Lavandin (Lavandula × intermedia Emeric ex Loiseleur) essential oil from Spain: determination of aromatic profile by gas chromatography–mass spectrometry, antioxidant and lipoxygenase inhibitory bioactivities

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Lavandin (Lavandula × intermedia Emeric ex Loiseleur) essential oils (EOs), from Abrial, Super and Grosso cultivars, cultivated and extracted in the South East of Spain, were analysed by using GC/MS to determine their composition, in both relative (peak area) and absolute (using standard curves) concentrations. Linalool (34–47%), linalyl acetate (17–34%), camphor (4–9%) and eucalyptol (3–7%) were determined as the main molecules. This characterisation was completed with the enantioselective gas chromatography, where (−)-linalool, (+)-camphor and (−)-linalyl acetate were determined as the main components. Antioxidant activity was evaluated positively by several methods: activity against free radicals, chelating and reducing power, probably due to linalool and linalyl acetate. Mild inhibitory activity on lipoxygenase was observed supporting potential anti-inflammatory activity, mainly due to linalool and camphor. These properties support the potential use of L. × intermedia essential oils as natural cosmetic and natural pharmaceutical ingredient to fight several skin diseases.

Keywords: Lavandula × intermedia; essential oil; GC–MS; enantiomeric ratio; antioxidant; LOX inhibitory activity

1. Introduction

Lavandula × intermedia Emeric ex Loiseleur, also known as lavandin, Lavandula hybrida or Dutch lavender is a hybrid between English lavender (Lavandula angustifolia Miller) and spike lavender (Lavandula latifolia Medikus), resulting in an aromatic flowering plant of the Lamiaceae family. The genus Lavandula, of the Lamiaceae family, consists of approximately
20 species with more than 100 varieties of lavender (Da Porto and Decorti 2008). *L. × intermedia* essential oil (EO) is one of the aromatic ingredients in the production of drinks, food, perfumes and soaps, especially important in the manufacture of ‘eau de cologne’ and perfumes (Torras-Claveria et al. 2007).

Some GC relative quantitation of the *L. × intermedia* composition have been reported (Papachristos et al. 2004; Bombarda et al. 2008; Seino et al. 2008; Baydar and Kineci 2009; Cosimi et al. 2009). None of them have studied the specific conditions of the Spanish Mediterranean coast, specifically the region of Murcia where the biggest aromatic plant diversity among all regions of Spain is found. Furthermore, there are few chiral studies on EOs of *L. × intermedia* (Flores et al. 2005) and it accounts for some specific molecules. Chiral distribution is an important aspect of the EO composition, it helps identifying natural EOs from those adulterated (Smelcerovic et al. 2013), even when the samples come from different places of the Earth (Tranchida et al. 2012). In addition, the chirality in biomolecules is highly important due to the different bioactivities and organoleptic properties of each of the enantiomers (Baser and Buchbauer 2010).

Apart from the aromatic usages of the *L. × intermedia* EO, some bioactivities may be relevant according to the popular uses of some plants of the *Lavandula* genus. The antioxidant potential of EOs can be determined using a representative selection of different antioxidant methods (Huang et al. 2005; Rubió et al. 2013; Bentayeb et al. 2014; Dawidowicz and Olszowy 2014). Many inflammatory processes are associated with leukotriene production catalysed by lipoxygenase (LOX) (Rubió et al. 2013), which can use molecular oxygen or hydrogen peroxide as oxidants (Anwar et al. 2014) Thus, the inhibition of soybean LOX as LOX model is a hint of anti-inflammatory activity of the EO.

The aim of the present study is to determine the relative, absolute and chiral distribution of each of the EO main components in five samples grown in Murcia (Spain). Then, five antioxidant methods [ORAC (Oxygen radical absorbance capacity), ABTS, DPPH, chelating power (ChP) and reducing power (RdP)] are applied to evaluate the antioxidant capacities of the *L. × intermedia* EOs. Furthermore, the inhibitory activity of these EOs on LOX is characterised. The experimental results are compared with those reported for *L. hybrida* EOs from other countries, and their potential biotechnological applications are discussed.

2. Results and discussion

2.1. Fast gas chromatography/mass spectrometry study

The essential oils were obtained by steam distillation in yields ranging from 0.2 to 1.3% (w/w). Fast gas chromatography/mass spectrometry was used, as described in Experimental section (shown in Supplementary material), to determine, using triplicate analysis of the samples (Sokal and Rohlf 2012), the components of the studied EOs (van Den Dool and Dec. Kratz 1963; IUPAC 1997; European-Pharmacopoeia 2011).

Abridal cultivar sample (Abridal1) is shown together with the two Super cultivar samples (Super1-2) because of its similarity in composition (Table S2A). Nevertheless, some components make the difference between cultivars acting as biomolecular markers. This is the case of the high relative concentration of β-pinene, cis- and trans-β-ocimene, camphor and E-β-caryophyllene in the Abridal cultivar. Focusing on the Super cultivar samples, some components perform as biomolecular markers accounting for the different environmental conditions (Rivas-Martínez 1987), i.e. high concentration of linalyl acetate in Super1 and Z-β-farnesene in Super2; and low concentration of terpinen-4-ol and α-terpineol in Super1. Results obtained about Grosso cultivar (Table S2B) (Grosso1-2) show some biomolecular markers. Grosso1 has low relative concentration of p-cymene. β-Pinene, linalyl acetate, Z-β-farnesene and γ-muurolene are found in low proportion in Grosso2. High concentrations are found in
Grosso1 for the constituents Z-β-ocimene and lavandulyl acetate; and in Grosso2 for hexyl alcohol, linalool and lavandulol.

Global results show five different L. × intermedia samples having the same 11 principal molecules, i.e. Z-β-ocimene, eucalyptol, linalool, camphor, borneol, terpinen-4-ol, α-terpineol, linalyl acetate, lavandulyl acetate, E-β-caryophyllene and Z-β-farnesene. Specific molecules are found in high concentration for each specific cultivar. In Abrial/Super cultivars, β-myrcene, limonene and E-β-ocimene arise; whereas in Grosso cultivar, lavandulol is found in higher concentration.

Despite the important differences found between all the three cultivars, using a dendrogram representation (Figure S5) of agglomerative hierarchical clustering based on Euclidean distance applied to the relative area of components, the high similarity between Grosso and Super cultivars was unveiled, whereas both cultivars show a higher distance to the Abrial cultivar. In the dendrogram, samples Super2 and Grosso2 are part of the same group, showing high similarity (74.81%); Super1 and Grosso1 show a similarity of 59.34% and Abrial1 has a similarity of 47.27% with the Super2/Grosso2 group. According to the results, the growing location (bioclimatic zone) becomes more important to composition than the cultivar of the plant material.

Oxygenated monoterpenes are highly predominant in the five samples (Table S2), accounting for more than 80%, in average, of the total molecules. Alcohol is the most abundant organic functional group, exceeding 40% of total molecules; the second organic functional group in abundance is ester, accounting for more than 20% in average. The hydrocarbon monoterpenes represent a maximum of 14%, in the case of Abrial/Super cultivar. Total terpene hydrocarbons were calculated as the sum of the monoterpenes and sesquiterpene hydrocarbons. Total oxygenated terpenes were calculated as the sum of the oxygenated monoterpenes and sesquiterpenes.

Several ingredients show peak area percentages similar to those reported in the literature for plant samples of L. × intermedia grown in different countries. Some main components such as linalool or camphor show a similar concentration to samples from Turkey (Baydar and Kineci 2009), borneol is similar to the Japanese (Seino et al. 2008) or French reports (Bombarda et al. 2008), linalyl acetate is similar to the one reported from Italy (Cosimi et al. 2009) while some others such as α-terpineol show similarities to Greece (Papachristos et al. 2004). Molecules such as cis- and trans-β-ocimene have been determined in higher concentrations in Spanish samples than in any other location.

2.2. International Standard comparative

The International Organization for Standardization (ISO) has published International Standards for L. × intermedia EOs of both Abrial (ISO 2001) and Grosso (ISO 2009) cultivars (French type) but not for the Super cultivar. The results shown in Table S3 were obtained taking ISO Standards for comparison and grouping up together Abrial and Super cultivars due to their similarities. Some constituents from the Spanish EOs exceed the maximum relative concentration allowed for the French type. The case of linalool is especially interesting, being found exceeding the limits in all samples. Linalyl acetate is another important molecule due to its pleasant aroma, it has been found in high concentration in Super1 whereas in Grosso2 has been found beneath limits.

2.3. Enantioselective gas chromatography/mass spectrometry study

The enantiomeric determinations of molecules of EOs from L. × intermedia are shown in Table S4. There are no adulterations with synthetic racemates of the main molecules, such as
linalool, linalyl acetate and camphor. The enantiomeric predominance is the same for the three types of cultivars. The (+)-enantiomeric excess is shown in the case of α-pinene, β-pinene, limonene, camphor, terpinen-4-ol, α-terpineol and borneol; while the (−)-enantiomeric excess is shown in camphene, linalool, linalyl acetate, (E)-β-caryophyllene and caryophyllene oxide. The enantiomeric distribution of α-terpineol is markedly different for each sample and could be useful for their characterisation. There are several biomolecular markers of the biochemotype origin: camphene for Abrial1 and Super1; β-pinene for Abrial1, Super1, Super2 and Grosso1; α-terpineol for Abrial1 and Grosso2; and limonene for Grosso2. These data could be useful to assess the origin and the authenticity of the EOs. To our knowledge, this is the first chiral wide characterisation of the EOs from *L. × intermedia* grown in Spain.

The high proportion of (−)-linalool and (−)-linalyl acetate in EOs grown in Spain (Table S4) is similar to those reported for the EOs of *L. × intermedia* worldwide. On the contrary, (−)-camphor was reported as the main enantiomeric species obtained from EOs but (+)-camphor was obtained from manufactured products declaring the presence of *Lavandula* EO (Flores et al. 2005). In this study, (+)-camphor was the main enantiomeric species of camphor observed. (1R)-(+) -Camphor has also been reported for some other varieties of lavender, such as *L. angustifolia* (Bicchi et al. 2010) or *Lavandula stoechas* (Ristorcelli et al. 1998), leading to a high probability of being (+)-camphor the predominant enantiomer in all the *Lavandula* genus. The enantiomeric species declared by this study is checked by commercial samples of (+) and (−)-camphor, thus showing a possible mistake in the already mentioned study of Flores et al.

### 2.4 Antioxidant activity

#### 2.4.1. ORAC

The ORAC antioxidant activity (Ou et al. 2001) of the five samples of *L. × intermedia* is expressed in TEAC (Trolox equivalent antioxidant capacity) units (μmol TE/μL EO) and resulted (Table 1) as follows:

\[
\text{Abrial1}^{\text{ORAC}} \approx \text{Super1}^{\text{ORAC}} \approx \text{Super2}^{\text{ORAC}} \approx \text{Grosso1}^{\text{ORAC}} \approx \text{Grosso2}^{\text{ORAC}}.
\]

The antioxidant activity of each EO is related to its composition and the intrinsic antioxidant activity of each of the compounds. In general, the rise in composition of oxygenated terpenes is correlated to higher ORAC antioxidant activity, which is lowest for Abrial1 and highest for Grosso2 EOs (Tables S2A, S2B and 1). Two oxygenated components are highly relevant to explain the ORAC value of the EO, namely linalool and linalyl acetate, the first and the most important molecule because of its high concentration and high ORAC value and the other for its moderate ORAC value and high concentration (Bentayeb et al. 2014).

| Antioxidant method (units)       | Abrial1 | Super1 | Super2 | Grosso1 | Grosso2 |
|---------------------------------|---------|--------|--------|---------|---------|
| ORAC (μmol TE/μL EO)            | 1.24 ± 0.08 | 1.27 ± 0.09 | 1.28 ± 0.09 | 1.26 ± 0.07 | 1.37 ± 0.09 |
| ABTS (μmol TE/mL EO)            | 1.7a ± 0.1 | 1.6a,b ± 0.1 | 1.4b,c ± 0.1 | 1.3c ± 0.0 | 1.6a,b ± 0.1 |
| DPPH (μmol TE/mL EO)            | 0.73a ± 0.05 | 0.56b,c ± 0.02 | 0.62b ± 0.04 | 0.48c ± 0.03 | 0.31d ± 0.01 |
| ChP (mg EDTA eq/mL EO)          | 0.9b,c ± 0.0 | 1.1b ± 0.1 | 0.8c ± 0.1 | 1.5a ± 0.1 | 0.5d ± 0.0 |
| RdP (mg Ascorbic acid eq/L EO)  | 1.5c ± 0.1 | 2.6b ± 0.1 | 2.8a,b ± 0.1 | 2.7a,b ± 0.1 | 2.9a ± 0.1 |

Note: a, b, c Different letters in the same antioxidant method mean statistically significant differences with \( p < 0.05 \).
Linalool is the principal ingredient of *L. × intermedia* EO and shows high intrinsic antioxidant activity, thus it holds the main contribution to the antioxidant activity of the EO. Other abundant molecules, such as linalyl acetate, exhibiting high ORAC values contribute significantly to the global oil ORAC value. However, the total ORAC value of the EO is determined not just by the main components but by the whole group of ingredients present in the EO.

2.4.2. **ABTS**

The ABTS antioxidant activity (Re et al. 1999) of the samples of *L. × intermedia* is expressed in TEAC units (μmol TE/mL EO) and resulted (Table 1) as follows:

\[
\text{Abrial1}^{\text{ABTS}} \simeq \text{Super1}^{\text{ABTS}} \simeq \text{Grosso2}^{\text{ABTS}} \simeq \text{Super2}^{\text{ABTS}} \simeq \text{Grosso1}^{\text{ABTS}}.
\]

As linalool and *E*-β-ocimene show the lowest concentrations in Grosso1 sample and show the highest concentrations in Grosso2 and Abrial1, respectively (Tables S2A and S2B), we can propose a remarkable antioxidant activity of *E*-β-ocimene and slightly lower antioxidant activity of linalool against ABTS radical cation.

2.4.3. **DPPH**

The DDPH antioxidant activity (Brandwilliams et al. 1995) of the samples of *L. × intermedia* is expressed in TEAC units (μmol TE/mL EO) and resulted (Table 1) as follows:

\[
\text{Abrial1}^{\text{DPPH}} > \text{Super2}^{\text{DPPH}} \simeq \text{Super1}^{\text{DPPH}} \simeq \text{Grosso1}^{\text{DPPH}} > \text{Grosso2}^{\text{DPPH}}.
\]

Different molecules, present in high quantity in Abrial1 and in low quantity in Grosso2, may explain the better performance in DPPH antioxidant assay (Dawidowicz and Olszowy 2014), i.e. ocimene and *E*-β-caryophyllene (Tables S2A and S2B).

2.4.4. **Chelating power**

The ChP activity (Miguel et al. 2010) is expressed in EDTA units (mg EDTA equivalents/mL EO) and resulted (Table 1) as follows:

\[
\text{Grosso1}^{\text{ChP}} > \text{Super1}^{\text{ChP}} \simeq \text{Abrial1}^{\text{ChP}} \simeq \text{Super2}^{\text{ChP}} > \text{Grosso2}^{\text{ChP}}.
\]

The highest concentrations of linalyl acetate and lavandulyl acetate (Tables S2A and S2B) were found in Grosso1 sample. The high electronic densities of the oxygen atoms of the carboxylic ester groups could be useful for complexation of cations.

2.4.5. **Reducing power**

The reducing power (RdP) antioxidant activity (Oyaizu 1986) of the samples of *L. × intermedia* is expressed in ascorbic acid units (mg ascorbic acid equivalents/L EO) and resulted (Table 1) as follows:

\[
\text{Grosso2}^{\text{RdP}} \simeq \text{Super2}^{\text{RdP}} \simeq \text{Grosso1}^{\text{RdP}} \simeq \text{Super1}^{\text{RdP}} > \text{Abrial1}^{\text{RdP}}.
\]

Some abundant alcoholic terpenoids, as the already reported linalool (Liu et al. 2012) (Tables S2A and S2B), might show a mild reducing power.
2.5. Inhibitory activity on LOX

The results of the LOX inhibitory activity (Christopher et al. 1970; Whent et al. 2010) were obtained as described in Experimental section (shown in Supplementary material). Inhibition degree (%) at 0.3 μL (EO)/mL was measured for Grosso2LOX (32.6 ± 0.9)a > Super2LOX (24.0 ± 0.8)b ≥ Grosso1LOX (20.9 ± 1.2)b,c ≥ Super1LOX (18.2 ± 1.1)c ≥ Abrial1LOX (17.5 ± 3.4)c. Tukey’s honest significant difference test revealed significant differences between the samples (different superscripts). For a deeper understanding of the LOX inhibitory activity of the EO, the main commercially available compounds, had their LOX inhibitory activity tested, obtaining their IC50 (μM) value: limonene (356 ± 30), p-cymene (486 ± 32), camphor (2743 ± 85) and linalool (3346 ± 44).

The inhibitory activity of the *L. × intermedia* EO is clearly due to a combination of compounds with high inhibitory activity, and more abundant compounds namely camphor and linalool (Tables S2A and S2B). The high inhibition of LOX by Grosso2 in comparison with the other samples can be explained by the fact that linalool is found in the biggest absolute concentration.

*L. × intermedia* is an economically key species within the *Lavandula* genus, due to its high yield EO with high proportion of linalool and linalyl acetate, thus it can emulate *L. angustifolia* EO aroma (Tongnuanchan and Benjakul 2014). Its biochemical composition is the key to understand the bioactivities of *L. × intermedia* EOs such as antibacterial, antifungal, antiparasitic, insecticidal, antioxidant and anti-inflammatory agents (Papachristos et al. 2004; Torras-Claveria et al. 2007; Jianu et al. 2013). These properties support the potential use of *L. × intermedia* EOs as natural cosmetics and natural pharmaceutical ingredients useful for relieving gastrointestinal disorders and for fighting human protozoal pathogens (Moon et al. 2006; Baker et al. 2012).

3. Conclusions

The different *L. × intermedia* EOs evaluated showed the same common 11 principal constituents, i.e. Z-β-ocimene, eucalyptol, linalool, camphor, borneol, terpinen-4-ol, α-terpineol, linalyl acetate, lavandulyl acetate, E-β-caryophyllene and Z-β-farnesene. Some concentrations of important odorant molecules such as linalool and linalyl acetate are found exceeding ISO normative limits, highlighting a new cheap raw material for industrial manufacturing of lavender aroma. A new source of pure enantiomers is unveiled showing (−)-linalool, (−)-linalyl acetate and (+)-camphor as highly representative compounds of the typical *L. × intermedia* EO. *L. × intermedia* EOs showed moderate antioxidant activities, especially due to linalool and linalyl acetate. Regarding potential anti-inflammatory properties, LOX inhibitory activities of *L. × intermedia* EOs, were especially due to linalool and camphor. *L. × intermedia* has a high yield of EO whose properties support the potential use of *L. × intermedia* EOs as natural cosmetics and natural pharmaceutical ingredients useful for relieving gastrointestinal disorders and for fighting diseases related to oxidative stress.

Supplementary material

Supplementary material is available online. It contains: Experimental section, Tables S1, S2A, S2B, S3 and S4 and Figure S5.

Disclosure statement

No potential conflict of interest was reported by the authors.
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