Vibrational and Thermal Studies of Essential Oils Derived from *Cistus ladanifer* and *Erica arborea* Shrubs

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Essential oils from the two most representative shrub species from the Iberian Peninsula (namely *Cistus ladanifer* L. and *Erica arborea* L.) have been characterized by Fourier transform infrared spectroscopy (FTIR) and thermoanalytical techniques (TG/DTG and DSC). Vibrational spectra have been compared with those of conserved in the hydrodistillation and that this extraction process ensures the recovery of the main constituents of both essential oils.

Pyrophytes are plants which have adapted to tolerate fire. “Passive pyrophytes” (e.g., *Asphodelus albus* Willd.) are able to resist the effects of fire, particularly when it passes over quickly, and hence can out-compete less resistant plants, which are damaged. “Active pyrophytes” (such as *Cistus ladanifer* L. and *Erica arborea* L.) have a similar competing advantage, but also contain volatile oils and hence encourage the incidence of fires which are beneficial to them. *C. ladanifer* (gum rockrose), native to the Western Mediterranean, is known in Spanish as ‘*Jara pringosa*’ (meaning ‘sticky shrub’) because the whole plant is covered with a sticky exudate of a fragrant resin. This resin, known as ‘*labdanun*’, ‘*ladanum*’, ‘*ladanum*’ or ‘*ladanon*’, is produced by boiling the leaves and twigs. It presents a relatively high content of ambrox (amber odor) and, consequently, it is valued in perfumery as a substitute for ambergris (from the sperm whale, which is an endangered species). *Cistus* essential oil is obtained by either hydrodistillation or steam distillation of the leaves and stems, reaching a price of over 200 €/L.

*Cistus* oil is relatively rich in monoterpenes (67%) and sesquiterpenes (20%) and poor in diterpenes (3%). The major constituents are α-pinene (a bronchodilator), *trans*-pinocarveol, bornyl acetate, terpinen-4-ol and camphene (monoterpenes); viridiflorol (estrogen mimic, also reported in propolis), ledol and globulol (sesquiterpene alcohols); and 15-nor-labd-8-ol (diterpene) [1-3].

*Erica arborea* (tree heath) is in the heather family, *Ericaceae*. It is native to the maquis shrublands (garrigue biome) of the Mediterranean Basin, Portugal and the Canary and Madeira Islands. The leaves and flowers of *E. arborea* have been popularly used as an anti-rheumatic, a diuretic, an astringent and in the treatment of urinary infections, while its wood (biar or bruyère in French) is used for making smoking pipes and jewelry.

*E. arborea* contains many active compounds such as flavonoids, monoterpenes, triterpenoids, phenylpropanoid glucosides and condensed tannins [4]. Pharmacological activities of the extracts from this plant have been reported to be anti-inflammatory, antioxidant and analgesic. In fact, the composition of its bark (with leaves) has been reported to include triterpenoids such as friedelin, lupool, betulin and ursolic acid [5, 6].

According to Bessah and Benyoussef [4], the water-distilled essential oil from leaves of *E. arborea* of Algerian origin (d=0.8587; [α] +2’44’) contains 75 components, amongst which palmitic acid (33.3%), (Z,Z,Z)-9,12,15-octadecatrien-1-ol, a fatty alcohol (6.6%), and nonacosane, a straight-chain hydrocarbon (6.1%), are the major constituents [4]. Other components, present in lower proportions, are β-fenchyl alcohol, β-caryophyllene, β-bourbonene, eugenol, ionol, geranylacetone and germacrene D [4].

To the best of the authors’ knowledge, no vibrational or thermal studies have been reported in the literature for any of the two aforementioned oils. The thermal behavior of *C. ladanifer* has been studied by TG and triple shot pyrolysis [7] of the entire plant, and the studies on *E. arborea* have been aimed at the assessment of the thermal behavior of the entire plant for its integration in wildland fire spread models [8] or to measure its ignitability, combustibility and sustainability as a forest fuel [9].

In the work presented herein, the physicochemical properties of the essential oils obtained from these two pyrophytic Mediterranean shrubs have been investigated by ATR-FTIR vibrational spectroscopy and by thermogravimetric (TG) and differential scanning calorimetric (DSC) thermoanalytical techniques. These data are of interest in extraction processes for the cosmetics industry, provided that they reveal differentiation patterns of the vegetable oil fingerprints.
The wavenumbers for the main bands in the ATR-FTIR vibrational spectra of the oils are listed in Table 1. It can be observed that they all shared the presence of bands at 145S cm⁻¹ (CH₂ cellulose, lignin) and 1375 cm⁻¹ (C-H cellulose, hemicellulose) [10]. Bands specific to the oils are those which appeared at 2960, 2873, 1678, 945 and 818 cm⁻¹. The bands at 2960 and 2873 cm⁻¹ are assigned to asymmetrically and symmetrically stretching vibration of C-H of aliphatic CH₂ groups, due to the alkyl rest of the triglycerides present in large quantities in vegetable oils, while the band at around 1678 cm⁻¹ can either be attributed to C=C stretching vibration of cis disubstituted olefins (RHC=CHR) or to an oxo group (α:β unsaturated) from terpenoids. The C-H out-of-plane deformation band observed between 952 and 939 cm⁻¹ is highly characteristic of trans double bonds, and the band at around 818 cm⁻¹ is related to an isopropylidene group (R₂C=CHR), usual in terpenoids [11-14].

The labdanum and tree heath oils spectra (Figure 1), as is the case for other oils, showed bands in common with resins and gums has led us to compare these spectra with those of rosehip and palm oils, myrrh and mastic resins and tragacanth gum (see Table 2). It is worth noting that, in the 2920-1160 cm⁻¹ region, there is a high correspondence with the bands of R. rubiginosa oil, moderate correspondence with the oils of some other species, and low correspondence with the oils of tragacanth gum.

In the case of C. ladanifer oil, whose terpene composition is favorable to low-number isoprenic units (monoterpenes and sesquiterpenes), the band at 1737 cm⁻¹ (attributable to ν(CO) ester carbonyl or to terpenic oxo groups) is shared with rosehip and palm oils, whereas the composition rich in terpenes with a high number of isoprenic units (triterpenoids) exhibited by E. arborea oil leads to a shift of this band towards lower wavenumbers (1721 cm⁻¹). As regards the band at around 2875 cm⁻¹ (attributed to symmetrical ν(C-H) from CH₃) in the E. arborea spectrum, it should be noted that it agrees with that of mastic resin. Another useful band in terms of differential identification of the oils is that which appears at 1681 cm⁻¹ in C. ladanifer oil and shifted to lower wavenumbers in tree heath oil (1674 cm⁻¹) and rosehip oil (1653 cm⁻¹). This band does not appear in palm pulp oil or in tragacanth gum.
Thermal analysis: TG plots of gum rockrose and tree heath oils showed mass loss in the 50-240°C and 100-220°C temperature range, respectively, with a more abrupt pattern for *E. arborea* (Figure 2). In both cases this mass loss corresponded to pyrolysis, which was sensitized in the DTG curves by endotherms at 151°C (*C. ladanifer*) and at 208°C (*C. arborea*). From these temperatures, it can be seen that *E. arborea* oil presents higher thermal stability than that of *C. ladanifer*. In any case, the decomposition point of both oils is high enough to ensure the preservation of terpenoids as main constituents. The chief products of pyrolysis were straight-chain alkanes and alklenes [16]. It should be noted that heating of isopropanol extracts (not shown) resulted, after solvent evaporation, in decomposition of the oils extending up to 400°C.

The low-temperature DSC thermograms of the two oils under study are depicted in Figure 3. Both *C. ladanifer* and *E. arborea* oils vitrify upon cooling. Upon heating, only devitrification is pyrolyzed. For *C. ladanifer* oil the glass transition is observed at \( T_g = (-113 \pm 1) \) °C and for *E. arborea* oil at \( T_g = (-79 \pm 1) \) °C.

As regards the FTIR results, this technique has proven useful to identify the different components of various parts of the plants and their derived products, serving as a fingerprint technique which provides insight into the biochemical composition of the samples. As noted by Huck [17], this method is particularly suitable for the fast and simultaneous qualitative and quantitative characterization of natural products and their constituents. Thus, the data presented herein can be used for quality control in the cosmetics industry.

With regard to the thermal stability of the oils under study, it is high enough to ensure that their thermolabile constituents would be preserved during hydro-distillation, which is the method recommended by the French Pharmacopoeia for the extraction of essential oils from dried spices and the quality control of essential oils in the laboratory [18].

On another topic, provided that the two shrub species under study are active pyrophytes, the results should also be put in relation to their impact on forest fires: the flammability of the shrubs under study (and, by extension, those of their resins and oils) must be referred (apart from ignition time and moisture) to their contents of terpenoids. The high flammability of *E. arborea* can be ascribed to its high emission of terpenes throughout all the year (which can reach up to 40 μg g\(^{-1}\) h\(^{-1}\)), in spite of the fact that its composition is relatively rich in low-volatile terpenoids, such as triterpenoids. Conversely, although *C. ladanifer* emits into the air modest amounts of terpenoids (<7 μg g\(^{-1}\) h\(^{-1}\)), it has a very high flammability in the summer due to the potentiated emission of α-pinene, a particularly volatile monoterpen, which, as noted above, is an important component of its essential oil [19].

The essential oils from two Western Mediterranean pyrophytes, namely *C. ladanifer* and *E. arborea*, have been characterized by ATR-FTIR spectroscopy and thermal analytical (TG/DTG and DSC) techniques. Their vibrational spectra have been compared with those of other parts of the plants (leaves, roots, capsules, etc.) and with those of oils, gums and resins from other species. The specific location of the bands from unsaturated αβ unsaturated oxogroups has been related to the different content of terpenoids of *C. ladanifer* oil (mono- and sesquiterpenoids) and *E. arborea* (triterpenoids). As regards the thermal behavior, *E. arborea* oil showed higher thermal stability than that of *C. ladanifer*, as evinced by the effects in the TG and DSC thermograms (at 210°C and
143°C, respectively). Thus, recognition of the TG and DSC characteristic patterns of both essential oils can also be helpful in identifying the type of oil. On the other hand, the delayed thermal decomposition of the oils under study, together with the characterization of terpenoids by ATR-FTIR, provide evidence that oil constituents that are thermolabile, such as terpenoids, are conserved in the extraction process, thus ensuring that hydrodistillation is a valuable method to recover the main constituents of these essential oils.

Experimental

Samples: C. ladanifer and E. arborea samples were collected from wild plants growing in the municipality of Ayoó de Vidriales (in the province of Zamora, Castilla y Leon, Spain) during the flowering period (Spring 2015). Oils from Rosa rubiginosa L. and Elaeis guineensis Jacq., myrrh from Commiphora, mastic from Pistacia lentiscus L. and tragacanth gum from Astragalus samples used for comparison purposes were of commercial origin.

Methods: Hydro-distillation of 100 g of fresh and whole leaves of C. ladanifer and E. arborea was carried out in a Clevenger-type apparatus for 3 h. The essential oils were collected, dried under anhydrous sodium sulfate and stored at 4°C until used. To confirm their purity, essential oil composition was determined by gas chromatography coupled to mass spectrometry (GC-MS) analysis, according to the experimental conditions specified by Bessah and Benyoussef [4]. A commercial sample E. arborea essential oil, supplied by Radhe Shyam (Barcelona, Spain), was also tested. An alternative extraction method consisting of suspending the vegetal dry matter in amyl alcohol in a 1:2 (w/v) ratio for 30 min, under constant shaking, at room temperature, was also used to isolate high-purity oil from both plants.

Apparatus: GC/MS analysis was conducted with an Agilent Technologies 7890A apparatus (Santa Clara, CA, USA). The vibrational spectra of the materials in the 400-4000 cm⁻¹ spectral range were measured using a Thermo Scientific (Waltham, MA, USA) Nicolet iS50 FT-IR spectrometer, equipped with an in-built diamond attenuated total reflection (ATR) system. Spectra of the oils were recorded at room temperature with a 1 cm⁻¹ spectral resolution, and 64 scans. TG and high-temperature DSC analyses were conducted with a Perkin-Elmer (Waltham, MA, USA) STA6000 simultaneous thermal analyser by heating the samples in a slow stream of N₂ (20 mL·min⁻¹) from room temperature up to 500°C, with a heating rate of 20°C·min⁻¹. Pyris v.11 software was used for data analysis. The low-temperature DSC experiments were performed in a Perkin-Elmer Pyris 1 power compensation calorimeter. A liquid nitrogen Cryofill cooling unit was used, and helium at a 20 mL·min⁻¹ flux was employed as the purge gas. Samples were cooled to -170°C and then heated to room temperature at a 50°C·min⁻¹ rate. The obtained data were analysed using TA Instruments Universal Analysis V4.1D software.

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