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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.

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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

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