajava* with 0.13–0.45% (Joseph and Priya, 2010; Nisha et al., 2011) and *Melaleuca alternifolia* with 1–2% yields (Carson et al., 2006). Indeed, *E. globulus* plant also presents economic poten-

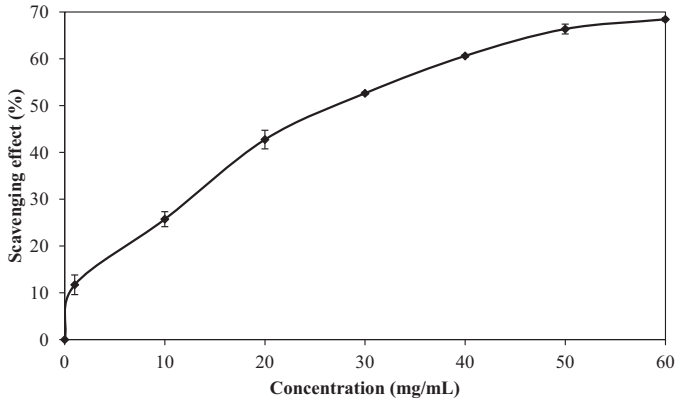

Fig. 1. Free radical scavenging activity (%) of the essential oil of *E. globulus* leaves.

tial, given that three to five thousand tonnes of Eucalyptus oils are traded every year on international markets (Batish et al., 2008).

3.3. GC/MS analysis of essentials oils

26 components have been identified in essential oils of of *E. globulus* leaves, which represent 98.82% of the total composition. They are mainly composed of monoterpenes (85.09%); their detailed composition is presented in Table 1. They consisted mostly of oxygenated monoterpenes and sesquiterpenes (78.58% and 13.39%, respectively), 1,8-Cineole (55.29%), Spathulenol (7.44%) and α-Terpineol (5.46%) being the main components (Table 1); similar amounts of the predominant compound (1,8-Cineole) have been reported in the literature (51.08 and 53.7%) (Boukhatem et al., 2014; Elaissi et al., 2012). These contents depend essentially on environmental, agronomic, age and geoclimatic factors and also on the used extraction techniques and the experimental extraction conditions. Table 1 lists the different compounds identified in the essential oils of *E. globulus* leaves obtained by hydrodistillation (HD), compared to those of Topiar et al. (2015) obtained by HD or supercritical fluid extraction (SFE). The difference in the essential oils extracted by HD and SFE is on the concentration of their components. In this study the content of the major compound (1,8-Cineole) is higher (55.29%) than those obtained by Topiar et al. (2015), which were about 36.68% and 21.01% for HD and SFE, respectively. Therefore, the HD extraction is the best method to extract 1,8-Cineole from *E. globulus* leaves. Besides 1,8Cineole, relative content of sesquiterpenes (S) and Oxygenated sesquiterpenes (OS) in *E. globulus* leaves essential oil extracted by the HD techniques (present study and Topiar's work, 2015) and SFE was 13.93, 8.75% and 2.77%, respectively. This indicates that HD remains the better technique for obtaining higher yields of sesquiterpenes and oxygenated sesquiterpenes. In addition, the HD used in the current study yielded more compounds (26 molecules) compared to the number of compounds (18 molecules) obtained by HD and SFE from Topiar's work (2015).

Table 2
Antioxidative capacities of the essential oil of *E. globulus* leaves and BHA.

Samples	IC 50 (mg/mL)		
	Reducing power	DPPH	β-carotene/linoleic acid
Essential oil	115.39 ± 1.45[b]	33.33 ± 0.55[b]	6.753 ± 0.39[b]
BHA	0.048 ± 0.015[a]	0.033 ± 0.002[a]	0.455 ± 0.19[a]

All the values are mean ± SD; SD: standard deviation.
[a] Column wise values with different superscripts of this type indicate significant difference ($p < 0.05$).
[b] Column wise values with different superscripts of this type indicate significant difference ($p < 0.05$).

3.4. Antioxidant activity

Fig. 1 shows the result of the scavenging effect on DPPH radical of the essential oils from *E. globulus* leaves. As we can see, the DPPH scavenging capacity of the tested oils increased by increasing the amount of sample, the inhibition percentage ranged from 11.72% to 60.63% according to the tested concentrations. Mishra et al. (2010) have reported a percentage of 79.55 ± 0.82 of leaf essential oils (80% 'v/v' concentration) from Indian *E. globulus*. The activity of the tested oils (Table 2) is lower (*IC*50 = 33.33 ± 055 mg/mL) than that of the standard BHA (*IC*50 = 0.033 ± 0.002 mg/mL). This result differs from values previously reported for the commercialized essential oils of the Tunisian *E. globulus* leaves with an *IC*50 of 57 μg/mL (Noumi et al., 2011), and that reported for the hydrodistillated essential oils from the Indian *E. citriodora* with an *IC*50 of 425.4 ± 6.79 μg/mL (Singh et al., 2012).

Concerning the reducing power activity, results are shown in Fig. 2, it increased with increasing concentrations. The *IC*50 values are depicted in Table 2, value of the standard BHA (0.048 ± 0.015 mg/mL) is significantly lower than that of the tested oils (115.39 ± 1.45 mg/mL). This activity is weak compared to that

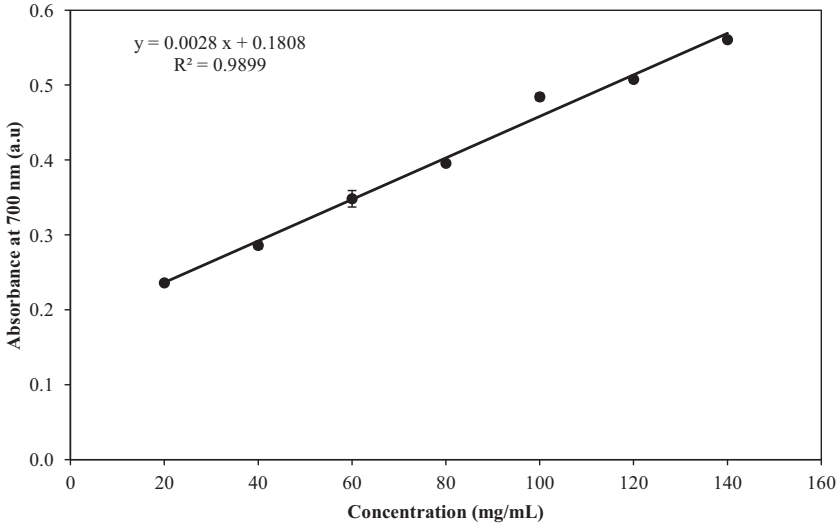

Fig. 2. Reducing power of the essential oil of *E. globulus* leaves.

Table 3
Minimum inhibitory concentration (MIC) of the essential oil of *E. globulus* leaves.

Gram-negative bacteria	MIC (mg/mL)	Gram-positive bacteria	MIC (mg/mL)
F. nucleatum ATCC 25586	1.14	*S. mutans* ATCC 35668	11.4
A. actinomycetemcomitans ATCC 29522	9.13	*S. mutans* ATCC 33535	11.4
P. gingivalis ATCC33277	0.28	*S. mutans* ATCC 25175	11.4
P. gingivalis ATCC49417	4.56	*S. sobrinus* ATCC 33478	11.4
P. gingivalis HW24D1	2.28	*S. sobrinus* ATCC 27607	11.4
P. gingivalis W83	2.28	*S. sobrinus* ATCC 27352	11.4

reported in the literature (48 μg/mL) (Noumi et al., 2011), this difference may be explained by the fact that these researchers used commercial essential oils rather than natural ones. On the other hand, an *IC*50 value of 87.3 ± 9.27 μg/mL has been reported for the essential oils of *E. citriodora* leaves (Singh et al., 2012), this value being less important than that found in our study.

The inhibition of the lipid peroxidation activity was assayed by the β-carotene bleaching test (Fig. 3), the activity of the oils was found to be dose dependent. Its *IC*50 value (6.75 ± 0.39 mg/mL) was significantly ($p < 0.05$) (Table 2) higher than that of BHA (0.455 ± 0.19 mg/mL). This value is also higher than that of the commercialized essential oils from *E. globulus* leaves (0.048 mg/mL) (Noumi et al., 2011). Compared to the DPPH scavenging effect and the reducing power, the essential oil of *E. globulus* leaves is more active on the inhibition of the lipid peroxidation, presumably due to the high specificity of the test for lypophilic compounds.

The difference found between our results and those reported in other studies about the antioxidant activity of essential oils from *E. globulus* plant, can be due to the difference in the mechanisms involved in the assays applied to evaluate the different tests and the extraction methods. The low activity of the tested oils can also be explained by the abundance of the ineffective compounds. Indeed, the tested oil is rich in monohydroxylated compounds such as 1,8-Cineole, which is not able to chelate ferrous ions (AidiWannes et al., 2010). AidiWannes et al. (2010) and MĎzami et al. (2013) have reported that essential oils with higher monoterpenic compounds are ineffective. Terpenes such as α-pinene, β-pinene, limonene, β-myrcene, sabinene and terpinolene are known to have a good antioxidant properties, however, depending on the mechanism involved in their action, some of them can exhibit low antioxidant activities (Martins et al., 2014). It has been reported that the β-carotene bleaching ability of monoterpene hydrocarbons may be due to the presence of methylene groups in their structure (AidiWannes et al., 2010). Consequently, the obtained results suggest that β-carotene antioxidant capacity of the oils may be lowered by its richness on monoterpene compounds. In the other hand, a recent study has confirmed the weak antioxidant activity of 1,8Cineole.

The low activity of the essential oil of *E. globulus* can also be explained by the degradation of the bioactive compounds during their extraction by hydrodistillation. Indeed, the hydrodistillation can lead to the thermal degradation, hydrolysis, and solubilisation of the bioactive compounds in water, thus changing their antioxidant capacity. Furthermore, the water used in hydrodistillation makes several antioxidants unstable or degrades them by enzymatic action in the wet plant material. In fact, in hydrodistillation, samples were usually extracted in boiling water over a long period of time, which could lead to thermal decomposition of thermolabile target compounds from *E. globulus* and thus lowering the antioxidant activity of the extract (Bagheri et al., 2014).

3.5. Antibacterial activity

Natural antibacterial substances, including essential oils, can be incorporated into mouthwash to control dental plaque. More specifically, Listerine™ which has been widely used for many years, contains thymol, eucalyptol, menthol and methyl salicylate (Allaker and Douglas, 2009).

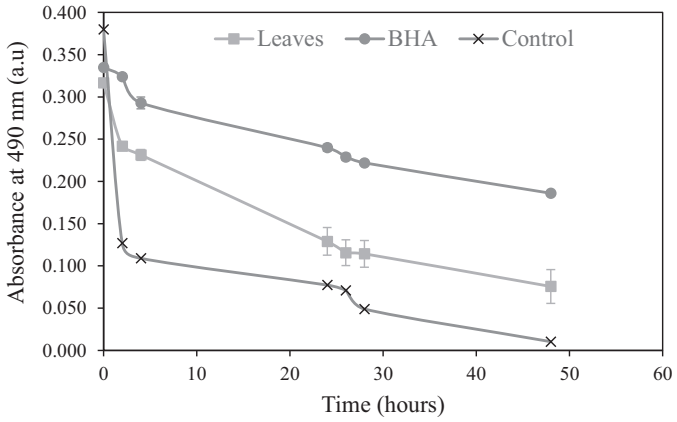

Fig. 3. Antioxidant activity of the essential oil of *E. globulus* leaves, BHA and control, measured by β-carotene/linoleic acid test.

As depicted in Table 3, Gram-negative bacteria were more sensitive to the essential oil of *E. globulus*, with the MIC ranged from 0.28 to 9.13 mg/mL. *P. gingivalis* ATCC 33277 was the most susceptible (MIC = 0.28 mg/mL) followed by *F.nucleatum* (MIC = 1.14 mg/mL). ; Goldbeck et al. (2014) have reported an MIC of 0.013 mg/mL for *S. mutans*, which is much lower than that found in our study, which was about 11.4 mg/mL for all tested strains of *S. mutans*. The divergence of our results and those of Goldbeck et al. (2014) can be related to the difference in the composition of the tested essential oils. Indeed, these two essential oils depict significant differences in their 1,8-Cineole and α-pinene concentrations (55.29% vs 71.05% and 8.30% vs 4.61%, respectively). Moreover, these two compounds were known by their antibacterial activity (Chikhoune et al., 2013; Elaissi et al., 2012). The sensitivity of *P. gingivalis* ATCC33277 bacteria (MIC = 0.28 mg/mL) to the essential oils of *E. globulus*, may be due to the presence of the oxygenated monoterpene compounds (α-Terpineol, MIC = 0.4 mg/mL) (Park et al., 2012).

Essential oils are slightly more active against Gram-positive than Gram-negative microorganisms, this can be explained by the presence of an outer membrane around their cell wall, which can limit the diffusion of hydrophobic compounds through its lipopolysaccharide covering. However, the sensitivity of Gram-positive bacteria to the essential oils has been reported (Wilkinson et al., 2003). Essential oils of *Mentha piperita* have shown a greater activity against *S. enteritidis* than against *L. monocytogenes,* when added to the Greek appetizers. In the other hand, no differences have been detected between the sensibilities of Gram-positives and Gram-negatives after 24 h. But, the inhibition effect was more pronounced with Gram-negative than with Gram-positive organisms after 48 h (Burt, 2004).

4. Conclusion

This study reports the antibacterial effect of the essential oil from *E. globulus* leaves against periodontopathogenic bacterial

species. This essential oil is found to be more active against Gram-negative bacteria, with weak antioxidant activity. The chemical identification of the different molecules characterizing the *E. globulus* essential oil evidenced the presence of oxygenated monoterpenes which can act as antibacterial agents. Essential oil of *E. globulus* leaves, being a significant antibacterial compounds, may thus have a potential application for pharmaceutical formulation, such as toothpaste and mouthwash.

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Further reading

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