Industrial Crops and Products 79 (2016) 274–282

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Chemical composition, antioxidant, antibacterial and anti-quorum sensing activities of *Eucalyptus globulus* and *Eucalyptus radiata* essential oils

Ângelo Luís[a], Andreia Duarte[a], Jorge Gominho[b], Fernanda Domingues[a], Ana Paula Duarte[a,*]

[a] CICS-UBI, Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal
[b] CEF-ISA, Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form 26 October 2015
Accepted 30 October 2015
Available online 21 November 2015

Keywords:
Essential oils
Eucalyptus globulus
Eucalyptus radiata
Antioxidant
Antimicrobial
Anti-quorum sensing

ABSTRACT

The interest in plant polyphenol antioxidants has increased remarkably over the last decade mostly because of their protective effects against different diseases, including cardiovascular, inflammatory and neurological diseases, cancer as well as for retarding aging. Many naturally occurring polyphenols found in plants and spices have also been shown to possess antimicrobial properties and could serve as a source of antimicrobial agents. *Eucalyptus globulus* and *Eucalyptus radiata* are well known species that provide essential oils. These oils are in great demand in the market, since they find a vast array of applications. The present study was performed to evaluate some bioactivities of the essential oils from *E. globulus* and *E. radiata*, namely their antioxidant, antibacterial and anti-quorum sensing properties. Moreover, its chemical composition was assessed and the potential synergistic activity with conventional antibiotics against *Acinetobacter baumannii* strains was also evaluated. The major component of the *E. globulus* oil was 1,8-cineole, also known as eucalyptol (63.81%), and in the *E. radiata* oil, the principal component was limonene (68.51%). It was possible to conclude that both eucalypt essential oils presented relevant radical scavenging properties and also had the capacity to inhibit the lipid peroxidation. The *E. globulus* oil antioxidant properties stand out when compared to the *E. radiata* oil. The *E. radiata* oil had a more pronounced antibacterial activity than *E. globulus* oil. The studied eucalypt essential oils can act as potential improving agents of antibiotics against *A. baumannii*, considering the synergic effect obtained between these oils and conventional antibiotics. Both eucalypt essential oils now studied can inhibit the quorum sensing phenomena, inhibiting quorum sensing-regulated violacein pigment production in bacteria without interfering with their growth.

1. Introduction

The interest in antioxidants from plants, namely polyphenols, has increased extremely over the last 10 years, mostly because of their benefic properties in several diseases, including cardiovascular, inflammatory and neurological diseases, cancer, as well as for retarding aging (Asgary et al., 2014; Bastianetto and Quirion, 2002; Gomes de Melo et al., 2012; Lu and Foo, 1997; Scalbert et al., 2005; Wang et al., 2008). The generally accepted mechanism of action of these compounds is that free radical-scavenging activity of polyphenols contributes to reduce the oxidative stress and to prevent the development of diseases (Huang et al., 2001; Wang et al., 2008).

Many plant and spices polyphenols, which naturally occurs, have also shown to have antimicrobial properties and could act as a source of antimicrobial agents (Kotzekidou et al., 2008; Luís et al., 2014a). The antimicrobial properties of plant extracts and essential oils (EOs) has been widely investigated against several human pathogenic microorganisms (Luís et al., 2014c; Andrade et al., 2014; Silva et al., 2011). Furthermore, the multidrug-resistant bacteria has coming out and it represents a challenge to treat the infections, which creates a true need to search for new substances with antimicrobial properties that can replace the conventional antibiotics to fight these microorganisms (Andrade et al., 2014). The emergence of resistance of Gram-negative strains (*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*) has been broadly recognized (Mulyaningsih et al.,

* Corresponding author. Fax: +351 275 329 099.
E-mail address: apcd@ubi.pt (A.P. Duarte).

http://dx.doi.org/10.1016/j.indcrop.2015.10.055
0926-6690/

2011). *A. baumannii* is an opportunistic pathogen that is usually related with nosocomial infections and is associated with infections acquired mainly in intensive care units (Duarte et al., 2013). This species have the ability to adhere to surfaces and then to form biofilms, and for this reason it can survive for extensive periods in hospital environments (Duarte et al., 2013). Multidrug-resistant pathogens like *A. baumannii*, make it particularly urgent to search and discover new antimicrobial compounds, such as EOs, which when used in combination with conventional antibiotics could improve the overall efficacy of the treatment creating a synergistic effect (Duarte et al., 2012).

Several Gram-negative bacterial strains use signal molecules, like *N*-acyl homoserine lactones (AHLs), to monitor their own population density (Singh et al., 2009). At a threshold population densities, AHLs interact with cellular receptors and trigger the expression of a set of target genes, including virulence, antibiotic production, biofilm formation, bioluminescence, mobility and swarming, in a process called quorum sensing (QS) (Singh et al., 2009). All these characteristics make the QS a novel approach for the development of new strategies to combat multidrug-resistant pathogens (Singh et al., 2009).

There are many reports relating the chemical composition and the antioxidant, antimicrobial and anti-QS activities of EOs, with their use in several commercial preparations such as antimicrobials and antioxidants (Castilho et al., 2012). The mainly constituents of EOs are terpenoids, which are a low molecular weight compounds that can be easily transported across the cell membranes and then induce a range of biological activities, including antioxidant, and antibacterial (Loizzo et al., 2009).

Among the EOs with antibacterial activity are the ones of *Eucalyptus* spp. (Goldbeck et al., 2014). These species are native from Australia, belong to the Myrtaceae family and are usually known as eucalypt, a name that represents more than 700 species worldwide (Goldbeck et al., 2014). The main component of the EOs from eucalypt is the terpene 1,8-cineole, also known as eucalyptol, being the amount of this compound dependent on the specific species (Goldbeck et al., 2014; Ishnava et al., 2013). The concentration of this compound varies between 44% and 84% and it is known to possess significant antimicrobial activity (Goldbeck et al., 2014). The EOs from eucalypt species are among the 18 most commonly traded EOs in the world (Goldbeck et al., 2014). Consequently, there is an increasing interest in their application as a natural additive for food, drugs and cosmetics, both in scientific research and industry (Brooker and Kleinig, 2006; Goldbeck et al., 2014; Ishnava et al., 2013). *Eucalyptus globulus* and *Eucalyptus radiata* are well known species that provide EOs which are in great demand by the consumers, since they can be used as anesthetic, antiseptic, astringent, deodorant, disinfectant, expectorant, febrifuge, fumigant, inhalant, insect repellant, and for a folk remedy for abscess, arthritis, asthma, boils, bronchitis, burns, flu, inflammation, rhinitis, worms, and wounds (Bachir and Benali, 2012; Elliot and Jones, 1986).

Based on this information, the present study was performed to evaluate some bioactivities of the EOs from *E. globulus* and *E. radiata*, namely their antioxidant, antibacterial and anti-QS properties. Moreover, its chemical composition was assessed and the potential synergistic activity with conventional antibiotics against *A. baumannii* strains was also evaluated.

2. Material and methods

2.1. Eucalypt essential oils

Both *E. globulus* and *E. radiata* EOs were acquired commercially from a local Pharmacy (Covilhã, Portugal). According to the accompanying leaflet, both these oils were obtained by hydrodistillation of leaves and small branches of the tree. The *E. globulus* EO has its origin in Spain, while the *E. radiata* EO in Australia. Both these EOs are marketed by the same company (Absolute Aromas Ltd., England) and are produced and certified as biological products to be used in humans ("Soil Association—Organic"), since this trademark belongs to "Aromatherapy Trade Council".

2.2. Gas chromatography-mass spectrometry (GC–MS) analysis

Both essential oils were analyzed in an Agilent 7890A gas chromatograph coupled with an ion trap spectrometer Agilent MS220. The compounds' identification was assessed using a time database and confronted to the NIST12 mass database. An Agilent VF50 column was used (30 m length, 0.25 mm diameter and 0.25 μm thickness).

The temperature was initiated at 50 °C and maintained for 5 min; afterwards, the temperature was raised to 180 °C at a rate of 2 °C min^{-1} and this temperature was maintained for 30 min. The temperature of the injection port and transfer line was set at 230 °C. The split injection mode (ratio 1:20) was adopted, and the carrier gas was helium at a constant flow rate of 1 mL min^{-1}. The mass spectrometer was operated in the electron ionization mode with an electron energy value of 10 μA. The identity of the components was ascertained based on their retention indices and their mass spectra which were compared with those obtained from available libraries. The analysis was repeated two times.

2.3. Antioxidant activity evaluation

2.3.1. DPPH scavenging assay

The antioxidant activity of the eucalypt EOs and standards (gallic acid and quercetin (Sigma–Aldrich, USA)) was determined by the free radical scavenging activity method using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Sigma–Aldrich, USA), previously implemented for plant extracts and slightly modified here for EOs (Luís et al., 2014b; Scherer and Godoy, 2009). In brief, aliquots of several concentrations of the EOs or standards (diluted in methanol) (0.1 mL) were added to three DPPH methanolic solutions with different concentrations (3.9 mL): 0.2000, 0.1242 and 0.0800 mM, which were prepared by dissolving 39.4, 24.5 and 15.8 mg of the compound in 500 mL of methanol (Fluka, Milwaukee), respectively. These concentrations were selected due to the linearity range of DPPH solutions: above 0.2 mM the concentration is very high, and below 0.5 mM due to the low concentration, the color is very weak, having a limited range of absorbance reading. The control sample was a solution of 0.1 mL of methanol mixed with 3.9 mL of DPPH. After the incubation period (90 min) at room temperature in the dark, the absorbance was measured at 517 nm using a spectrophotometer (Helios–Omega, Thermo Scientific, USA). The radical scavenging activity was calculated using the following formula:

$$\text{Inhibition}\,(\%) = \left[\frac{(Ab_{s0} - Ab_{s1})}{Ab_{s0}}\right] \times 100,$$

where Abs_0 was the absorbance of the control and Abs_1 was the absorbance in the presence of the test sample at different concentrations. The IC_{50} (%) (concentration providing 50% of inhibition) was determined using a calibration curve in the linear range of the graphic, by plotting the EO concentration *vs.* the corresponding scavenging effect. The antioxidant activity was expressed as the Antioxidant Activity Index (AAI), calculated as follows: AAI = (final concentration of DPPH in the control sample)/(IC_{50}) (Scherer and Godoy, 2009).

As a result, the AAI was determined considering the mass of DPPH and the mass of the EO in the reaction, resulting in a