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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 components of the plants, and with those of oils, gums and resins from other species. The different content in terpenoids of *C. ladanifer* oil (mainly mono- and sesquiterpenoids) and *E. arborea* oil (mainly triterpenoids) is reflected in the ATR-FTIR by the position of the bands at around 2873 cm^{-1}, 1730 cm^{-1} and 1678 cm^{-1}. As regards their thermal behavior, *C. ladanifer*-derived oil evinced higher thermal stability than that of obtained from *E. arborea*: the pyrolysis of the former was sensitized at 210ºC, whereas for the later it occurred at 143ºC. These temperatures are high enough to state that thermolabile constituents such as terpenoids are conserved in the hydrodistillation and that this extraction process ensures the recovery of the main constituents of both essential oils.

Keywords: *Cistus ladanifer*, *Erica arborea*, Oils, TG/DTG, DSC, FTIR.

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 ‘labdanum’, ‘ladanum’, ‘laudanum’ 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-labdan-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 (briar root 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, lupeol, 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. ladafiner* 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.

ATR-FTIR spectra: The wavenumbers for the main bands in the ATR-FTIR spectra of the oils of the two plants under study, together with those from leaves, roots, capsules, fine and coarse-texture components for each of the species, are summarized in Table 1. A close similarity can be observed, on the one hand, between the leaves and roots spectra and, on the other hand, between the fine and coarse components spectra. The capsules vibrational pattern for *E. arborea* exactly matched that of the fine components and is listed in the same column.

Table 1: Main bands in the ATR-FTIR vibrational spectra of *C. ladanifer* and *E. arborea* oils and various plant components. All wavenumber values are in cm^{-1}.

Cistus ladanifer					*Erica arborea*				
Oil	Leaves	Roots	Fine	Coarse	Oil	Leaves	Roots	Capsules / Fine	Coarse
3465	3412	3416	3419	3421	3360	3396	3405	3421	3412
2953	×	×	×	×	2970	×	×	×	×
2918	2919	2920	2922	2920	2931	2923	2929	2918	2919
2871	2850	×		×	2874	2852	×	2850	
×	2360	2361	2355	2361	×	2360	2361	2360	2360
×	≠	2344	2339	2343	×	≠	2344	2342	2343
1737	1733	1735	1732	1735	1721	1733	1736	1739	1740
1681	×	×	×	×	1674	×	×	×	×
×	1615	1617	1621	1617	1614	1616	1617	1616	1616
×	1515	1508	1505	1508	×	1516	1513	1508	1510
1448	1455	1452	1455	1457	1454	1455	1451	1464	1459
1375	1367	1375	1373	1375	1374	1373	1374	1375	1375
1328	≠	1320		1320	1329	≠		1331	
1245	1232	1243	1242	1243	1249	1243	1247	1246	1247
1164	1164				1152		1151		
1125									
1111					1085		1081		
×	1035	1035	1031	1035	1046	1035	1031	1050	1039
1015					1007				
952					939				
886					860				
815					821				
787					758				
635					665				
618	617	559	602	610	559		579	608	607

The oils spectra differed from those of the leaves, roots and other plant components from which they were obtained by the absence of the absorption bands at 2360 cm^{-1} (absorbed CO_2), 2344 cm^{-1} (glucose ring stretching), 1515 cm^{-1} (benzene ring stretching in lignin) and 1035 cm^{-1} (lignin), present in the latter components. Nonetheless, they all shared the presence of bands at 1455 cm^{-1} (CH_2 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 CH_3 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 isopropylidine group (R_2C=CHR), usual in terpenoids [11-14].

The fact that 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, a moderate correspondence with those of other oils and resins, and low agreement with those 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 CH_3) 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.

Table 2: Comparison of the vibrational spectra of *C. ladanider* and *E. arborea* oils with rosehip and palm oils, myrrh and mastic resins and tragacanth gum.

C. ladanifer		*E. arborea*		Myrrh resin	Mastic resin	Rosehip oil [15]	Palm pulp oil	Tragacanth gum	Assignments
extract	oil	extract	oil						
	3465								OH group
3362		3366	3360	3348				3335	
2969	2953	2969	2970		2949	3009	3004		asymmetric *ν*(CH) from CH_3
2915	2918	2930	2931	2925		2923	2914	2932	asymmetric *ν*(CH) from CH_2
	2871	2875	2874		2874				symmetric *ν*(CH) from CH_3
2848	2834			2854		2853	2850		
1732	1737	1735	1721	1732		1742	1736	1716	*ν*(CO) ester carbonyl of triglycerides
1712	1710				1707				
1655	1681	1674	1674	1635	1650	1653	×	×	ν(C=C) disubstituted olefins; olefinic terpenoids
1607		1614	1614	1601				1600	*ν*(C=C) aromatic ring
1462	1448	1454	1454		1459	1456	1467		*δ*(CH_2) bending deformation
				1435				1410	*δ*(C-H) from CH_2 or CH_3 groups; CH_2 cellulose/lignin
1378	1375	1374	1374	1378		1377	1389	1371	*δ*(CH_2)$_2$ bending deformation CH hemicellulose, cellulose
1341	1328	1329	1328	1335				×	*δ*(CH_3) bending deformation
			1298						
1227	1245	1249	1249	1238	1245	1238	1243	1242	*δ*(C-H), *ν*(C-O-H).
1160	1164	1152	1142		1161	1160	1170	1145	methyl ester, *ν*(CO)
1128	1125							1120	*ν*(C-C)
1106	1111				1115				
	1083	1085	1084			1098	1100		stretching vibration of C–O ester groups
	1048	1046	1045		1046				starch OH, cellulose
	1032			1023				1034	
	993	1007	1007		1008				trans double bond
950	952	939	988						*δ*(C-H) bending
876	886	860	860	841	837			892	*δ*(C-O-C)
816	815	821	838					846	R_2C=CHR isopropylidine
	787								
729		758	758	725		723	717	×	*ρ*(CH_2)$_n$
719	698		701						1,2-*cis*-disubstituted olefin
638	635	665	662	597	580				*δ*(C-H) in the furan ring
	563	559	559					595	
			527					521	

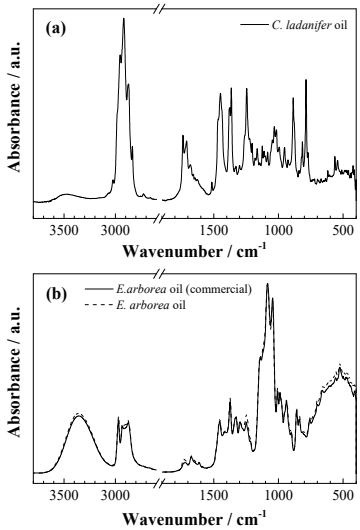

Figure 1: FTIR spectra of (a) *Cistus ladanifer* oil and (b) *Erica arborea* oil.

Thermal analysis: TG plots of gum rockrose and tree heath oils showed mass loss in the 50-240ºC and 100-220ºC 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 151ºC (*C. ladanifer*) and at 208ºC (*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 alkenes [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 ºC.

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 observed. For *C. ladanifer* oil the glass transition is observed at T_g = (-113 ± 1) ºC and for *E. arborea* oil at T_g = (-79 ± 1) ºC.

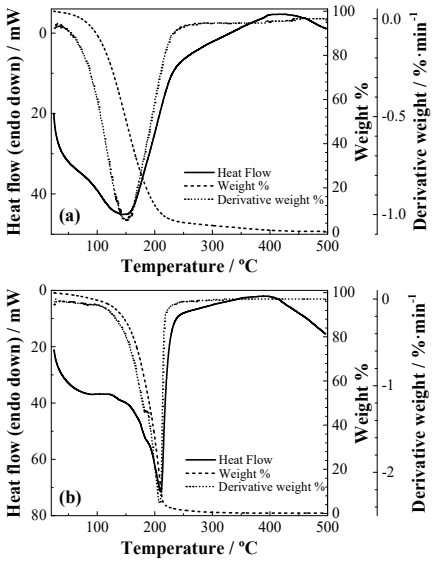

Figure 2: Thermograms for (*a*) *C. ladanifer* oil, *m* = 21.14 mg; and (*b*) *E. arborea* oil, m = 17.77 mg. The left *y* axis corresponds to the DSC curve, while the two *y* axes on the right correspond to TG/DTG curves.

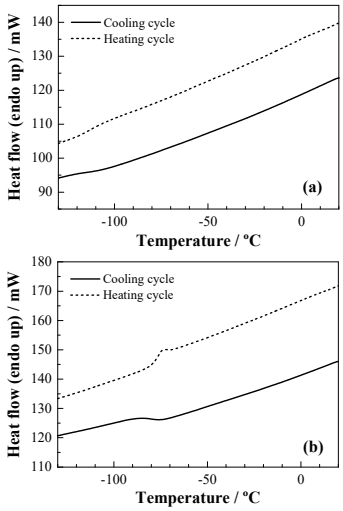

Figure 3: Low-temperature DSC thermograms for (*a*) *C. ladanifer* oil, *m* = 4.1 mg; and (*b*) *E. arborea* oil, *m* = 5.7 mg. The cooling/heating rate was $|\beta|$=50ºC·min^{-1}.

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 $\mu g \cdot g_{DM}^{-1} \cdot h^{-1}$), 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 $\mu g \cdot g_{DM}^{-1} \cdot h^{-1}$), it has a very high flammability in the summer due to the potentiated emission of α-pinene, a particularly volatile monoterpene, 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 and α:β 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 210ºC and

143ºC, 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 4ºC until used. To confirm their purity, essential oil composition was determined by gas chromatography coupled to mass spectrometric (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 N_2 (20 $mL \cdot min^{-1}$) from room temperature up to 500ºC, with a heating rate of 20ºC·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 $mL \cdot min^{-1}$ flux was employed as the purge gas. Samples were cooled to -170ºC and then heated to room temperature at a 50ºC·min^{-1} rate. The obtained data were analysed using TA Instruments Universal Analysis V4.1D software.

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