Extraction of essential oils from the residues of two shrub species aiming for their revalorization: chemical characterization and antioxidant, antimicrobial and cytotoxic activities

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In recent years, essential oils have been subject of research for their bioactive properties, such as antimicrobial, fungitoxic, anti-viral, anti-inflammatory and antioxidant activities. Owing to these properties they are potentially interesting for diverse industries including the food industry since one of its main problems concerns microbes and associated toxins that are responsible for food spoilage. Although the application of essential oils in the food industry may have some limitations, such as impact on the organoleptic properties and low solubility, different delivery strategies such as nanoencapsulation, active packaging and coatings are promising technologies that may overcome these issues without compromising nutritional properties in food systems [1]. In this view, increased knowledge on the composition and activity of different essential oils is needed, particularly regarding novel potential sources of essential oils such as agricultural wastes or species grown in marginal lands, on a perspective of circular economy. Therefore, in the scope of the BeonNAT project, biomass from different tree and shrub species are being screened as possible sources of essential oils and respective bioactivity evaluated.

In this work, the essential oil extracted by steam distillation from the branches (>20mm) of two shrub species grown in Spain, *Juniperus communis* L. and *Cistus ladanifer* L., was characterized for their chemical composition by gas chromatography coupled with mass spectrometry (GC-MS) as well as for their antioxidant, antimicrobial, anti-inflammatory and cytotoxic activities. GC-MS analysis allowed the identification of 98.1% of compounds in *J. communis* EO, corresponding to a total of 63 identified compounds, with α-pinene being the major compound (35.1%), followed by limonene (15.0%), sabinene (6.7%), cis-tujopsene (8.0%), β-myrcene (3.2%) and β-caryophyllene (3.5%). In general, the chemical composition is in agreement with that of juniper berry EO, defined in the European Pharmacopoeia and ISO 8897 standard, except for limonene (15.0%) which was slightly higher than the defined range (Eur. Ph of 2-12% and ISO standard of 2-8%). For *C. ladanifer* EO, 61 compounds were identified corresponding to 92.8% of the total compounds, with viridiflorol being the main compound (24.0%), followed by α-pinene (19.3%), ledol (6.9%), camphene (6.7%) and bornyl acetate (5.0%), which is in good agreement with previous data [2]. Both oils showed potential against the panel of bacteria selected according to their importance in public health and foodborne diseases, highlighting the rock-rose EO that showed interesting activity against *Escherichia coli*, *Morganella morganii*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Listeria monocytogenes* and methicillin resistant *Staphylococcus aureus* in a concentration range of 0.039-2.5%(v/v). Regarding the antioxidant activity, both oils showed promising results, with EC_{50} values of 1.35 ± 0.19 mg/mL and 1.30 ± 0.07 mg/mL in the reducing power assay and 68% and 83% inhibition of oxidation according to the cellular antioxidant activity assay, for *J. communis* and *C. ladanifer*, respectively. The essential oils showed anti-inflammatory (IC_{50} of 24±1 µg/mL and 21±2 µg/mL for juniper and rock-rose, respectively) and cytotoxic activity, with the best results obtained with the rock-rose EO in the inhibition of stomach-AGS, colon-CaCo, breast-MCF-7 and lung-NCI-H460 cancer cell lines (GI_{50} between 47±5 µg/mL and 58±1 µg/mL). Juniper EO did not evidenced cytotoxicity in non-tumoral Vero cells at the highest tested concentration (400 µg/mL) which can be an indicator of its safety. Overall, the results demonstrated that shrubs biomass can be a source of EO with similar composition to that reported for respective berries and leaves. The EOs showed interesting antibacterial and antioxidant activity thus being potential candidates for further studies on their safety and potential application in food systems.

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