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Article *in* Industrial Crops and Products · October 2016

Impact Factor: 2.84 · DOI: 10.1016/j.indcrop.2016.05.018

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Industrial Crops and Products 89 (2016) 167–175

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Essential oils composition, antibacterial and antioxidant activities of hydrodistillated extract of *Eucalyptus globulus* fruits

Zakia Bey-Ould Si Said [a], Hayate Haddadi-Guemghar [a], Lila Boulekbache-Makhlouf [a,*], Peggy Rigou [b], Hocine Remini [a], Abdennour Adjaoud [a], Nabyla Khaled Khoudja [a,c], Khodir Madani [a]

[a] *Laboratoire de Biomathématiques, Biophysique, Biochimie, et Scientométrie (L3BS), Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000, Bejaia, Algeria*
[b] *Faculté des Sciences Biologiques et des Sciences Agronomiques, Université de Tizi-Ouzou, 15000, Tizi-Ouzou, Algeria*
[c] *INRA, UMR 1083 Sciences pour l'œnologie, Montpellier, France*

ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form 8 May 2016
Accepted 9 May 2016

Keywords:
Eucalyptus globulus
Fruits
Essential oils
GC/MS analysis
Antioxidant activity
Antibacterial activity

ABSTRACT

Aromatic plants and their essential oils have been used since antiquity in flavor and fragrances, as condiments or spices, in medicines, as antimicrobial/insecticidal agents, and to protect stored products. The present study was undertaken: to determine (:) the chemical composition of essential oils extract from *Eucalyptus globulus* (*E. globulus*) fruits, using Gas-Chromatography coupled with Mass Spectrometry (GC/MS) method, to examine their antioxidant activity (DPPH', reducing power and lipid peroxidation inhibition assays) compared to that of Butylated hydroxyanisole (BHA) standard, and to estimate their antibacterial effects against reference pathogenic strains: *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Listeria innocua* (*L. innocua*), *Escherichia coli* (*E. coli*), *Pseudomonas aerugenosa* (*P. aerugenosa*), compared to that of two antibiotics (tetracycline and gentamicin). Twenty eight volatile compounds were identified, with the predominance of sesquiterpenes and oxygenated sesquiterpenes compounds (61.2%). The results of the antioxidant activities (DPPH scavenging activity, reducing power and inhibition of lipid peroxidation activity) of essential oils extract revealed weak activities with IC_{50} values of 27.0 ± 0.2 mg mL^{-1}, 32.9 ± 1.8 mg mL^{-1} and 4.9 ± 0.2 mg mL^{-1}, respectively; as compared to those of Butylated hydroxyanisole (BHA) standard that were about 0.05 ± 0.0 mg mL^{-1}, 0.03 ± 0.0 mg mL^{-1} and 0.5 ± 0.2 mg mL^{-1}, respectively. The antibacterial activity shows an inhibition effect of essential oils extracts against all the tested bacteria with MIC of 3 and 4 mg mL^{-1}. A bactericidal effect is observed, with MBC varying between 3.6 and 9.0 mg mL^{-1}, which demonstrates the sensibility of all tested bacteria to the essential oils of *E. globulus* fruits.

1. Introduction

In the light of scientific development, the medicinal properties of plants have reached a great interest, due to their pharmacological activities, low toxicity and economic viability (Auddy et al., 2003). These studies have focused on the benefits of phytochemicals extracted from plants and their impact on human health. Natural additives from plants can be compounds, groups of compounds or essential oils. More recently, food industry's interest in natural compounds for direct addition or to be used in synergy with other compounds has been increasing. Several studies report direct addition of aromatic plants essential oils and extracts to foodstuffs to exert an antimicrobial or antioxidant effect (Costa et al., 2015).

Among natural compounds, essential oils from aromatic and medicinal plants have shown biological activities and receive particular attention due to their radical scavenging properties (de Sousa Barros et al., 2015). Herbal substances are used against free radicals which are related to several pathologies such as cancer and neurodegenerative diseases. They are also involved in the deterioration of the organoleptic and hygienic quality of food (Hale et al., 2008).

Another problem affecting public health is the emergence of antibiotic resistance, following their massive use (De Billerbeck, 2007). This led to the strong demand of consumer for new antibiotics against pathogens (Fisher and Phillips, 2008) and has

* Corresponding author.
E-mail address: lilaboulekbachemakhlouf@yahoo.fr (L. Boulekbache-Makhlouf).

http://dx.doi.org/10.1016/j.indcrop.2016.05.018
0926-6690/

prompted scientists to use herbal medicine with antimicrobial properties.

Indeed, it is well known that essential oils have a significant antiseptic activity (Kaloustian et al., 2008). They exhibit antibacterial (Benjilali and Ayadi, 1986), antiviral, antifungal, antioxidant, antiparasitic, and insecticidal effects (Burt, 2004). Therefore, they can be a powerful tool to reduce the bacterial resistance (Stefanakis et al., 2013). Moreover, essential oils are recognized as safe substances by Health and Human Services Public Health Services (2005) and some contain compounds which can be used as antibacterial additives (Stefanakis et al., 2013). Several studies have reported their efficacy against pathogens and contaminants in food (Djenane et al., 2012; Gutierrez et al., 2008). This suggests their application in the food industry (Benjilali and Ayadi, 1986), thus reducing the problem of the food poisoning, despite the improved hygiene conditions (Burt, 2004).

Eucalyptus globulus plant is known for its richness on bioactive compounds such as essential oils, phenolic acids, flavonoids and hydrolysable tannins (Boulekbache-Makhlouf et al., 2010; Boulekbache-Makhlouf et al., 2013; Chinnarasu et al., 2015; Harkat-Madouri et al., 2015). Because of their multiple biological activities (Hasegawa et al., 2008; Tyagi and Malik, 2011) essential oils of *E. globulus* are used in medicine, perfume and food industry.

This study is part of research on antioxidants and natural antibiotics. Indeed, essential oils of the leaves of *E. globulus* are widely studied, but related works about its fruits are extremely scarce. To the best of our knowledge, this is the first report on their antioxidant activity and the second one about their antibacterial effect. Thus the objectives of this study are (*i*) to evaluate for the first time the antioxidant capacity of essential oils extracts from *E. globulus* fruits plant, and (*ii*) to confirm their antibacterial activity with a view of their pharmaceutical and industrial applications; as it was the case for the natural food additive, "Eucalyptus leaf extract", which is included in the list of Existing Food Additives [Notification No. 120 (16 April 1996), Ministry of Health and Welfare, Japan] (Amakura et al., 2009).

2. Materials and methods

2.1. Plant materials and chemicals

Eucalyptus globulus fruits were harvested from Bejaia (36°31′13.56″ N, 5°17′ 18.43″ E, North-east of Algeria), in February 2013. All solvents and reagents were of analytical grade.

2.2. Essential oils extraction

The harvested samples were washed with tap water, and dried at 30 °C during 6 days. A sample of 150 g boorishly crushed fruits was subjected to extraction by hydrodistillation for 3 h 500 mL^{-1} distilled water using a Clevenger type apparatus (Harkat-Madouri et al. 2015). The extractedoil was recovered and stored at 4 °C and its extraction yield was calculated as the ratio of the weight of oil to the weight of fruits using Eq. (1).

$$\%\,\text{yield of oil} = \frac{\text{Weight of oil}}{\text{Weight of dried fruits}} \times 100 \quad (1)$$

2.3. Gas chromatography mass spectrometry (GC/MS) analysis

Essential oils extracts from *E. globulus* fruits were analyzed 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). The GC instrument equipped with a DB–5 ms capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent J&W, Santa Clara, CA, USA). The analysis was performed using helium (purity > 99.99 vol.%) as a carrier gas at 1.2 mL min^{-1}. The temperature of GC oven was held at 40 °C for 2 min, then increased to 250 °C at 5 °C min^{-1} and to 300 °C at 30 °C min^{-1} and maintained at this temperature for 10 min. One μL of diluted sample (in Acetone 1:100, v/v) was injected at a constant temperature of 250 °C via a split injector (1:20 during 1 min). The scan range was between 40–650 amu. Peaks in the total ion chromatogram profiles tentatively identified by matching their mass spectra data to NIST/EPA/NIH mass spectral library and confirmed by comparison with Kovat's index on DB–5 ms column. Concentration of the identified compounds, expressed as a percentage, was directly calculated from peak areas without FID response factor correction.

2.4. Antioxidant activity

The antioxidant activity was compared to that of a Butylated hydroxyanisole (BHA) standard and assessed by three different tests, i.e. DPPH assay, the reducing power test, and β-carotene/linoleic acid method. All assays were performed in triplicate and in the dark using amber light flasks.

2.4.1. Free radical- scavenging activity

The effect of essential oil extracts on DPPH degradation was estimated according to the Noumi et al. (2011) method. Different concentrations of the sample were prepared in pure methanol, after that, one mL of each of them was added to 0.25 mL of a 0.2 mmol/L (v/v) DPPH solution. The mixture was then left at room temperature for 30 min. The absorbance of the resulting solutions was measured at 517 nm after 30 min. The DPPH radical scavenging ability of essential oil extracts was calculated according Eq. (2).

$$DPPH\ \text{scavenging effect}(\%) = \frac{[(A_0 - A_t) \times 100]}{A_0} \quad (2)$$

where A_0 is the control absorbance after 30 min, and A_t is the sample absorbance after 30 min. The antiradical scavenging activity was expressed as IC_{50} value (mg mL^{-1}).

2.4.2. Reducing power

The reducing power was assessed according to Singh et al. (2012) protocol. One mL of the extract at different dilutions in pure methanol (10, 20, 30, 40 and 50 mg mL^{-1}) was mixed with phosphate buffer (1 mL, 0.2 M'w/v', pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1%'w/v'). The obtained mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (TCA) (1 mL, 10%'w/v') was then added to the solution and was centrifuged for 10 min at 3000*g*. The supernatant was gathered and mixed with distilled water (1.5 mL) and $FeCl_3$ (150 μL, 0.1%'w/v'), and the absorbance was recorded at 700 nm and compared against BHA standard. An increase in the absorbance corresponds to an increase in the reducing power. The reducing power was expressed as IC_{50} value (mg mL^{-1}).

2.4.3. Inhibition of lipid peroxidation activity

In this assay, antioxidant capacity is determined using ß-carotene/linoleic assay described by Tepe et al. (2006). A stock solution of ß-carotene/linoleic acid was prepared by mixing 0.5 mg of ß-carotene (purity ≥ 97.0%), 1 mL of chloroform (HPLC grade, purity ≥ 99.9%), 25 μL linoleic acid (purity ≥ 99%), and 200 mg Tween 40. Chloroform was evaporated; then, distilled water (100 mL) was added. An aliquot of this solution (2.5 mL) was dispersed into test tubes added with 350 μL of the prepared sample at 2 mg mL^{-1} in pure methanol. The obtained emulsion was incubated up to 48 h at room temperature along with two controls, one containing the antioxidant BHA (positive control) and the other one without BHA or extract (Blank). The sample absorbance was

immediately measured at 490 nm after 0 min, 2 h, 4 h, 13 h, and 48 h. Relative antioxidant activity was calculated using Eq. (3).

$$\text{Antioxidant activity}(\%) = \frac{A_t}{A_0} \times 100 \quad (3)$$

where A_0 is the absorbance essential oil extracts at the beginning of incubation; A_t is the absorbance essential oil extracts at the end of incubation. The antioxidant activity was expressed as IC_{50}value ($mg\,mL^{-1}$).

2.5. Antibacterial activity

The most methods used for the antibacterial activity evaluation are: discs diffusion, agar dilution and broth dilution. These methods are relatively rapid, inexpensive and do not require sophisticated laboratory equipment. All assays were performed in triplicate in two independent experiments to ensure reproducibility.

2.5.1. Origin and selection of microbial strains

The target bacteria strains were selected for their high frequency to contaminate food stuffs and to their pathogenicity. They are maintained onto nutrient agar favorable to their growth for 24 h at 37 °C. The used microorganisms were: three Gram positive (*S. aureus* ATCC 43300; *B. subtilis* ATCC 6633; *L. innocua* CLIP 74915) and two Gram negative (*E. coli* ATCC 25922; *P. aerugenosa* ATCC 27853) bacteria.

2.5.2. Preparation of the inoculums

On nutrient agar, identical colonies were scraped from 24 h pure culture bacteria with a sealed Pasteur pipette. A volume of 10 mL was discharged into a sterile saline solution, the bacterial suspension was homogenized, and then its opacity was reduced to 0.5 McFarland corresponding to 10^8 CFU mL^{-1}. After that, the suspension was diluted to obtain an inoculum of 10^6 CFU mL^{-1} on nutrient agar (Tyagi and Malik, 2011).

2.5.3. Diffusion method on agar

The antibacterial activity was tested by a diffusion method using discs impregnated with essential oils at different concentrations in DMSO (100%, 75%, and 50%'w/v'). Sterilized paper discs (6 mm) were impregnated with 10 μL of each concentration, and placed onto nutrient agar. In parallel, controls are used to verify the growth of different strains. The plates were incubated for 30 min at temperature of 25–30 °C and then at 37 °C/24 h. Antibacterial activity was assessed by measuring the diameters of the light areas (mm) around discs including discs diameter.

2.5.4. Determination of the minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) of the essential oils extract was determined using the microdilution broth method in 96-well microplates. Inocula of the different strains was obtained from a pre-culture of 18 h; the microbial load was adjusted to 10^6 CFU mL^{-1} using a 0.5 McFarland turbidity standard. Plates containing 96 wells were prepared by dispersing, into each well, volumes from 187 to 194 μL of Mueller Hinton broth, and from 1 to 12 μL of each sample of essential oils; then 5 μL of inoculum was poured into the wells to obtain a final volume of 200 μL (Djenane et al., 2011).

2.5.5. Determination of minimum bactericidal concentration (MBC)

For the determination of the minimal bactericidal concentration, a sample from the MIC is performed on Mueller Hinton agar and then incubated at 37 °C for 18–24 h. The minimal bactericidal concentration is defined as the lowest concentration which shows no bacterial growth (Cherrat et al., 2014).

2.6. Statistical analysis

Assays on antioxidant activity were carried out in triplicates and results were reported as mean ± standard error. IC_{50} values were calculated using the linear regression equation obtained from the curve, i.e. absorbance = *f* (essential oils extract concentrations). The analysis of variance (ANOVA) was performed using XLSTAT Release 10 (Addinsoft, Paris, France). Tukey's multiple range test (HSD) was used to compare means of each parameter. Evaluations were based on the $p < 0.05$ significance level.

3. Results and discussion

3.1. Extraction yield of essential oils

The volatile oil extracted from fruits of *E. globulus* plant was yellow, having camphor like smell and pleasant odor. The extraction yield was about3.1 ± 0.4%, which is higher than those found by Mulyaningsih et al. (2010), Pereira et al. (2005) and Selvakumar (2012) (0.7%, 1.6% and 0.7%, respectively). As reported by Gilles et al. (2010), environmental, agronomic, age, genotype and geoclimatics factors can all affect the total essential oil content of plants. In addition, the method and the extraction conditions may influence the percentage recoveries (Bagheri et al., 2014).

3.2. Chemical characterization of the extracted essentials oils

The GC/MS analysis of the essential oils from *E. globulus* fruits extracts allowed the identification of 28 volatile compounds (Table 1). They are mainly composed of sesquiterpenes and oxygenated sesquiterpenes (61.2%), with globulol (23.6%) as the major compound of the identified oils. Indeed, Tan et al. (2008) have focused their works on the same major compound from *E. globulus* fruits. However, the identified monoterpene compounds (about 31.2%) were composed by eucalyptol (19.8%), α-pinene (3.8%), isovaleradehyde (2.4%) and α-phellandrene (1.9%).

These results are similar to those reported in the literature about the predominance of sesquiterpenes in *E. globulus* fruits. Indeed, the fresh fruits of *E.globulus* from California have shown a predominance of this class of essential oils (Nishimura and Calvin, 1979). In the other hand, (Mulyaningsih et al., 2010) have reported their abundance (with the predominance of aromadendrene: 31.2% and globulol: 10.7%), followed by monoterpene (1,8-cineole: 14.5%, α-phellandrene: 2.6% and α Pinene: 1.5%). However, in the study conducted on the *E. globulus* fruits from Portugal, equal amounts have been found between monoterpenes (50.4%) (α-phellandrene: 17.2%, 1,8-cineole: 11.7%) and sesquiterpenes (49.6%) (aromadendrene: 25.1%, ledene: 5.8%, globulol: 5.2%) (Pereira et al., 2005). In the study conducted on the fruits essential oils of *E. camaldulensis* var. *brevirostris w*, the main class of volatile compounds was also sesquiterpenes (33.8%) with the predominance of aromadendrene (18.0%), followed by monoterpenes (20.6%) with the predominance of α-pinene (12.7%) (El-Ghorab et al., 2002). In the other hand, the main compounds found in the volatile oils of four *E. camaldulensis* fruit samples, obtained from different geographical areas in Turkey were sesquiterpenes followed by monoterpenes (aromadendrene: 6.4–15.0%, eucalyptol: 0.2–12.6%, γ-gurjunene: 8.4–10.0%, terpinolene: 2.0–8.4%, spathulenol: 1.4–8.3%, α-pinene: 0.8–6.8%, ledene: 0.9–6.7%, and longifonene: 0.1–6.2%) (Özel et al., 2008). However, Koundal et al. (2016) have reported the predominance of monoterpenes (72.5%) (α-pinene being the major compound) in *E. citriodora* fruits growing in the Northwestern Himalaya (India),

Table 1
Chemical composition of essential oil extracts from *E. globulus* fruits.

Compounds	KI	Composition (%)
Monoterpenes (M)		
α-pinene	920	3.8
β-pinene	1122	0.1
β-myrcene	990	0.2
α-phellandrene	1024	1.9
α-terpinene	1015	0.2
O-cymene	1026	0.5
γ-Terpinene	1065	0.2
Limonene	1035	0.3
Total (M)		**7.3**
Oxygenatedmonoterpenes (OM)		
Eucalyptol (1,8-cineole)	**1033**	**19.8**
Isovaleraldehyde	660	2.4
2-pentanone-4-hydroxy-4- methyl	837	0.9
4-terpineol	1181	0.4
α-Terpineol	1196	0.2
Cis-Sabinol	1043	0.1
Carvenone	1250	0.1
Total (OM)		**23.7**
TOTAL (M + OM)		**31.2**
Sesquiterpenes (S)		
β-gurjunene	1430	0.4
β-humulene	1440	0.2
α-gurjunene	1409	1.4
Aromadendrene	**1462**	**19.7**
Allo-Aromadendrene	1440	2.5
γ-gurjunene	1473	0.5
Ledene	1031	3.1
β-Selinene	1480	0.2
δ-cadinene	1510	0.7
Total (S)		**28.9**
Oxygenated Sesquiterpenes (OS)		
Epiglobulol	1561	6.4
Globulol	**1589**	**23.6**
Eudesmol	1626	2.1
δ-cadinol	1640	0.2
Total (OS)		**32.3**
Total (S + OS)		**61.2**
TOTAL		**92.3**

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 DB-5MS column.
Values in bold correspond to that of the major compounds, and that of the sum of the compounds in each class, as well as that of the sum of all compounds identified in the analyzed oil.

and Silva et al. (2011) have reported the predominance of 1,8-Cineole (81.0%) in fruit of *E.cinerea* from Brazil. Essential oil from aerial parts (leaves and juvenile branches and flowers or fruits) of *Eucalyptus camaldulensis* Dehnh., growing wild in different localities of Sardinia (Italy), is mainly composed by monoterpenes such as *p*-cymene (27.8–42.7%), 1,8-cineole (4.1–39.5%), β-phellandrene (3.9–23.8%) and ketone such as cryptone (3.2–10.2%). The oils possessed moderate amounts (1.4–4.7%) of two uncommon aldehydes, cuminal and phellandral. the only sesquiterpinene present in these oils is spathulenol (2.1–15.5%) (Barra et al., 2010). In the essential oil of fruits of *E. oleosa*, the sesquiterpenes constituted 45.7% and the monoterpenes constituted 40.4%, of which the oxygenated monoterpenes had the most important contributions (35.9%). 1,8-Cineole (29.1%), γ-eudesmol (16.4%), α-selinene (10.0%) and *p*cymene (9.0%) were the major compounds in fruits essential oil (Marzoug et al., 2011). Several studies have been conducted on the chemical composition of leaves of the studied plant. So, in our recent work (Harkat-Madouri et al., 2015) we have reported the predominance of monoterpenes (78.6%), and a recent study has reported that 1,8-cineole was the principal component (56.5%) found in *E. globulus* leaves (Ghaffar et al., 2015). Similarly, monoterpenes were the major compounds in leaves of leaves of *E. globulus* from Blida (Algeria) and Herval City (southern Brazil) (with the predominance of 1,8-Cineole: 51.1 and 71.0%, respectively) (Boukhatem et al., 2014; Goldbeck et al., 2014). These contents depend essentially on environmental, agronomic, age and geoclimatics factors and also on the used extraction techniques and the experimental extraction conditions (Boukhatem et al., 2014).

Essential oils can be present both in different organs but its composition may vary from organ to organ. Indeed, the biosynthesis of monoterpenes and sesquiterpenes is compartmentally separated in many plants. However, there is significant cross-talk between the two pathways in the form of isopentenyl pyrophosphate transport across the chloroplast membrane, and it has been shown that both pathways are capable of using substrates from the alternate compartment. So, the covariation among groups of compounds may result from co-regulation of multiple biosynthetic genes, controlling the complex terpene profiles in the different parts of the plant (Andrew et al., 2013).

3.3. Antioxidant activity

As shown in Fig. 1, 2 and 3, about the inhibition of DPPH radical, the reducing power and the inhibition of lipid peroxidation activities of the essential oils extracts from *E. globulus* fruit, respectively, are dose dependent. As can be seen in Table 2, the IC_{50} values of the essential oils extracts from the three antioxidant activity tests (27.0 ± 0.2; 32.8 ± 1.8 and 4.9 ± 0.2 mg mL^{-1}, respectively) are significantly higher than that of the tested BHA standard (0.05 ± 0.0; 0.03 ± 0.0; 0.5 ± 0.2 mg mL^{-1}, respectively). In other words, the activity of the tested essential oils is lower than that of the BHA standard.

According to the DPPH assay results obtained in the present study (IC_{50} values of 27.0 ± 0.1 mg mL^{-1}); the radical scavenging activity of the essential oils was more active than those obtained from *E. oleosa* fruits by Marzoug et al. (2011) with moderate antioxidant activity (IC_{50} value of 441.1 ± 12.7 mg L^{-1}). Furthermore, El-Ghorab et al. (2002) showed moderate antioxidant activity by inhibiting the oxidative degradation of linoleic acid (20%) after 12 days for the volatile fruit oil of *E. camaldulensis var. brevirostris*. On the other hand, essential oil from aerial parts of *Eucalyptus camaldulensis* Dehnh., growing wild in different localities of Sardinia (Italy), have shown an antioxidant activity (DPPH assay) ranged between 0.5 and 5.8 mmol L^{-1} (Barra et al., 2010). While, the mechanisms involved in the inhibition of lipid peroxidation and DPPH assay are different, consequently, the results of the present study are not comparable with those previously reported by El-Ghorab et al. (2002) and Barra et al. (2010), since the standard and units used in both studies for the expression of the IC_{50} values are differents.

As it is the first work, no available data exists on the antioxidant activity of the essential oils extracts from *E. globulus* fruit. So, the obtained results will be solely compared with those reported for *E. globulus* leaves (Table 2). The three antioxidant activity tests revealed that essential oils of *E. globulus* fruit are more effective than those of its leaves (IC_{50} values were 33.3 ± 0.5; 115.4 ± 1.4; 6.7 ± 0.4 mg mL^{-1}, respectively) under the same conditions (Harkat-Madouri et al., 2015). However, it remains lower compared to those reported in the literature (Noumi et al., 2011; Singh et al., 2012). Our results differ from that of the commercialized essential oils of the Tunisian *E. globulus* leaves (IC_{50} values were 0.057, 0.048 mg mL^{-1} and 0.048 mg mL^{-1}) (Noumi et al., 2011) and that of the hydrodistillated essential oils of the Indian *E. citriodora* leaves (IC_{50} values were 0.425 ± 0.006; 0.087 ± 0.009; 0.01 ± 0.008 μg mL^{-1}) (Singh et al., 2012) for the three tested antioxidant activities, respectively. This difference in activity may be assigned to the difference in chemical composition, environmental, agronomic, age and geoclimatics factors, extrac-

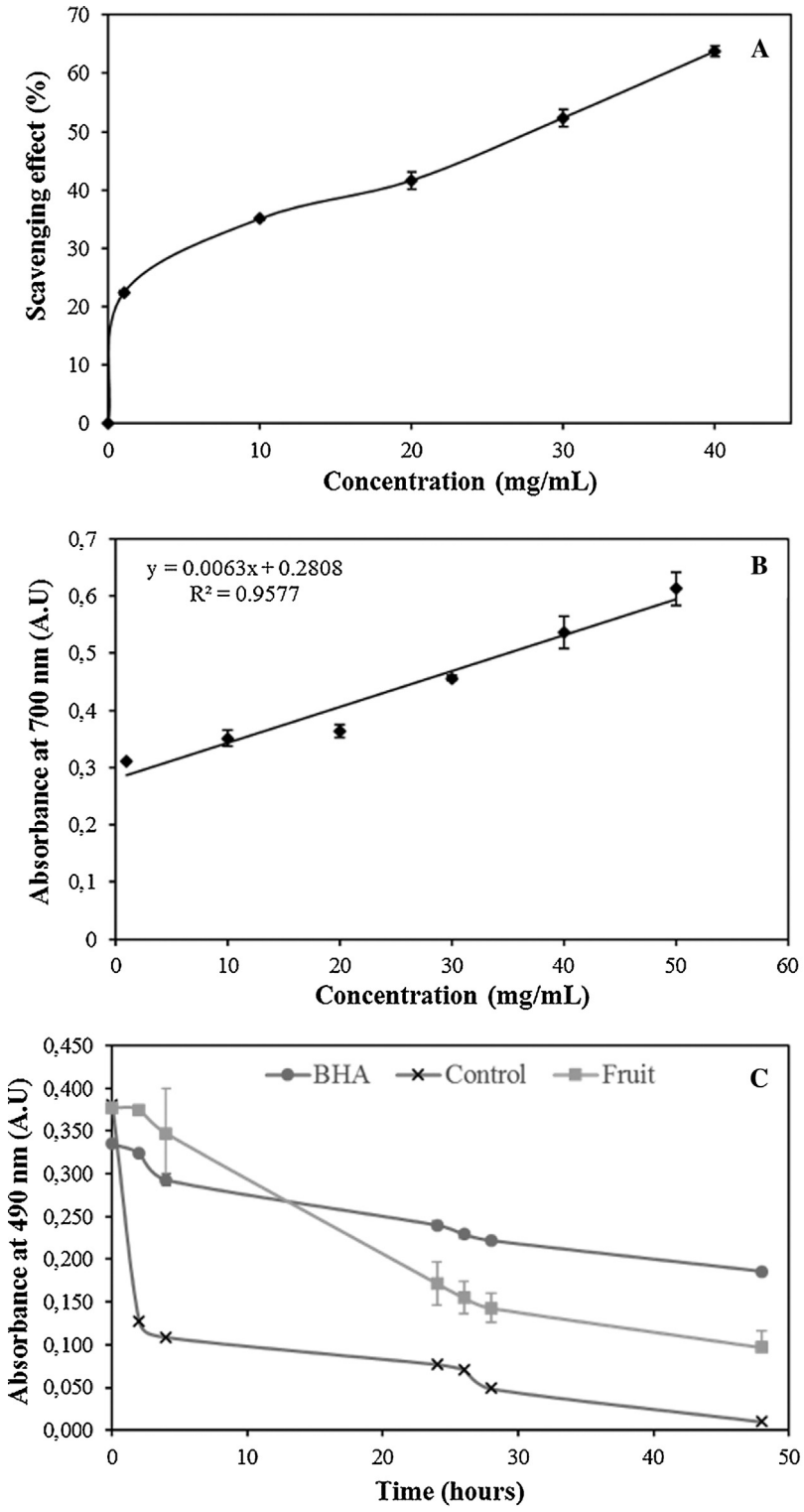

Fig. 1. Free radical scavenging activity (%) (A), Reducing power (B) and inhibition of linoleic acid peroxidation capacity (C) of the essential oil extracts from *E. globulus* fruits.

tion techniques, storage condition and the experimental extraction conditions (Boukhatem et al., 2014).

3.4. Antibacterial activity

The study of antibacterial activity was performed by measuring the diameter of the inhibition zones of growth bacteria, the determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC).The used DMSO for dissolving the essential oils did not show any antibacterial effect on the strains.

3.4.1. Agar diffusion test

According to the values of the inhibition zones diameter (Table 3), and to the classification established by De Billerbeck (2007), all the tested strains showed sensitivity to the tested oils.

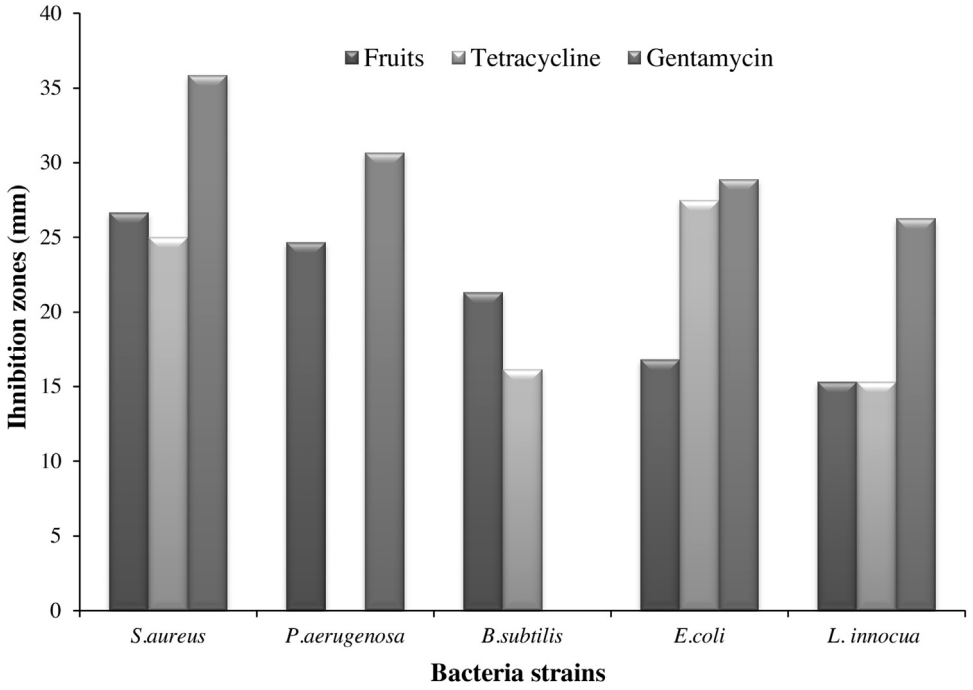

Fig. 2. Comparison of the antibacterial activity of the essential oil extracts from *E. globulus* fruits with antibiotics (10 μg/disc for gentamicin, and 30 μg/disc for tetracycline).

Table 2
Antioxidative capacities (IC_{50} values) of essential oil extracts from *E. globulus* fruits, BHA compared to the results reported in the literature about its leaves.

Samples	DPPH	RP	LP	References
Fruits	27.0± 0.2	32.8 ± 1.8	4.9 ± 0.2	Present study
BHA	0.05± 0.0	0.03 ± 0.0	0.5 ± 0.2	Present study
	33.3 ±0.5	115.4 ± 1.4	6.7 ± 0.4	Harkat-Madouri et al. (2015)
Leaves	0.057	0.048	0.048	Noumi et al. (2011)
	0.425 ± 0.006	0.087 ± 0.009	0.01 ± 0.008	Singh et al. (2012)

All the values are mean ± SD; SD: standard deviation; all results are expressed as mg mL^{-1}. DPPH: for Free radical-scavenging activity method; RP: for Reducing Power activity method; LP: for Inhibition of lipid peroxidation activity method.

Table 3
The effect of essential oil of *E. globulus* fruits on the growth of various strains.

Strains	Essential oils Concentrations 100% Inhibition zone diameter (mm)	Essential oils Concentrations 75%	Essential oils Concentrations 50%
B. subtilis	21.3	20.3	19.0
S. aureus	26.7	21.5	19.0
E. coli	16.8	16.0	14.5
P. aerugenosa	24.7	23.3	20.7
L. innocua	15.3	14.0	13.0

Indeed, this researcher has proposed a classification of antibiotics on the basis of their inhibition diameter values (Resistant: D < 6 mm; intermediate: 13 mm > D > 6 mm; sensitive: D > 13 mm).

Comparison of bacteria susceptibility to essential oils of *E. globulus* fruit is shown in Fig. 2. *E. coli* and *L. innocua* have shown similar sensitivity, but a significant difference has been observed between the other strains. Statistical analysis of the effect of essential oils on bacteria allowed us to classify them according to their sensitivity: *S. aureus* > *P. aeruginosa* > *B. subtilis* > *E. coli.* = *L.innocua*. *Staphylococcus aureus* is the most sensitive strain, which is consistent with results obtained by Traore et al. (2013) on *E. houseana* and Elaissi et al. (2011) on *E. odorata*. The resistance of *B. subtilis* to the tested essential oils extracts compared to *S. aureus*, can be explained by the fact that under unfavorable conditions, this bacterium has the ability to form an endospore which leads to its adaptation to the environmental conditions, which is not the case for *S. aureus* (Ahmad and Beg, 2001; Boulekbache-Makhlouf et al., 2013; Nascimento et al., 2000). The antibacterial activity of the tested oils could be due to the presence of aromadendrene, 1,8 cineole and globulol. Indeed, Mulyaningsih et al. (2010) reported a synergistic effect between aromadendrene and 1,8 cineole against *B. subtilis* and *S. aureus* by combining 0.12 mg mL^{-1} and 16 mg mL^{-1}, respectively. These researchers have reported that aromadendrene has better antimicrobial activity than 1,8 cineol and globulol. The activity of aromadendrene can be explained by its lipophilic nature, which allows it to cross the phospholipidic cell membrane; it has also a reactive methylene group and a cyclopropane ring which can alkylate proteins and thus disturb their conformation (Mulyaningsih et al., 2010). However, globulol, the major compound of the obtained oils (23.6%), previously isolated from *E. globulus* fruits has been reported to be effective against *B. subtilis* with an IC_{50} value of 737.2 μg mL^{-1} (Tan et al., 2008).

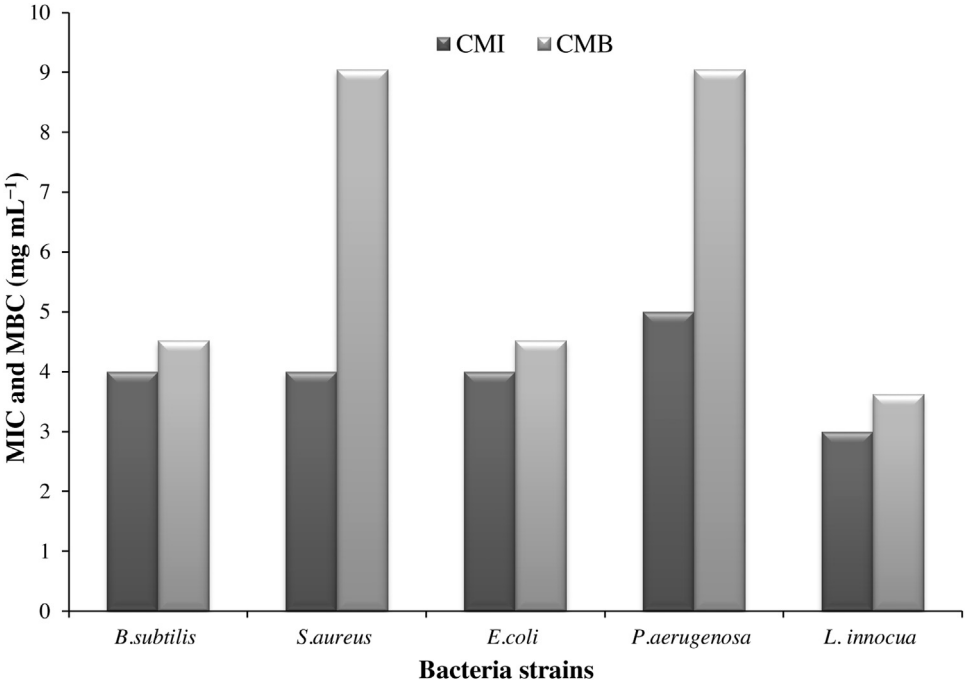

Fig. 3. MIC and MBC of essential oil extracts from *E. globulus* fruits.

3.4.2. Comparison of the antibacterial activity of the essential oils with antibiotics

The comparative analysis of the effects of the studied essential oils extracts from *E. globulus* fruit and antibiotics (Fig. 2) revealed the resistance of *P. aerugenosa* to tetracycline, and *B. subtilis* to gentamicin. The resistance of *P. aerugenosa* to the antimicrobial agents is reported in the literature (Lambert, 2002). Resistance of *P. aerugenosa* and *B. subtilis* could be due to the absence of the porin gaps which are required for the input of the antibiotics (Carle, 2009). A significant difference between the effect of the essential oils and the two antibiotics on the same bacterial strain was observed. However, similar activity of tetracycline and essential oils has been exercised on *S. aureus*.

The sensitivity of *B. subtilis* to tetracycline and essential oils of *E. globulus* plant can be explained by the fact that they have the same action mode on Gram-positive bacteria. Even the sensitivity of *E. coli* and *L.innocua* for tetracycline and gentamicin is due to their inhibition of the protein synthesis. In fact, the Gram-negative and Gram-positive bacteria are sensitive to gentamicin (Van Der Mee-Marquet et al., 2004). A similar sensitivity to gentamicin was noted for *E.coli* and *P.aerugenosa*. The variation of the antibacterial activity of different antimicrobial agents could be explained by the structural differences among bacteria (Rather et al., 2012).

3.4.3. Determination of MIC and MBC

The MIC and MBC values are shown in Fig. 3; they reflect results of the diffusion method and confirm the sensitivity of the tested bacteria to the essential oils extracts from *E. globulus* fruit. The highest MIC was shown by *P. aerugenosa* (9 mg mL^{-1}), the same finding was reported by Wilkinson and Cavanagh (2005) about the resistance of this strain to the essential oils of *E. staigeriana* and several other Australian plants. Thus, higher concentration of essential oils of fruit of *E. globulus* plant will be required for the inhibition of this bacterium. However, Gram-positive bacteria are more susceptible and the lowest MIC was observed for *L. innocua* (3 mg mL^{-1}). This could be explained by the fact that Gram-positive bacteria have only a peptidoglycan layer which is not a selective barrier to these compounds (Bachir and Benali, 2012; Tyagi and Malik, 2011). The MIC of essential oils against *S. aureus* and *B. subtilis* are higher than that reported by Mulyaningsih et al. (2010) (0.25 mg mL^{-1}). As against *P. aerugenosa* and *E. coli*, we obtained an MIC lower (4 mg mL^{-1}) than those reported by the same authors (8 mg mL^{-1} and > 8 mg mL^{-1}, respectively).

Since there is only two studies on the antibacterial activity of the essential oils extracts from *E. globulus* fruit (Mulyaningsih et al., 2010; Tan et al., 2008), the obtained results was solely compared with those reported in the literature about *E. globulus* leaves. Indeed, Bharti et al. (2012) have reported an MIC of 0.75 μL mL^{-1} and 1.56 μL mL^{-1} for *S. aureus* and *P. aerugenosa*, respectively. In the other hand, (Đakov, 2011) have found 0.09 mg mL^{-1} for *S. aureus* and *E. coli,* and 1.57 mg mL^{-1} for *P. aerugenosa*. The MIC obtained in our study was higher than those obtained by Tyagi and Malik (2011) which were 2.25 mg mL^{-1} for S. *aureus* and *B. subtilis*, 4.5 mg mL^{-1} for *E. coli*, and 9 mg mL^{-1} for *P. aerugenosa*. The antimicrobial activity of essential oils is related to their composition, functional groups (alcohol, phenols, terpenes and ketones) (Tyagi and Malik, 2011), nature of the chemical structures, and their proportions (Imène, 2012). The percentage of major and minor chemical constituents are considered as chemical composition of each essential oil. Furthermore, chemotypes of essential oils are depends on the levels of the major chemical components (A.L-Jabri and Hossain, 2014; Nasser Al-Jabri and Hossain, 2014).

It has been reported that the minor compounds act synergistically on bacteria strains (Tyagi and Malik, 2011). Indeed, Barel et al. (1991) have reported the antimicrobial activity of minor compounds such as α-terpineol and terpinen-4-ol which are identified in the studied essential oils extracts from *E. globulus* fruit (0.25%, 0.39%, respectively). Accordingly, Shunying et al. (2005) have found a bacteriostatic effect of these two compounds against the bacteria responsible for urinary tract infections; and Silva et al. (2011) have confirmed the high antibacterial activity of terpineol against *S. aureus*, this compound is detected in the studied oil with a percentage of 0.39 for 4-terpineol and 0.25 for α-terpineol. On the other hand, Rather et al. (2012) ranked in descending order the antibacterial activity of α-pinene, β-pinene, limonene, which are detected in essential oils of the studied plant (3.83%, 0.1% and 0.29%, respec-

tively). Concerning the MBC values of all bacteria; they are higher than those reported in the literature.

The mechanism of action of the essential oils on microorganisms is complex and remains unknown. The activity of the essential oils extracts from *E. globulus* fruit can be due to its oxygenated compounds such as oxygenated monoterpenes (23.7%) and oxygenated sesquiterpenes (32.3%) with globulol as the main sesquiterpene compound with 23.63% (de Sousa Barros et al., 2015; Tan et al., 2008). Bajpai et al. (2013) confirm the efficacy of *Cudrania tricuspidata* fruit essential oil (CTEO) as natural antimicrobial agent, which contain oxygenated sesquiterpenes and their respective hydrocarbons, revealed by its inhibitory effect as confirmed by the severe physical and morphological alterations on the cell wall of the tested microorganisms. As outlined by Ghannoum (1988), these morphological alterations consist on the lysis of bacterial cell wall followed by the loss of intracellular dense material on the surface of treated cells. Sikkema et al. (1995) explain that changes in membrane fluidity usually occur due to alterations in membrane lipid composition and are supposed to be a compensatory mechanism to counter the lipid disordering effects of the essential oil. Other works suggest that the active components of the essential oil might bind to the cell surface and then penetrate to the target sites possibly the plasma membrane and membrane-bound enzymes, resulting in the disruption of cell wall structure (Bajpai et al., 2013).

4. Conclusion

The use of extracts from plant in the pharmaceutical and food industries involves the determination of their composition and their activities such as antioxidant capacity for the prevention of the lipid oxidation leading to rancidity, and antibacterial activity for the prevention of proliferation of microorganisms. The extraction yield of essential oils extract from *E. globulus* fruit is high with the predominance of sesquiterpene and oxygenated sesquiterpene. A part from its poor antioxidant activity, the studied essential oils extracts from *E. globulus* fruit have exhibited an interesting antibacterial activity against pathogenic and spoilage microorganisms. The results showed the sensitivity of all strains to essential oils extracts from *E. globulus* fruit espacially *S. aureus* and *P. aerugenosa*, which are known for their resistance to the antibiotics. Thus, the high yield and the results of antibacterial activity found for the studied essential oils extracts from *E. globulus* fruit made them a good candidates for their use in pharmaceutical and food industries.

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