Aromatic plants as a source of important phytochemicals: vitamins, sugars and fatty acids in *Cistus ladanifer*, *Cupressus lusitanica* and *Eucalyptus gunnii.* leaves

RAFAELA GUIMARÃES, LILLIAN BARROS, ANA MARIA CARVALHO, MARIA JOÃO SOUSA, JORGE SÁ MORAIS, AND ISABEL C.F.R. FERREIRA*

CIMO/Escola Superior Agrária, Instituto Politécnico de Bragança, *Campus* de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.

* Author to whom correspondence should be addressed (e-mail: iferreira@ipb.pt telephone +351-273-303219; fax +351-273-325405).

ABSTRACT

The phytochemical composition of three aromatic plants (*Cistus ladanifer* L., *Cupressus lusitanica* Mill. and *Eucalyptus gunnii* Hook. f) was evaluated, in order to valorize them as sources of nutraceuticals. Samples were analyzed for ascorbic acid by spectrophotometric assay, tocopherols by high performance liquid chromatography (HPLC) coupled to a fluorescence detector, sugars by HPLC coupled to a refraction index detector (RID), and fatty acids by gas-chromatography (GC) coupled to a flame ionization detector (FID). The analysed plants contain very useful compounds such as vitamins (ascorbic acid and tocopherols), reducing sugars (glucose and fructose) and polyunsaturated fatty acids (linoleic, α-linoleic and eicosadienoic acids). Their vitamins and sugars could be useful in diseases related to oxidative stress, in dermatological applications or in cosmetics. The polyunsaturated fatty acids, including omega-3 and omega-6 families, detected in the plants constitute another important class of phytochemicals due to their generalized beneficial health effects.

***Keywords*:** Aromatic plants; phytochemicals; vitamins; polyunsaturated fatty acids; nutraceuticals

1. Introduction

There is emerging interest in the use of naturally occurring antioxidants for the preservation of foods and in the management of a number of pathophysiological conditions, most of which involve free radical damage. The implication of oxidative and nitrosative stress in the etiology and progression of several acute and chronic clinical disorders has led to the suggestion that antioxidants can have health benefits as prophylactic agents (Sobrattee et al., 2005). The number of individual phytochemicals already identified in fruits and vegetables is estimated in >5,000, but a large percentage still remain unknown and need to be identified before we can fully understand the health benefits of phytochemicals in whole foods (Liu, 2004). However, more and more convincing evidence suggests that the benefits of phytochemicals may be even greater than is currently understood, because the oxidative stress induced by free radicals is involved in the etiology of a wide range of chronic diseases (Ames and Gold, 1991). Phytochemicals can include secondary metabolites such as phenolic compounds but also vitamins, sugars and fatty acids, and these compounds can be used as nutraceuticals. The term "nutraceutical" was coined in 1989 by the Foundation for Innovation in Medicine (New York, US), to provide a name for this rapidly growing area of biomedical research. A nutraceutical was defined as any substance that may be considered a food or part of a food and provides medical or health benefits including the prevention and treatment of disease (DeFelice, 1992). Nutraceuticals may range from isolated compounds or dietary supplements to genetically design foods, plant extracts or processed products such as cereals, soups and beverages.

Cistus ladanifer L. (gum rockrose) is a Mediterranean shrub from the Cistaceae family, having white flowers and viscid stems and leaves producing a fragrant oleoresin used in

perfumes especially as a fixative. It is widely distributed in Portugal, being one of the most abundant species in the southern part of the country, occurring in large areas as pure dense stands (Teixeira et al., 2007). *Cistus ladanifer* and other species of Cistaceae are used as general remedies in folk medicine for treatment of various skin diseases, as antidiarrheics, and as anti-inflammatory agents (Attaguile et al., 2000). Several research works have been reported in the literature on *C. ladanifer* L. volatile compounds, due to the great importance of this raw material for the fragrance industry (Teixeira et al., 2007). *Cupressus lusitanica* Mill., commonly known as white Mexican cedar, Mexican cypress or Portuguese cypress, belongs to the Cupressaceae family. Fast growing to 30m tall, and drought tolerant it is very popular in Portugal and widely cultivated, both as an ornamental tree and for timber production since a long time, and thus naturalised in several Portuguese regions. The leaves are scale-like, 1.5-2mm long, and produced on rounded shoots (Castroviejo, 1998). According to literature the cedar foliage is used in indigenous practices to treat catarrh, headache and dermatitis (Kuiate et al., 2006; Tene et al., 2007). The essential oil of the leaves is used against rheumatism, whooping cough, and styptic problems (Kuiate et al., 2006). In general, *Eucalyptus* species (Myrtaceae) are fast growing trees exploited mainly for paper pulp but also as a source for various essential oils and as ornamental plants. For the production of phytopharmaceuticals, essential oils rich in 1,8-cineole (called also ‘‘eucalyptol’’), are of special importance. These products are applied for relief of head colds, rheumatism, muscular pain, and as expectorant in cases of bronchitis (added to cough syrups) (Lassak and McCarthy, 1983). *Eucalyptus gunnii* Hook. f (cider gum) is commonly planted as an ornamental tree across the northern Portugal due to his exceptional cold tolerance and sometimes may become naturalized. The foliage changes as the tree

matures from a round opposite leaf of waxy blue colour to alternate and more elongated rich green foliage in the older trees (Castroviejo, 2007).

Recent research demonstrated that these aromatic plants are a good source of compounds with a positive impact in human health, such as phenolic compounds (Vázquez et al., 2009), flavonoids (Danne et al., 1994) and terpenes (Adams et al., 1997). In this work, a phytochemical characterization was performed, in order to find other important compounds; samples were analyzed for tocopherols by HPLC-fluorescence, sugars by HPLC/RID, and fatty acids by GC-FID.

2. Materials and methods

2.1. Samples

Cistus ladanifer fresh leaves from flowering steams were collected randomly, from wild plants growing in the Natural Park of Montesinho (Northeastern Portugal) in July 2008. *Cupressus lusitanica* foliage and *Eucalyptus gunnii* matured alternate leaves were randomly gathered from the crown of trees cultivated in the experimental farm of the school of agriculture (Escola Superior Agrária, ESA), in Bragança (Northeastern Portugal) in July 2008.

Morphological key characters from the Flora Iberica were used for plant identification: *Cistus ladanifer* (Castroviejo, 2005); *Cupressus lusitanica* (Castroviejo, 1998); *Eucalyptus gunnii* (Castroviejo, 2007). Voucher specimens from each plant were numbered and deposited in the Herbarium of the ESA. The vegetal material was lyophilized (Ly-8-FM-ULE, Snijders, HOLLAND) and kept in the best conditions for subsequent use.

107

108 *2.2. Standards and Reagents*

109 Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from
110 Lab-Scan (Lisbon, Portugal). All the other solvents were of analytical grade purity:
111 methanol and diethyl ether were supplied by Lab-Scan (Lisbon, Portugal), while toluene
112 and sulphuric acid were supplied by Sigma Chemical Co. (St. Louis, MO, USA). The
113 fatty acids methyl ester (FAME) reference standard mixture 37 (fatty acids C4 to C24;
114 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other
115 individual fatty acid isomers and tocopherol standards (α, β, γ and δ). Racemic Tocol,
116 50 mg/mL, was purchased from Matreya (PA, USA). All other chemicals were obtained
117 from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Milli-Q water
118 purification system (TGI Pure Water Systems, USA).

119

120 *2.3. Phytochemicals composition*

121 *2.3.1. Vitamins*

122 Tocopherols content was determined according to the following procedure. BHT
123 (butylated hydroxytoluene) solution in hexane (10 mg/mL; 100 μL) and IS (internal
124 standard) solution in hexane (tocol; 50 μg/mL; 400 μL) were added to the sample prior
125 to the extraction procedure. BHT was used as antioxidant protector to minimize
126 tocopherols loss. The samples (~500 mg) were homogenized with methanol (4 mL) by
127 vortex mixing (1 min). Subsequently, hexane (4 mL) was added and again vortex mixed
128 for 1 min. After that, saturated NaCl aqueous solution (2 mL) was added, the mixture
129 was homogenized (1 min), centrifuged (5 min, 4000g) and the clear upper layer was
130 carefully transferred to a vial. The sample was re-extracted twice with hexane. The

131 combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 mL
132 of n-hexane, dehydrated with anhydrous sodium sulphate, filtered through a 0.22 μm
133 disposable LC filter disk, transferred into a dark injection vial and analysed by HPLC.
134 The HPLC equipment consisted of an integrated system with a Smartline pump 1000
135 (Knauer, Germany), a degasser system Smartline manager 5000, an AS-2057 auto-
136 sampler and a 2500 UV detector at 295 nm (Knauer, Germany) connected in series with
137 a FP-2020 fluorescence detector (Jasco, Japan) programmed for excitation at 290 nm
138 and emission at 330 nm. Data were analysed using Clarity 2.4 Software (DataApex).
139 The chromatographic separation was achieved with a Polyamide II (250 x 4.6 mm)
140 normal-phase column from YMC Waters (Japan) operating at 30ºC (7971 R Grace
141 oven). The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v)
142 at a flow rate of 1 ml/min, and the injection volume was 20 μL. The compounds were
143 identified by chromatographic comparisons with authentic standards. Quantification
144 was based on the fluorescence signal response, using the internal standard method.
145 Tocopherol contents in the samples are expressed in μg per g of dry sample.
146 For ascorbic acid determination, a fine powder (20 mesh) of sample (150 mg) was
147 extracted with metaphosphoric acid (1%, 10 mL) for 45 min at room temperature and
148 filtered through Whatman Nº 4 filter paper. The filtrate (1 mL) was mixed with 2,6-
149 dichloroindophenol (9 mL) and the absorbance was measured within 30 min at 515 nm
150 against a blank (Analytikijena 200-2004 spectrophotometer). Content of ascorbic acid
151 was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.006-
152 0.1 mg/mL; $y = 3.0062x + 0.007$; $R^2 = 0.9999$), and the results were expressed as μg of
153 ascorbic acid per g of dry sample.
154

155 *2.3.2. Sugars*

156 Free sugars were determined by high performance liquid chromatography coupled to a
157 refraction index detector (HPLC-RID). Dried sample powder (1.0 g) was spiked with
158 the melezitose as internal standard (IS, 5 mg/ml), and was extracted with 40 mL of 80%
159 aqueous ethanol at 80 ºC for 30 min. The resulting suspension was centrifuged
160 (Centorion K24OR- 2003 refrigerated centrifuge) at 15,000 g for 10 min. The
161 supernatant was concentrated at 60 ºC under reduced pressure and defatted three times
162 with 10 mL of ethyl ether, successively. After concentration at 40 ºC, the solid residues
163 were dissolved in water to a final volume of 5 mL. Soluble sugars were determined by
164 using HPLC (Knauer, Smartline system) at 35 ºC. The HPLC system was equipped with
165 a Knauer Smartline 2300 RI detector and with a Eurospher 100-5 NH_2 column (4.6 x
166 250 mm, 5 mm, Knauer). The mobile phase was acetonitrile/deionized water, 7:3 (v/v)
167 at a flow rate of 1 mL/min. The results are expressed in mg per g of dry weight,
168 calculated by internal normalization of the chromatographic peak area. Sugar
169 identification was made by comparing the relative retention times of sample peaks with
170 standards. The sugar standards used for identification were purchased from Sigma
171 Chemical Co. (St. Louis, USA): L(+)-arabinose, D(-)-fructose, L-fucose, D(+)-
172 galactose, D(+)-glucose anhydrous, lactose 1-hydrate, maltose 1-hydrate, maltulose
173 monohydrate, D(+)-mannitol, D(+)-mannose, D(+)-melezitose, D(+)-melibiose
174 monohydrate, D(+)- raffinose pentahydrate, L(+)-rhamnose monohydrate, D(+)-sucrose,
175 D(+)-trehalose, D(+)- turanose and D(+)-xylose.

176

177 *2.3.3. Fatty Acids*

178 Fatty acids were determined by gas-liquid chromatography with flame ionization
179 detection (GC-FID)/capillary column based on the following trans-esterification
180 procedure: fatty acids (obtained after Soxhlet extraction) were methylated with 5 mL of
181 methanol:sulphuric acid:toluene 2:1:1 (v:v), during at least 12 h in a bath at 50 ºC and
182 160 rpm; then 3 mL of deionised water were added, to obtain phase separation; the
183 FAME were recovered with 3 mL of diethyl ether by shaking in vortex , and the upper
184 phase was passed through a micro-column of sodium sulphate anhydrous, in order to
185 eliminate the water; the sample was recovered in a vial with Teflon, and before injection
186 the sample was filtered with 0.2 µm nylon filter from Milipore. The fatty acid profile
187 was analyzed with a DANI model GC 1000 instrument equipped with a split/splitless
188 injector, a flame ionization detector (FID) and a Macherey-Nagel (OPTIMA 225: 50%
189 cyanopropyl-methyl – 50% phenylmethylpolysiloxane) column (30 m x 0.32 mm ID x
190 0.25 µm d_f). The oven temperature program was as follows: the initial temperature of
191 the column was 50 ºC, held for 2 min, then a 10ºC/min ramp to 240 ºC and held for 11
192 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50
193 ºC. Split injection (1:40) was carried out at 250 ºC. For each analysis 1 µL of the sample
194 was injected in GC. Fatty acid identification was made by comparing the relative
195 retention times of FAME peaks from samples with standards. The results were recorded
196 and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative
197 percentage of each fatty acid.
198
199 *2.4. Statistical analysis*
200 For each one of the species three samples were analysed and also all the assays were
201 carried out in triplicate. The results are expressed as mean values and standard deviation

(SD) or standard errors (SE). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 16.0 program.

3. Results and discussion

Tocopherols composition in the studied aromatic plants showed important variations in their content (**Table 1**). α-Tocopherol was the major compound in all the samples, and δ-tocopherol was only detected in *Eucalyptus*. This sample presented the highest content of tocopherols (1558.27 µg/ g of dry weight; **Table 1**) while *Cistus ladanifer* leaves revealed the lowest content (76.90 µg/g).

Ascorbic acid was the most abundant vitamin in *Cupressus lusitanica* and *Cistus ladanifer* leaves, and particularly for the latter sample it presented a very high level (647.64 mg/g dry weight; **Table 1**). Plants produce large amounts of vitamins to facilitate resistance to the oxidative stresses. Particularly vitamin E and vitamin C with potent antioxidant activities have recently received a great deal of attention because of their action on immunity and disease etiology. They are naturally-occurring antioxidant nutrients exerting a protective role against various oxidative stress-related diseases such as heart disease, stroke, cancer, several neurodegenerative diseases and cataractogenesis (Halliwell, 1996). Cooperative interactions exist among vitamin C and vitamin E. They interact synergistically at the membrane-cytosol interface to regenerate membrane-bound oxidized vitamin E (Li and Schellhorn, 2007). Water-soluble chain radicals, such as vitamin C, function as a primary defence against aqueous radicals, while vitamin E acts as lipophilic chain-breaking antioxidant and is responsible for scavenging lipid peroxyl radicals (Liu et al., 2008). As far as we know, nothing is described in literature

226 concerning vitamins contents in the studied aromatic plants. Being important sources of
227 these powerful antioxidants, they can be used in the treatment of diseases related to the
228 production of free radicals, but also in cosmetics for anti-ageing treatments. For the
229 latter application, the studied pants have the advantage of being aromatic and, therefore,
230 possess volatile compounds with good fragrances.
231 Other compounds that can act as antioxidants are sugars, mainly reducing sugars such as
232 glucose and fructose. In what concerns sugar composition (**Table 2**) the aromatic plants
233 presented fructose, glucose, sucrose and raffinose as main sugars. The present study
234 describes for the first time the sugars composition in these aromatic plants. For *Cistus*
235 *ladanifer* (48.21 mg/ g) and *Cupressus lusitanica* (40.32 mg/g) fructose was the most
236 abundant sugar, while sucrose predominates in *Eucalyptus gunnii*. (29.84 g/100 g).
237 *Cupressus lusitanica* revealed the highest total sugars content, and highest levels of
238 fructose and raffinose. Otherwise, *Eucalyptus gunni*i. showed the lowest levels in total
239 sugars (61.92 mg/g).
240 The total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and
241 polyunsaturated fatty acids (PUFA) showed significant variation on their content (**Table**
242 **3**). The major fatty acids found in *Cupressus lusitanica* and *Eucalyptus gunnii* samples
243 were α-linolenic acid (C18:3), followed by linoleic acid (C18:2) and palmitic acid
244 (C16:0). For *Cistus ladanifer* leaves, eicosadienoic acid (C20:2) was the most abundant
245 fatty acid, and was followed by arachidic acid (C20:0) and α-linolenic acid.
246 In all the cases UFA predominate over SFA, ranging from 62 to 64%, and particularly
247 PUFA predominated over MUFA. Twenty four fatty acids were identified and
248 quantified. As far as we know, nothing has been reported on fatty acid composition of
249 the leaves of the three aromatic plants, despite the extensive reports on their essential

250 oils composition (Adams et al., 1997; Kuiate et al., 2006; Teixeira et al., 2007). The
251 present study proved that they are sources of beneficial fatty acids such as the
252 polyunsaturated fatty acids: linoleic, α-linolenic and eicosadienoic acids.
253 Linoleic acid is a member of the group of essential fatty acids called omega-6 fatty
254 acids, so called because they are an essential dietary requirement for all mammals,
255 allowing the biosynthesis of arachidonic acid and thus some prostaglandins. Linoleic
256 acid is used in making soaps, emulsifiers, and quick-drying oils. It has become
257 increasingly popular in the cosmetic industry because of its beneficial properties on the
258 skin, including anti-inflammatory, acne reduction and moisture retention properties
259 (Letawe et al., 1998; Darmstadt et al., 2002).
260 α-Linolenic acid is a polyunsaturated omega-3 fatty acid and studies have found
261 evidence that α-linolenic acid is related to a lower risk of cardiovascular disease
262 (William et al., 2000).
263 Prostaglandins and thromboxanes are related compounds known as eicosanoids, which
264 have a large variety of biological activities including mediation in anti-inflammatory
265 processes, lowering of blood pressure, relax of coronary arteries and inhibition of
266 platelet aggregation (Zubay et al., 2006). They are biosynthesised from C20
267 polyunsaturated fatty acids, and therefore the abundance of eicosadienoic acid in *Cistus*
268 *ladanifer* leaves support its popular use as anti-inflammatory (Attaguile et al., 2000).
269 Overall, the combination of the useful phytochemicals found in the analysed aromatic
270 plants with the precious contribution of tocopherols, ascorbic acid and reducing sugars
271 make them a possible source of compounds to be used as remedies for diseases related
272 to oxidative stress, or dermatological applications, as also for cosmetics. The
273 polyunsaturated fatty acids including omega-3 and omega-6 families detected in the

274 plants constitute another important class of phytochemicals due to their generalised
275 beneficial health effects.
276
277 **Acknowledgements**
278 The authors are grateful to the Foundation for Science and Technology (Portugal) for
279 financial support to the research centre CIMO and L. Barros grant
280 (SFRH/BPD/4609/2008).
281
282 **References**
283 Adams, R.P., Zanoni, T.A., Lara, A., Barrero, A.F., Coll, L.G., 1997. Comparisons
284 among *Cupressus arizonica* Greene, *C. benthamii* Endl., *C. lindleyi* Klotz. ex Endl.
285 and *C. lusitanica* Mill. using leaf essential oils and DNA fingerprinting. J.
286 Essential Oil Res. 9, 303–309.
287 Ames B.N., Gold L.S., 1991. Endogenous mutagens and the causes of aging and cancer.
288 Mutat. Res. 250, 3-16.
289 Attaguile, L.G., Russo, A., Campisi, A., Savoca, F., Acquaviva, R., Ragusa, N., Vanella,
290 A., 2000. Antioxidant activity and protective effect on DNA cleavage of extracts
291 from *Cistus incanus* L. and *Cistus monspeliensis* L. Cell Biol. Toxicol. 16, 83-90.
292 Castroviejo S. (coord.), 1998. Flora Iberica, Vol I, 4ª reimpresión. Madrid: Real Jardín
293 Botánico, CSIC.
294 Castroviejo S. (coord.), 2005. Flora Iberica, Vol III, 2ª editión. Madrid: Real Jardín
295 Botánico, CSIC.
296 Castroviejo S. (coord.), 2007. Flora Iberica, Vol VIII, 3ª reimpresión. Madrid: Real
297 Jardín Botánico, CSIC.

298 Danne, A., Petereit, F., Nahrstedt, A., 1994. Flavan-3-ols, prodelphinidins and further
299 polyphenols from *Cistus salvifolius*. Phytochemistry 37, 533-538.
300 Darmstadt, G.L., Mao-Qiang, M., Chi, E., Saha, S.K., Ziboh, V.A., Black, R.E.,
301 Santosham, M., Elias, P.M., 2002. Impact of topical oils on the skin barrier:
302 possible implications for neonatal health in developing countries. Acta Paediatrica
303 91, 546–554.
304 DeFelice, S.L., 1992. The nutraceutical initiative: a recommendation for U.S. Economic
305 and regulatory reforms. Gen. Eng. News 12, 13–15.
306 Halliwell, B., 1996. Antioxidants in human health and disease. Annu. Rev. Nutr. 16, 33-
307 50.
308 Kuiate, J.R., Bessière, J.M., Zollo, P.H.A., Kuate, S.P., 2006. Chemical composition and
309 antidermatophytic properties of volatile fractions of hexanic extract from leaves of
310 *Cupressus lusitanica* Mill. from Cameroon. J. Ethnopharmacol. 103, 160-165.
311 Lassak, E.V., McCarthy, T., 1983. In Australian Medicinal Plants; North Ryde, NSW,
312 Reed New Holland.
313 Letawe, C., Boone, M., Pierard, G.E., 1998. Digital image analysis of the effect of
314 topically applied linoleic acid on acne microcomedones. Clin. Exp. Dermatol. 23,
315 56–58.
316 Li, Y., Schellhorn, H.E., 2007. New developments and novel therapeutic perspectives for
317 vitamin C. J. Nutr. 137, 2171–2184.
318 Liu, R.H., 2004. Potential synergy of phytochemicals in cancer prevention: mechanism
319 of action. J. Nutr. 134, 3479S–3485S.

320 Liu, D., Shi, J., Ibarra, A.C., Kakuda, Y., Xue, S.J., 2008. The scavenging capacity and
321 synergistic effects of lycopene, vitamin E, vitamin C, and b-carotene mixtures on
322 the DPPH free radical. LWT 41, 1344 – 1349.
323 Soobrattee, M.A., Neergheen, V.S., Luximon-Ramma, A., Aruoma, O.I., Bahorun, T.,
324 2005. Phenolics as potential antioxidant therapeutic agents: mechanism and
325 actions. Mut. Res. 579, 200–213.
326 Teixeira, S., Mendes, A., Alves, A., Santos, L., 2007. Simultaneous distillation-
327 extraction of high volatile compounds from *Cistus ladanifer* L. Anal. Chim. Acta
328 584, 439-446.
329 Tene, V., Malagón, O., Vita Finzi, P., Vidari, G. Armijos, C., Zaragoza, T., 2007. An
330 ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe,
331 Ecuador. J. Ethnopharmacol. 111, 63–81.
332 Vázquez, G., González-Alvarez, J., Santos, J., Freire, M.S., Antorrena, G., 2009.
333 Evaluation of potential applications for chestnut (*Castanea sativa*) shell and
334 eucalyptus (*Eucalyptus globulus*) bark extracts. Ind. Crop. Prod. 29, 364-370.
335 William, E. Connor, 2000. Importance of n−3 fatty acids in health and disease. Am. J.
336 Clin. Nutr. 71, 171S–175S.
337 Zubay, G. (2006). Biochemistry, 5th edition, Wm. C. Brown Publishers.
338

339 **Table 1.** Tocopherols and ascorbic acid composition (μg/g dry weight) of the aromatic
340 plants (mean ± SD; n=3).
341

	Cistus ladanifer	*Cupressus lusitanica*	*Eucalyptus gunnii*
α-tocopherol	68.15 ± 1.28 c	115.04 ± 3.65 b	1362.36 ± 7.84 a
β-tocopherol	1.02 ± 0.12 c	9.41 ± 0.16 b	31.29 ± 0.31 a
γ-tocopherol	7.73 ± 0.31 b	81.09 ± 1.17 a	83.46 ± 2.79 a
δ-tocopherol	*nd*	*nd*	81.16 ± 3.86
Total tocopherols	76.90 ± 1.32 c	205.54 ± 2.98 b	1558.27 ± 11.22 a
Ascorbic acid	647.64 ± 8.90 a	431.70 ± 7.55 b	339.16 ± 8.91 c

342 *nd*- not detected. In each row different letters mean significant differences ($p<0.05$).
343

Table 2. Sugars composition (mg/g of dry weight) of the aromatic plants (mean ± SD; n=3).

	Cistus ladanifer	*Cupressus lusitanica*	*Eucalyptus gunnii*
Fructose	48.21 ± 0.56 a	40.32 ± 0.34 b	5.45 ± 0.11 c
Glucose	26.00 ± 0.57 a	11.90 ± 1.19 b	5.33 ± 0.28 c
Sucrose	2.23 ± 0.35 c	27.90 ± 1.08 b	48.48 ± 1.99 a
Raffinose	1.07 ± 0.57 b	2.85 ± 0.33 a	2.65 ± 0.07 a
Total sugars	77.51 ± 1.04 b	82.96 ± 1.51 a	61.92 ± 2.15 c

In each row, different letters mean significant differences (p<0.05).

356 **Table 3.** Fatty acids composition of the aromatic plants (mean ± SD; n=3).
357

	Cistus ladanifer	*Cupressus lusitanica*	*Eucalyptus gunnii*
C8:0	0.10 ± 0.01	0.12 ± 0.00	0.10 ± 0.01
C11:0	0.09 ± 0.01	0.04 ± 0.00	0.17 ± 0.02
C12:0	0.10 ± 0.00	0.31 ± 0.00	2.37 ± 0.18
C14:0	0.71 ± 0.05	4.50 ± 0.55	2.31 ± 0.07
C14:1	*nd*	0.35 ± 0.01	0.23 ± 0.02
C15:0	0.02 ± 0.00	0.13 ± 0.02	0.27 ± 0.02
C16:0	1.93 ± 0.04	12.39 ± 0.48	12.57 ± 0.03
C16:1	0.03 ± 0.00	0.09 ± 0.01	*nd*
C17:0	0.10 ± 0.00	0.42 ± 0.01	4.99 ± 0.04
C17:1c	*nd*	0.03 ± 0.00	0.56 ± 0.03
C18:0	3.93 ± 0.03	3.25 ± 0.18	0.95 ± 0.02
C18:1n9c	0.95 ± 0.10	4.03 ± 0.12	4.61 ± 0.29
C18:2n6c	3.57 ± 0.23	12.75 ± 0.36	18.03 ± 0.30
C18:3n6	12.63 ± 2.91	*nd*	*nd*
C18:3n3	4.56 ± 0.06	42.11 ± 0.10	37.53 ± 0.93
C20:0	16.42 ± 0.33	6.19 ± 0.66	1.16 ± 0.01
C20:1c	*nd*	0.69 ± 0.09	*nd*
C20:2c	40.63 ± 2.21	0.28 ± 0.02	0.37 ± 0.02
C20:3n6	*nd*	*nd*	0.24 ± 0.00
C20:4n6	*nd*	*nd*	0.35 ± 0.05
C22:0	3.40 ± 0.13	3.39 ± 0.40	2.89 ± 0.11
C22:2c	*nd*	1.76 ± 0.17	1.89 ± 0.21
C23:0	9.65 ± 0.38	1.48 ± 0.00	1.61 ± 0.12
C24:0	1.17 ± 0.06	5.69 ± 0.26	6.81 ± 0.10
Total SFA	37.63 ± 0.89 a	37.91 ± 0.44 a	36.18 ± 0.77 b
Total MUFA	0.98 ± 0.02 b	5.19 ± 0.43 a	5.40 ± 0.27 a
Total PUFA	61.39 ± 0.99 a	56.90 ± 0.01 c	58.41 ± 0.50 b

358 *nd*- not detected. In each column different letters mean significant differences (p<0.05).
359 Caprylic acid (C8:0); Undecanoic acid (C11:0); Lauric acid (C12:0); Myristic acid
360 (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid (C15:0); Palmitic acid (C16:0);
361 Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); *cis*-10Heptadecenoic acid
362 (C17:1c); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); γ-
363 Linolenic acid (C18:3n6); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0);

Eicosenoic acid (C20:1c); *cis*-11,14-Eicosadienoic acid (C20:2c); *cis*-8,11,14-Eicosatrienoic acid (C20:3n6); Arachidonic acid (C20:4n6); Behenic acid (C22:0); *cis*-13,16-docosadienoic acid (C22:2c); Tricosanoic acid (C23:0); Lignoceric acid (C24:0).