



**HAL**  
open science

## **Analysis of individual anthocyanins, flavanols, flavonols and other polyphenols in *Pistacia lentiscus* L. fruits during ripening**

Aghiles Karim Aissat, Nassima Chaher-Bazizi, Tristan Richard, Dina Kilani-Atmani, Eric Pedrot, Elodie Renouf, Djebbar Atmani, Josep Valls Fonayet

### ► To cite this version:

Aghiles Karim Aissat, Nassima Chaher-Bazizi, Tristan Richard, Dina Kilani-Atmani, Eric Pedrot, et al.. Analysis of individual anthocyanins, flavanols, flavonols and other polyphenols in *Pistacia lentiscus* L. fruits during ripening. *Journal of Food Composition and Analysis*, 2022, 106, pp.104286. 10.1016/j.jfca.2021.104286 . hal-03699495

**HAL Id: hal-03699495**

**<https://hal.inrae.fr/hal-03699495>**

Submitted on 5 Jan 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Analysis of individual anthocyanins, flavanols, flavonols and other**  
2 **polyphenols in *Pistacia lentiscus* L. fruits during ripening**

3  
4

5 Aghiles Karim AISSAT<sup>1</sup>, Nassima CHAHER-BAZIZI<sup>1</sup>, Tristan RICHARD<sup>2</sup>, Dina KILANI-  
6 ATMANI<sup>1</sup>, Eric PEDROT<sup>2</sup>, Elodie RENOUF<sup>2</sup>, Djebbar ATMANI<sup>1,\*</sup>, Josep VALLS  
7 FONAYET<sup>2,\*</sup>

8  
9  
10  
11  
12

13 <sup>1</sup> *Laboratoire de Biochimie Appliquée, Faculté des Sciences de la Nature et de la Vie,*  
14 *Université de Bejaia, 06000, Algeria*

15  
16  
17  
18

<sup>2</sup> *Université de Bordeaux, UR Œnologie, MIB, EA 4577, USC 1366 INRAE, Bordeaux INP,*  
*ISVV, F-33140 Villenave d'Ornon, France. Bordeaux Metabolome, MetaboHUB, 33140*  
*Villenave d'Ornon, France*

19  
20  
21  
22  
23  
24  
25  
26  
27

28 \* Authors to whom correspondence should be addressed; e-mail:

29 Josep Valls-Fonayet: [josep.valls-fonayet@u-bordeaux.fr](mailto:josep.valls-fonayet@u-bordeaux.fr)

30 Djebbar Atmani: [djebbar.atmani@univ-bejaia.dz](mailto:djebbar.atmani@univ-bejaia.dz)

31

1 **Analysis of individual anthocyanins, flavanols, flavonols and other**  
2 **polyphenols in *Pistacia lentiscus* L. fruits during ripening**

3  
4  
5 **Abstract**

6 *Pistacia lentiscus* L. is a shrub of the Anacardiaceae family whose fruits are used in Tunisian  
7 and Algerian diets. The phenolic composition at 5 different physiological stages of the fruit  
8 was investigated using two different targeted methodologies: ultra-high-performance liquid  
9 chromatography–UV/visible detection (UHPLC-UV/vis) for anthocyanins and UHPLC  
10 coupled with tandem mass spectrometry (UHPLC-MS/MS) for the other polyphenols. For the  
11 specific analysis of anthocyanins, compound identification was confirmed by UHPLC-  
12 MS/MS and LC-NMR analysis. This study revealed the identification of 30 phenolic  
13 compounds including 9 anthocyanins, 7 flavanols, 7 flavonols, 2 phenolic acids, 1 stilbene, 2  
14 flavanones, 1 flavanonol and 1 dihydrochalcone. Quantification showed significant qualitative  
15 and quantitative variation in phenolic content during the ripening of *P. lentiscus* fruits,  
16 flavonols being the main compounds for the unripe berries and anthocyanins for ripe berries.  
17 To the best of our knowledge, our study reports the presence of piceid and protocatechuic acid  
18 in *P. lentiscus* L. fruits, as well as several anthocyanins in *Pistacia*, for the first time. The  
19 results indicate potential applications of *P. lentiscus* L. fruits as a source of phenolic  
20 compounds to be used as nutraceuticals and as food colorants.

21  
22  
23  
24  
25  
26  
27  
28 **Key words:** targeted metabolomics, *Pistacia lentiscus* L.fruits, ripening, HPLC-MS/MS, NMR  
29

## 30 **1. Introduction**

31 The genus *Pistacia*, which belongs to the Anacardiaceae family, is divided into eleven species  
32 and is largely distributed and cultivated from the Mediterranean basin to Central Asia (Milia  
33 et al., 2021). Among these species, *P. lentiscus* is a wild and cultivated species, known for its  
34 aromatic natural resin (Pachi et al., 2020), traditionally used as food by the population of the  
35 Mediterranean region. The resin is used as a mastic gum and also as flavouring in bread  
36 preparation and rice dough (Burešová et al., 2017). Fruits are commonly eaten, either raw or  
37 roasted. Moreover, the fruit oil represents a source of vegetable oils traditionally consumed in  
38 Tunisian and Algerian diets (Trabelsi et al., 2012; Mezni et al., 2016; Yosr et al., 2018; Milia  
39 et al., 2021). In addition, fruits and other aerial parts of *P. lentiscus* are used in folk medicine  
40 (Bozorgi et al., 2013; Milia et al., 2021). Interesting pharmacological properties of the plant  
41 including antioxidant (Atmani et al., 2009), anti-diabetic (Mehenni et al., 2016), anti-tumor  
42 (Remila et al., 2015), and anti-microbial (Mezni et al., 2015) effects have been described in  
43 different parts of the plant such as leaves and fruit extracts (Milia et al., 2021).

44 Even if the majority of studies have been focused on fruit oil (Mezni et al., 2016; Trabelsi et  
45 al., 2012) and essential oil composition (Ben Khedir et al., 2016; Yosr et al., 2018), a few  
46 investigations indicated that *P. lentiscus* hydro-alcoholic fruit extracts constitute a rich source  
47 of phenolic compounds including phenolic acids, flavanols, flavonols and anthocyanins (Elez  
48 Garofulić et al., 2020; Longo et al., 2007).

49 The chemical and biological properties of *P. lentiscus* fruits can be affected by the  
50 development stage, as reported for many other berries (Benbouguerra et al., 2021b). In fact,  
51 the influence of fruit maturity on polyphenol and tocopherol constituents of *P. vera* kernels, a  
52 closely related species to *P. lentiscus*, has already been reported (Ballistreri et al., 2009). In  
53 addition, changes in the sterol and lipid composition with the ripening of fruits has been  
54 reported (Trabelsi et al., 2012). Therefore, harvesting time could be an important factor  
55 affecting food quality and the potential use of the fruits. To our knowledge, studies describing  
56 the evolution of the phenolic composition of *P. lentiscus* fruits during ripening are scarce.

57 Hence, the present study was focused on the characterization of the phenolic composition of  
58 *P. lentiscus* fruits during the developing stages. Phenolic content was analysed by a  
59 combination of LC-NMR and UHPLC-MS experiments to provide a detailed polyphenols  
60 description in the fruit, which could assist in the identification of potential nutraceutical  
61 applications from *P. lentiscus* fruits and extracts.

## 62 **2. Materials and methods**

### 63 **2.1 Chemicals and reagents**

64 Methanol (laboratory and UHPLC grades), Hexane (laboratory grade), Formic acid LC-MS  
65 grade (> 99%) were purchased from Fisher Scientific (France). Acetonitrile (HPLC and  
66 UHPLC grades) was obtained from VWR Chemicals (United Kingdom). 4-hydroxybenzoic  
67 acid and protocatechuic acid were purchased from Sigma-Aldrich (France). Quercetin,  
68 quercetin-3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucuronide, quercetin 3-*O*-  
69 *O*-rhamnoside, quercetin 3-*O*-rutinoside, myricetin, catechin, epicatechin gallate,  
70 gallic acid, procyanidin B1, naringenin, naringenin 7-*O*-glucoside, taxifolin, *trans*-piceid,  
71 epigallocatechin gallate, delphinidin 3-*O*-glucoside and cyanidin 3-*O*-glucoside were  
72 purchased from Extrasynthese (France). MilliQ water was obtained with a Millipore system.

73

### 74 **2.2 Plant material and extract preparation**

75 *P. lentiscus* fruits were harvested from the forest of Tizi Neftah province of Amizour, Bejaia,  
76 Algeria (GPS coordinates 36.644°N and 4.921°E). The botanical identification was confirmed  
77 with support from the Laboratory of Botany, University of Bejaia (Algeria), according to a  
78 voucher herbarium specimen (N° 970704) deposited at the National Institute of Agronomy,  
79 Algiers, Algeria. The stage of maturity was determined by fruit colour and harvest month.  
80 The fruits were air-dried at 30°C in the dark and then crushed with an electric grinder and  
81 finally, defatted thrice with hexane (5 g: 40 mL). The defatted powder was then mixed with  
82 100% methanol (5 g: 40 mL). Five extraction cycles were carried out in an ultrasonic bath for  
83 10 min. The extracts were dried in a rotatory evaporator and solubilized in methanol/water  
84 (1:1, v/v) to obtain a final concentration equivalent to 5 g of dried fruit in 10 ml of solvent,  
85 and then they were stored at – 20°C until analysis.

86

87

### 88 **2.3 Individual compound analysis**

#### 89 **2.3.1 Anthocyanins analysis**

90 Individual anthocyanin identification was performed by a combination of HPLC-MS and  
91 HPLC-NMR analysis following the method developed by Acevedo et al. (2012). For  
92 anthocyanin identification, the extract reconstituted in water was passed through an XAD  
93 column, cleaned with water, recovered using methanol as mobile phase and dried in a rotary  
94 vacuum evaporator. HPLC-NMR experiments were performed on a BRUKER AVANCE III

95 600 MHz spectrometer (Wissembourg, France) equipped with a  $^1\text{H}$ - $^{13}\text{C}$  inverse-detection  
96 flow probe.  $^1\text{H}$ -NMR spectra were obtained in stopped-flow mode. For 2D-NMR  
97 experiments, individual anthocyanins were collected after on-flow  $^1\text{H}$ -LC-NMR analysis onto  
98 a FOXY collector from Teledyne ISCO (Lincoln, USA), lyophilized and analyzed by using  
99 classical COSY and NOESY 2D-NMR experiments (Acevedo De la Cruz, Alexander et al.,  
100 2012).

101 Individual anthocyanin quantification was performed on a UHPLC-MWL-MS/MS system  
102 (Thermo Scientific, France) composed of an Accela 1250 system coupled to a TSQ Quantum  
103 Access Max triple quadrupole equipped with an H-ESI ion source. Anthocyanins were  
104 separated on a Zorbax SB-C18 column (2.1 x 100 mm, 1.8  $\mu\text{m}$ , Agilent) with 1  $\mu\text{L}$  of volume  
105 injection. Mobile phase A was deionized water containing 2.5% formic acid (v/v) and mobile  
106 phase B was methanol (UHPLC grade) containing 2.5% formic acid (v/v). The elution  
107 gradient was for solvent B: 2.5% (0-2 min); 15% (7 min); 23% (9.3 min); 55% (10 min); 95%  
108 (11-12,5 min); 2.5% (13-15 min). The temperature and the flow were fixed to 35°C and 0.4  
109 mL/min, respectively. The mass detector operated in the positive mode while the source  
110 parameters were as follows: Sheath gas 60 a.u.; Auxiliary Gas 20 a.u., Voltage 1500V,  
111 Vaporizer Temperature 450°C, Capillary Temperature 150 °C. All compounds were  
112 quantified using the MWL detector at 520 nm using a calibration curve built with Dp3glu  
113 (range 0.97-1000 mg/L). Results were expressed as mg delphinidin 3-*O*-glucoside (Dp3glu)  
114 equivalents per 100 g of dry weight fruits (mg DGE/100g DW). The limits of detection (LOD  
115 = 0.08 mg DGE/100g DW) and quantification (LOQ = 0,23 mg DGE/100g DW) were  
116 calculated as  $\text{LOD}=3.3 \times s/b$  and  $\text{LOQ}=10 \times s/b$ , where “b” is the slope of the curve and “s”  
117 the standard deviation of the signal determined as the residual standard deviation of the  
118 calibration line in the LOD region (Kruve et al., 2015).

119

### 120 **2.3.2 UHPLC-QqQ-MS/MS analysis of other phenolic compounds**

121 Individual phenolic acids and flavonoid compounds were quantified by a targeted UHPLC-  
122 QqQ-MS/MS approach according to a previously developed methodology (Loupit et al.,  
123 2020; Djemaa-Landri et al., 2020) using an Infinity UHPLC 1260 system coupled to a 6430  
124 triple quadrupole (QqQ) mass spectrometer (Agilent Technologies, France). Separation was  
125 achieved with an Agilent Poroshell 120 EC-C18 column (150 x 2.1 mm, 2.7  $\mu\text{m}$ ) thermostated  
126 at 40°C. Acidified water and acidified acetonitrile (both containing formic acid 0.1%, v/v)  
127 were used as solvents A and B, respectively. The elution parameters consisted of a 0.4

128 mL/min flow and a solvent B gradient as follows: 1-10% (0-4 min); 20% (12 min); 33% (13-  
129 16 min); 40% (21 min); 95% (22-24 min); 95% (25 min); 1% (28 min). Measurements were  
130 based on Multiple Reaction Monitoring (MRM) in positive or negative mode depending on  
131 the compounds (Supplementary Figure 1). Collision energies applied are shown in Table 2.  
132 Quantification was done by comparison with a calibration curve with standards in the range of  
133 0.03 to 15.4 mg/L. All compounds were quantified with their corresponding standard except  
134 procyanidin B3 and an unknown trimer, expressed as procyanidin B1 and C1, respectively.  
135 Calibration parameters can be found in Supplementary Table 1.

136

137

## 138 **2.4 Statistical analysis**

139 All results were expressed as mean  $\pm$  standard deviation ( $n \geq 3$ ). One-way analysis of variance  
140 (ANOVA) tests was performed using Origin.

141

142

## 143 **3. Results and discussion**

144 *Pistacia lentiscus* fruits were collected at 5 different stages of maturity (Figure 1): September  
145 2017 (less red unripe fruits, stage 1), October 2017 (red unripe fruits, stage 2), November  
146 2017 (Red unripe fruits, stage 3), December 2017 (majority black ripe fruits, stage 4), January  
147 2018 (black fully ripe fruits, stage 5).

148

### 149 **3.1. Identification of phenolic compounds**

#### 150 **3.1.1 Anthocyanins**

151 An UHPLC-UV/vis-MS/MS methodology was used to obtain more details of the individual  
152 anthocyanin composition of the fruits. Classic methods of anthocyanin analysis usually  
153 require a very low acidic pH to assure the presence of the flavylum cationic form, which  
154 facilitates a good separation of anthocyanins and is easily detected at red wavelengths (520  
155 nm). This low pH is commonly achieved by using a high percentage of formic acid (5%-10%)  
156 in the mobile phases. However, coupling with MS instruments usually requires lower  
157 concentrations of acid. After several tested gradients (data not shown) reducing the amount of  
158 acid, a method using 2.5% formic acid was established allowing a good separation leading to  
159 the detection of 9 major anthocyanin peaks (Figure 2). A series of different scan modes of  
160 tandem mass spectrometry was used to identify these compounds. First of all, a full MS scan

161 was applied to determine the molecular weight of the molecules. Afterwards, MS/MS  
162 fragmentation analysis was performed by using a product ion scan, which selectively isolated  
163 the parent ions in the first quadrupole, and fragmented them in the collision cell. The data  
164 obtained were consistent with cyanidin and delphinidin derivatives conjugated with sugars.  
165 The results were confirmed with a precursor ion scan, which screened the presence of  
166 precursors of cyanidin ( $m/z$ : 287) and delphinidin ( $m/z$ : 303), and by a neutral loss scan,  
167 which screened the presence of pentosides (-132 u loss), hexosides (-162 u loss), and  
168 dihexosides (-324 u loss). To complete the identification, compounds were analysed by  
169  $^1\text{H-LC-NMR}$  (Acevedo De la Cruz, Alexander et al., 2012). The identified compounds are  
170 listed in Table 1.

171 Peak 1 was identified as a delphinidin dihexoside. The full scan MS revealed a molecular ion  
172 at  $m/z$  627, while the MS/MS product ion scan showed the characteristic  $m/z$  303 of the  
173 delphinidin aglycon. The precursor ion scan was consistent with delphinidin and the neutral  
174 loss ion scan indicated the loss of two hexoses. Peak 2 was also identified as a delphinidin  
175 dihexoside. The full scan MS revealed a molecular ion at  $m/z$  627, while the MS/MS product  
176 scan showed the delphinidin aglycon at  $m/z$  303. For peaks 1 and 2,  $^1\text{H-LC-NMR}$  data  
177 confirmed the identification of delphinidin dihexoside; nevertheless, signal complexity  
178 precluded complete annotation. MS and NMR data of peak 3 were consistent with delphinidin  
179 3-*O*-galactoside (Table 1). MS spectra exhibited fragment ions at  $m/z$  465 and 303. 2D-NMR  
180 NOESY spectrum confirmed the position of the galactose moiety. Similarly, peak 4 was  
181 identified as delphinidin 3-*O*-glucoside, with further confirmation by coinjection with the  
182 pure standard. Based on MS and NMR data, peak 5 was identified as cyanidin 3-*O*-  
183 galactoside. This compound presented a parent ion at  $m/z$  449 and a fragment ion at 287  
184 (cyanidin aglycon). The position of the galactose was confirmed by a NOESY experiment.  
185 Peak 6 was attributed to delphinidin pentoside, presenting a molecular ion at  $m/z$  435 and a  
186 fragment ion at  $m/z$  303. Using MS, NMR data, and comparison with injection of a standard,  
187 peak 7 was identified as cyanidin 3-*O*-glucoside (Table 1). Peak 8 and peak 9 were attributed  
188 to cyanidin and delphinidin pentoside (molecular ions at  $m/z$  419 and 435, respectively), loss  
189 of pentoside moiety providing typical ions of cyanidin and delphinidin aglycones ( $m/z$  287  
190 and 303, respectively). Unfortunately, NMR spectra complexity precludes the complete  
191 identification of peaks 6, 8 and 9.

192 Regarding the growing interest in anthocyanins as natural dyes in foods, several studies have  
193 pointed out the richness of different plants in these compounds (Krga & Milenkovic, 2019).



194 However, few data have been reported for *P. lentiscus* fruits. So far, research carried out on  
195 anthocyanins in *P. lentiscus* fruits had unravelled the presence of just 3 compounds:  
196 delphinidin 3-*O*-glucoside, cyanidin 3-*O*-arabinoside and cyanidin 3-*O*-glucoside (Longo et  
197 al., 2007). Indeed, most studies on anthocyanins in the whole *Pistacia* genus only report 3  
198 anthocyanins (El Bishbishy et al., 2020; Erşan et al., 2016; Ojeda-Amador et al., 2019). In our  
199 study, the presence of 9 anthocyanins was observed: 3 cyanidin derivatives (cyanidin 3-*O*-  
200 galactoside, 3-*O*-glucoside and pentoside) and 6 delphinidin derivatives (2 delphinidin  
201 dihexosides, 2 delphinidin pentosides, delphinidin 3-*O*-galactoside and 3-*O*-glucoside).

202

### 203 **3.1.2 Other phenolic compounds**

204 Besides the anthocyanin profile, the phenolic composition of *P. lentiscus* fruit was analysed  
205 by UHPLC-QqQ mass spectrometry. The approach was based on a multiple reaction  
206 monitoring (MRM) method previously developed for the analysis of phenolic compounds in  
207 natural extracts (Gabaston et al., 2020; Loupit et al., 2020). The identification relied on the  
208 comparison of the retention time with the pure standard and the presence of at least 2  
209 fragment ions. To assure the good attribution of the chromatographic peaks, samples were  
210 also spiked with a solution of standards at a concentration of 2 mg/L. A total of 21  
211 compounds were retained as being identified in the different stages of maturation (Table 2)  
212 including 2 phenolic acids, 1 stilbene, 7 flavanols, 7 flavonols, 2 flavanones, 1 flavanonol and  
213 1 dihydrochalcone.

214 This study revealed the presence of 4-hydroxybenzoic acid and protocatechuic acid, the latter  
215 being detected for the first time in *P. lentiscus* fruit. Stilbenes, a family of polyphenols with  
216 interesting potential and found in several plants, particularly in grapevines (Benbouguerra et  
217 al., 2021a; Gabaston et al., 2020) were also screened using 9 different stilbene standards  
218 following the MRM methodology (Loupit et al., 2020), but only *trans*-piceid, a glucosidic  
219 derivative of *trans*-resveratrol, was detected unequivocally in the sample. Indeed, *trans*-piceid  
220 was detected for the first time in *P. lentiscus* fruit. Seven flavonols were detected, mainly  
221 quercetin derivatives (quercetin 3-*O*-rutinoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-  
222 glucuronide, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, quercetin) and myricetin.  
223 Our screening did not indicate the presence of kaempferol derivatives in *P. lentiscus* fruits.

224 In the case of flavanols, 7 compounds were detected including catechin, gallic catechin,  
225 procyanidin B1, epigallocatechin gallate and epicatechin gallate, procyanidin B3 and an  
226 unidentified trimer. As previously commented, all polyphenols were identified by the MS/MS

227 data and further confirmed by spiking with pure compounds, but because of lack of standards  
228 two compounds (procyanidin B3 and the unknown trimer) were tentatively identified by their  
229 MS/MS signal and retention time. Finally, 4 other phenolic compounds were detected  
230 including 2 flavanones (naringenin 7-*O*-glucoside and naringenin), 1 flavanonol (taxifolin)  
231 and 1 dihydrochalcone (phloretin).

232

### 233 **3.2 Phenolic content**

234 The individual content of the identified phenolic compounds in each maturity stage is  
235 reported in Table 3. A significant variation in phenolic content is observed during the ripening  
236 of *P. lentiscus* fruits, both in terms of quality and quantity. While the anthocyanin content  
237 increased during ripening, most other phenolic compounds presented the highest content at  
238 stage 1 (less red unripe fruits), followed by an overall decrease by half in the rest of the stages  
239 of maturity.

240 The presence of anthocyanins was observed at all stages but their content was significant only  
241 for the two last stages (majority black ripe and black fully ripe fruits). At these stages 4 and 5,  
242 anthocyanins reached levels of 1373 and 1273 mg/100 g DW respectively, becoming the main  
243 polyphenol constituents of the extract (53 and 59% of the total polyphenols content,  
244 respectively). The delphinidin derivatives were predominant in all cases reaching  $1038 \pm 30$   
245 (stage 4) and  $1116 \pm 20$  mg/100 g DW (stage 5), which represented 76 and 88% of total  
246 anthocyanins, respectively. In contrast to *P. lentiscus*, only cyanidin derivatives had been  
247 reported in *P. vera* (Bellomo & Fallico, 2007), with a similar increase of anthocyanin content  
248 during maturity (Ballistreri et al., 2009). Based on the results obtained in this study and those  
249 found in the literature, we can say that the ripe fruits of *P. lentiscus* represent a rich and  
250 interesting source of anthocyanins, in general, and in delphinidin derivatives in particular.

251 While anthocyanins are the most abundant polyphenols in the ripe fruit, flavonols constitute  
252 the main phenolic family at the early stages of maturity (until stage 3), with levels of  $216 \pm 23$   
253 mg/100 g DW (representing 58% of the total polyphenol content) at stage 1 and  $108 \pm 5$   
254 mg/100 g DW (61% of the total polyphenols) at stage 3. Afterwards, their content decreased  
255 to just  $34 \pm 2$  mg/100 g DW at stage 5. Among the 9 flavonols determined, quercetin-*O*-  
256 galactoside was the predominant flavonol at all stages of maturity (except in stage 5), but  
257 decreasing from  $93 \pm 5$  mg/100 g DW (stage 1) to  $10 \pm 1$  mg/100 g DW. It was followed in  
258 terms of abundance by quercetin-*O*-glucoside, varying from  $64 \pm 8$  mg/100 g DW (stage 1) to  
259  $11 \pm 1$  mg/100 g DW (stage 5). Significant amounts of quercetin 3-*O*-glucuronide and

260 quercetin 3-*O*-rutinoside were also observed. The presence of quercetin derivatives had  
261 previously been reported in *P. lentiscus* fruits (Mehenni et al., 2016) and other *Pistacia*  
262 species such as *P. vera* (Romani et al., 2002) and *P. atlantica* (Khallouki et al., 2017). In  
263 agreement with our results, flavonol glycosides are considered the most abundant phenolic  
264 family in *P. lentiscus* fruits at the early fruiting stage (Elez Garofulić et al., 2020).  
265 In the case of flavanols, galliccatechin was the main compound detected, which is in  
266 agreement with other studies on *P. vera* (Ojeda-Amador et al., 2019). Levels of this monomer  
267 decreased substantially between stages 1 and 2, but then increased during fruit ripening to  
268 recover the initial values (over 100 mg/100 g DW). The monomer catechin was the second  
269 most abundant flavanol in all cases, varying from  $37 \pm 1$  mg/100 g DW (stage 1) to  
270  $18 \pm 1$  mg/100 g DW (stage 5). A phytochemical study carried out on *P. lentiscus* ripe fruits  
271 had also shown a high concentration of catechin (Mehenni et al., 2016). The amount of  
272 catechin decreases during ripening similar to the rest of flavanols, appearing to be stable or  
273 increase slightly at the latest stages. A study carried out on *P. vera* also showed a decrease of  
274 catechin concentration with fruit maturation (Kelebek et al., 2020), as observed in other fruits  
275 such grape berry (Benbouguerra et al., 2021b). Interestingly, levels of epicatechin and  
276 epigallocatechin were much lower than their isomers catechin and galliccatechin. Epicatechin  
277 was under the LOD for the three first stages and under the LOQ of the method (0.04 mg/100  
278 g) at the 4th and 5th stages. Epigallocatechin was under the LOD (0.02 mg/100 g) for all  
279 cases. The main procyanidin dimer was tentatively identified as B3, which is a catechin dimer  
280 (catechin-(4 $\alpha$ →8)-catechin), which seems to confirm the prevalence of catechin forms in *P.*  
281 *lentiscus* fruits over epicatechin forms. In fact, the other dimer identified, procyanidin B1, is  
282 an epicatechin-catechin dimer (epicatechin-(4 $\beta$ →8)-catechin), but it accounted for much  
283 lower amounts than procyanidin B3 and could not be quantified at the ripe stages. Levels of  
284 the epicatechin dimer B2 (epicatechin-(4 $\beta$ →8)-epicatechin) were not quantifiable at any stage  
285 (under the LOD of 0.1 mg/100 g), which is again in agreement with the low abundance of  
286 epicatechin forms over catechin in the fruit. Other flavanols such as epicatechin gallate and  
287 epigallocatechin gallate were observed in small amounts regardless of the stage of maturity.  
288 Previous observation of the prevalence of catechin forms seems to indicate that it cannot be  
289 ruled out that these minor forms are actually catechin gallate and galliccatechin gallate, since  
290 catechin gallate and epicatechin gallate share the same pattern of MS/MS fragmentation,  
291 which is also the case between galliccatechin gallate and epigallocatechin gallate.

292 Three other flavonoids were detected and quantified in *P. lentiscus* fruits: naringenin 7-*O*-  
293 glucoside and small amounts of naringenin and taxifolin. Previous studies have shown the  
294 presence of taxifolin in high quantities in the leaves of *P. lentiscus* (Vaya & Mahmood, 2006).  
295 Naringenin was also detected in minor quantities in *P. vera* hulls (Barreca et al., 2016).  
296 The present investigation also revealed the presence of 2 phenolic acids: 4-hydroxybenzoic  
297 acid and protocatechuic acid. The latter was detected for the first time as the main phenolic  
298 acid in *P. lentiscus* fruits. The highest concentration was observed at stage 1 ( $49 \pm 4$  mg/100 g  
299 DW) and reached its lowest level at stage 5 ( $2 \pm 1$  mg/100 g DW). In addition to phenolic  
300 acids, 2 other non-flavonoid compounds were identified and quantified: phloretin and piceid.  
301 Significant amounts of phloretin were observed in *P. lentiscus* fruits up to  $47 \pm 5$  mg/100 g  
302 DW at stage 1. Piceid was observed in small amounts in all stages. The presence of stilbenes  
303 in *Pistacia* genus was previously reported in *P. vera*: *trans*-resveratrol (Ballistreri et al., 2009;  
304 Tokuşoglu et al., 2005) and *trans*-piceid (*trans*-resveratrol-3-*O*- $\beta$ -glucoside) were found in *P.*  
305 *vera* peanuts (Grippi et al., 2008). However, to the best of our knowledge, this is the first time  
306 that piceid has been reported in *P. lentiscus*.

307

308 The variation in the content of phenolic compounds during maturation implies variations in  
309 therapeutic potential, nutritional benefits and industrial interest of *P. lentiscus* L. fruits.  
310 Several studies have previously highlighted the involvement of these fruits in the protection  
311 and/or prevention of several pathologies, indicating a correlation between total polyphenols  
312 and the antioxidant capacity of *P. lentiscus* fruits (Remila et al., 2015; Yemmen et al., 2017).  
313 Antidiabetic potential of ripe fruits of *P. lentiscus* via  $\alpha$ -amylase inhibitory capacities has also  
314 shown a positive correlation with the concentration of total phenolic compounds (Mehenni et  
315 al., 2016). However, most of these studies are based on colorimetric tests for determining the  
316 polyphenol content, which cannot be enough to fully understand the potential uses of *P.*  
317 *lentiscus* fruits.

318 Our results show that the black ripe fruits represent an excellent source of anthocyanins. This  
319 class of polyphenols have shown human health benefits linked to a decrease of inflammation  
320 and oxidative stress biomarkers and improved cardiovascular risk and metabolic diseases  
321 incidence (Ockermann et al., 2021). They have also shown neuroprotective potential effects  
322 (Ullah et al., 2019; Zhang et al., 2021), and daily consumption of anthocyanins improved the  
323 condition of subjects with diabetes (Fallah et al., 2020). Moreover, anthocyanins are widely  
324 exploited in the food industry as natural colorants and preservatives due to their antioxidant

325 potential (Echegaray et al., 2020). Our results indicate levels of anthocyanins around 1200-  
326 1300 mg/100 g DW for *P. lentiscus* fruits, which are in the same range of other fruits widely  
327 recognised as sources of these flavonoids. For example, in a study comparing the  
328 concentrations of anthocyanins in common foods by a similar HPLC-DAD methodology,  
329 blackberries reached levels of 300 mg/100 g FW, wild blueberries had 486 mg/100 g FW,  
330 black currant 476 mg/100 g FW, red grapes 120 mg/100 g FW, and strawberries 21 mg/100 g  
331 FW (Wu et al., 2006).

332 On the other hand, unripe *P. lentiscus* fruits can be a valuable source of other flavonoids,  
333 namely flavonols, whose remarkable health benefits are associated with their promising  
334 anticancer, antioxidant, antimicrobial and antiviral properties (Barreca et al., 2021). Our study  
335 reveals maximum amounts of 200 mg/100 g DW at the 1<sup>st</sup> stage of maturity. For comparison,  
336 yellow onions contain 27–119 mg of flavonols per 100 g FW, whereas red onions contain 41–  
337 192 mg of flavonols per 100 g FW (Slimestad et al., 2007). Comparing these values, the fruits  
338 of *P. lentiscus* could be an interesting source of these flavonoids. Unripe stages could also  
339 provide a significant amount of flavanols, mostly in the form of catechin gallate, although  
340 other sources such as tea leaves can provide a higher amount of these compounds. In fact,  
341 some tea species can reach in some cases over 200 mg total catechins per gram of tea leaves  
342 (DW), with epicatechin gallate as the major compound (Deka et al., 2021). Anyway, unripe *P.*  
343 *lentiscus* fruits could be used as an alternative source of flavonols and flavanols, but in that  
344 case, they should be better considered as sources for extraction of flavonoids to be formulated  
345 in nutraceuticals rather than being directly consumed as foods. Such a strategy has also been  
346 proposed for other unripe fruits whose green stages contain more polyphenols than the mature  
347 ones, and which are considered a very good raw material for the production of polyphenol-  
348 based nutraceuticals with high antioxidant potential (Wojdyło & Oszmiański, 2020). It is  
349 worth mentioning that in the case of preparing extracts for nutraceutical purposes, green *P.*  
350 *lentiscus* fruits could also be combined with other parts of the *P. lentiscus* plant such as  
351 leaves, which have also proven to contain high amounts of flavonols showing health benefits  
352 (Azib et al., 2019).

353

354

#### 355 **4. Conclusion**

356 Chemical analyses of the various extracts of *P. lentiscus* fruits during ripening led to the  
357 identification of 30 compounds, including 9 anthocyanins, detected and identified by a  
358 combination of UHPLC-UV/vis, UHPLC-MS/MS and LC-NMR methodologies. To the best  
359 of our knowledge, this is the most detailed study on the polyphenolic composition in *P.*  
360 *lentiscus* fruits, especially concerning the anthocyanin composition, indicating the presence of  
361 3 cyanidin derivatives and 6 delphinidin derivatives in the ripe stages of the fruit, with  
362 different glycoside moieties (glucosides, galactosides, dihexosides and pentosides). Among  
363 anthocyanins, delphinidin galactoside was the main compound, while quercetin galactoside  
364 and quercetin glucoside were the main flavonols detected and gallocatechin was the main  
365 flavanol. Protocatechuic acid and *trans*-piceid were quantified for the first time in *P. lentiscus*  
366 fruits.

367 The contents of each family of polyphenols were highly influenced by the fruit maturation  
368 stage. Flavonols were the main polyphenols at the beginning of the fruit ripening, but their  
369 levels decreased during the maturation. Flavanols followed a similar pattern, except for  
370 gallocatechin. On the opposite side, anthocyanins increased enormously in the last stages of  
371 ripening to become the main polyphenols in the mature fruit, followed by gallocatechin. The  
372 increase in anthocyanin content during ripening is relevant for using ripe *P. lentiscus* fruits as  
373 an important source of this kind of molecules in the diet or anthocyanin-based nutraceuticals  
374 and for food applications such as dye additives. Unripe fruits would be interesting as a source  
375 of other flavonoids to produce natural extracts enriched in flavonols and flavanols.

376

#### 377 **Acknowledgments**

378 This work was supported by the Bordeaux Metabolome Facility and MetaboHUB (ANR-11-  
379 INBS-0010 project), the algero-français program of doctoral fellowships (PROFAS B+) 2019  
380 and the DGRSDT (MESRS, Algeria, Grant D01N01UN060120180003).

381

382

#### 383 **5. Bibliography**

384

385 Acevedo De la Cruz, Alexander, Hilbert, G., Rivière, C., Mengin, V., Ollat, N., Bordenave, L., Decroocq,  
386 S., Delaunay, J.-C., Delrot, S., Mérillon, J.-M., Monti, J.-P., Gomès, E., & Richard, T. (2012).  
387 Anthocyanin identification and composition of wild *Vitis* spp. accessions by using LC-MS and LC-  
388 NMR. *Analytica chimica acta*, 732, 145–152.

389 Atmani, D., Chaher, N., Berboucha, M., Ayouni, K., Lounis, H., Boudaoud, H., Debbache, N., & Atmani,  
390 D. (2009). Antioxidant capacity and phenol content of selected Algerian medicinal plants. *Food*  
391 *Chemistry*, *112*, 303–309.

392 Azib, L., Debbache-Benaidia, N., Costa, G. D., Atmani-Kilani, D., Saidene, N., Ayouni, K., Richard, T., &  
393 Atmani, D. (2019). Pistacia lentiscus L. leaves extract and its major phenolic compounds reverse  
394 aluminium-induced neurotoxicity in mice. *Industrial Crops and Products*, *137*, 576–584.

395 Ballistreri, G., Arena, E., & Fallico, B. (2009). Influence of Ripeness and Drying Process on the  
396 Polyphenols and Tocopherols of Pistacia vera L. *Molecules*, *14*, 4358–4369.

397 Barreca, D., Laganà, G., Leuzzi, U., Smeriglio, A., Trombetta, D., & Bellocco, E. (2016). Evaluation of  
398 the nutraceutical, antioxidant and cytoprotective properties of ripe pistachio (Pistacia vera L.,  
399 variety Bronte) hulls. *Food Chemistry*, *196*, 493–502.

400 Barreca, D., Trombetta, D., Smeriglio, A., Mandalari, G., Romeo, O., Felice, M. R., Gattuso, G., &  
401 Nabavi, S. M. (2021). Food flavonols: Nutraceuticals with complex health benefits and  
402 functionalities. *Trends in Food Science & Technology*.

403 Bellomo, M. G., & Fallico, B. (2007). Anthocyanins, chlorophylls and xanthophylls in pistachio nuts  
404 (Pistacia vera) of different geographic origin. *Journal of Food Composition and Analysis*, *20*, 352–  
405 359.

406 Ben Khedir, S., Mzid, M., Bardaa, S., Moalla, D., Sahnoun, Z., & Rebai, T. (2016). In Vivo Evaluation of  
407 the Anti-Inflammatory Effect of Pistacia lentiscus Fruit Oil and Its Effects on Oxidative Stress.  
408 *Evidence-Based Complementary and Alternative Medicine: eCAM*, *2016*, 6108203.

409 Benbougerra, N., Hornedo-Ortega, R., Garcia, F., El Khawand, T., Saucier, C., & Richard, T. (2021a).  
410 Stilbenes in grape berries and wine and their potential role as anti-obesity agents: A review.  
411 *Trends in Food Science & Technology*, *112*, 362–381.

412 Benbougerra, N., Valls-Fonayet, J., Krisa, S., Garcia, F., Saucier, C., Richard, T., & Hornedo-Ortega, R.  
413 (2021b). Polyphenolic Characterization of Merlot, Tannat and Syrah Skin Extracts at Different  
414 Degrees of Maturity and Anti-Inflammatory Potential in RAW 264.7 Cells. *Foods*, *10*, 541.

415 Bozorgi, M., Memariani, Z., Mobli, M., Salehi Surmaghi, M. H., Shams-Ardekani, M. R., & Rahimi, R.  
416 (2013). Five *Pistacia* species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk* and *P. lentiscus*): A Review of Their  
417 Traditional Uses, Phytochemistry, and Pharmacology. *The Scientific World Journal*, *2013*, 1–33.

418 Burešová, I., Salek, R. N., Varga, E., Masaříková, L., & Bureš, D. (2017). The effect of Chios mastic gum  
419 addition on the characteristics of rice dough and bread. *LWT - Food Science and Technology*, *81*,  
420 299–305.

422 Deka, H., Barman, T., Dutta, J., Devi, A., Tamuly, P., Kumar Paul, R., & Karak, T. (2021). Catechin and  
423 caffeine content of tea (*Camellia sinensis* L.) leaf significantly differ with seasonal variation: A  
424 study on popular cultivars in North East India. *Journal of Food Composition and Analysis*, *96*,  
425 103684.

426 Djemaa-Landri, K., Hamri-Zeghichi, S., Valls, J., Cluzet, S., Tristan, R., Boulahbal, N., Kadri, N., &  
427 Madani, K. (2020). Phenolic content and antioxidant activities of *Vitis vinifera* L. leaf extracts  
428 obtained by conventional solvent and microwave-assisted extractions. *Journal of Food*  
429 *Measurement and Characterization*, *14*, 3551–3564.

430 Echegaray, N., Munekata, P. E. S., Gullón, P., Dzuovor, C. K. O., Gullón, B., Kubi, F., & Lorenzo, J. M.  
431 (2020). Recent advances in food products fortification with anthocyanins. *Critical Reviews in Food*  
432 *Science and Nutrition*, *0*, 1–15.

433 El Bishbishy, M. H., Gad, H. A., & Aborehab, N. M. (2020). Chemometric discrimination of three  
434 Pistacia species via their metabolic profiling and their possible in vitro effects on memory  
435 functions. *Journal of Pharmaceutical and Biomedical Analysis*, *177*, 112840.

436 Elez Garofulić, I., Kruk, V., Martić, A., Martić, I., Zorić, Z., Pedisić, S., Dragović, S., & Dragović-Uzelac,  
437 V. (2020). Evaluation of Polyphenolic Profile and Antioxidant Activity of Pistacia lentiscus L.  
438 Leaves and Fruit Extract Obtained by Optimized Microwave-Assisted Extraction. *Foods*, *9*, 1556.  
439 Erşan, S., Güçlü Üstündağ, Ö., Carle, R., & Schweiggert, R. M. (2016). Identification of Phenolic  
440 Compounds in Red and Green Pistachio (*Pistacia vera* L.) Hulls (Exo- and Mesocarp) by HPLC-DAD-  
441 ESI-(HR)-MS(n). *Journal of Agricultural and Food Chemistry*, *64*, 5334–5344.  
442 Fallah, A. A., Sarmast, E., & Jafari, T. (2020). Effect of dietary anthocyanins on biomarkers of glycemic  
443 control and glucose metabolism: A systematic review and meta-analysis of randomized clinical  
444 trials. *Food Research International*, *137*, 109379.  
445 Gabaston, J., Valls Fonayet, J., Franc, C., Waffo-Teguo, P., Revel, G. de, Hilbert, G., Gomès, E., Richard,  
446 T., & Mérillon, J.-M. (2020). Characterization of Stilbene Composition in Grape Berries from Wild  
447 Vitis Species in Year-To-Year Harvest. *Journal of Agricultural and Food Chemistry*, *68*, 13408–  
448 13417.  
449 Grippi, F., Crosta, L., Aiello, G., Tolomeo, M., Oliveri, F., Gebbia, N., & Curione, A. (2008).  
450 Determination of stilbenes in Sicilian pistachio by high-performance liquid chromatographic  
451 diode array (HPLC-DAD/FLD) and evaluation of eventually mycotoxin contamination. *Food*  
452 *Chemistry*, *107*, 483–488.  
453 Kelebek, H., Sonmezdag, A., Güçlü, G., Cengiz, N., Uzlasir, T., Kadiroglu, P., & Selli, S. (2020).  
454 Comparison of phenolic profile and some physicochemical properties of Uzun pistachios as  
455 influenced by different harvest period. *0145-8892*.  
456 Khallouki, F., Breuer, A., Merieme, E., Ulrich, C. M., & Owen, R. W. (2017). Characterization and  
457 quantitation of the polyphenolic compounds detected in methanol extracts of Pistacia atlantica  
458 Desf. fruits from the Guelmim region of Morocco. *Journal of Pharmaceutical and Biomedical*  
459 *Analysis*, *134*, 310–318.  
460 Krga, I., & Milenkovic, D. (2019). Anthocyanins: From Sources and Bioavailability to Cardiovascular-  
461 Health Benefits and Molecular Mechanisms of Action. *Journal of Agricultural and Food Chemistry*,  
462 *67*, 1771–1783.  
463 Kruve, A., Rebane, R., Kipper, K., Oldekop, M.-L., Evard, H., Herodes, K., Ravio, P., & Leito, I. (2015).  
464 Tutorial review on validation of liquid chromatography-mass spectrometry methods: part I.  
465 *Analytica chimica acta*, *870*, 29–44.  
466 Longo, L., Scardino, A., & Vasapollo, G. (2007). Identification and quantification of anthocyanins in  
467 the berries of Pistacia lentiscus L., Phillyrea latifolia L. and Rubia peregrina L. *Innovative Food*  
468 *Science & Emerging Technologies*, *8*, 360–364.  
469 Loupit, G., Prigent, S., Franc, C., Revel, G. de, Richard, T., Cookson, S. J., & Fonayet, J. V. (2020).  
470 Polyphenol Profiles of Just Pruned Grapevine Canes from Wild Vitis Accessions and Vitis vinifera  
471 Cultivars. *Journal of Agricultural and Food Chemistry*, *68*, 13397–13407.  
472 Mehenni, C., Atmani-Kilani, D., Dumarçay, S., Perrin, D., Gérardin, P., & Atmani, D. (2016).  
473 Hepatoprotective and antidiabetic effects of Pistacia lentiscus leaf and fruit extracts. *Journal of*  
474 *Food and Drug Analysis*, *24*, 653–669.  
475 Mezni, F., Aouadhi, C., Khouja, M. L., Khaldi, A., & Maaroufi, A. (2015). In vitro antimicrobial activity  
476 of Pistacia lentiscus L. edible oil and phenolic extract. *Natural product research*, *29*, 565–570.  
477 Mezni, F., Labidi, A., Khouja, M. L., Martine, L., Berdeaux, O., & Khaldi, A. (2016). Diversity of Sterol  
478 Composition in Tunisian Pistacia lentiscus Seed Oil. *Chemistry & Biodiversity*, *13*, 544–548.  
479 Milia, E., Bullitta, S. M., Mastandrea, G., Szoťáková, B., Schoubben, A., Langhansová, L., Quartu, M.,  
480 Bortone, A., & Eick, S. (2021). Leaves and Fruits Preparations of Pistacia lentiscus L.: A Review on  
481 the Ethnopharmacological Uses and Implications in Inflammation and Infection. *Antibiotics*, *10*,  
482 425.



483 Ockermann, P., Headley, L., Lizio, R., & Hansmann, J. (2021). A Review of the Properties of  
484 Anthocyanins and Their Influence on Factors Affecting Cardiometabolic and Cognitive Health.  
485 *Nutrients*, *13*.

486 Ojeda-Amador, R. M., Salvador, M. D., Fregapane, G., & Gómez-Alonso, S. (2019). Comprehensive  
487 Study of the Phenolic Compound Profile and Antioxidant Activity of Eight Pistachio Cultivars and  
488 Their Residual Cakes and Virgin Oils. *Journal of Agricultural and Food Chemistry*, *67*, 3583–3594.

489 Pachi, V. K., Mikropoulou, E. V., Gkiouvetidis, P., Siafakas, K., Argyropoulou, A., Angelis, A., Mitakou,  
490 S., & Halabalaki, M. (2020). Traditional uses, phytochemistry and pharmacology of Chios mastic  
491 gum (*Pistacia lentiscus* var. Chia, Anacardiaceae): A review. *Journal of Ethnopharmacology*, *254*,  
492 112485.

493 Remila, S., Atmani-Kilani, D., Delemasure, S., Connat, J.-L., Azib, L., Richard, T., & Atmani, D. (2015).  
494 Antioxidant, cytoprotective, anti-inflammatory and anticancer activities of *Pistacia lentiscus*  
495 (*Anacardiaceae*) leaf and fruit extracts. *European Journal of Integrative Medicine*, *7*, 274–286.

496 Romani, A., Pinelli, P., Galardi, C., Mulinacci, N., & Tattini, M. (2002). Identification and quantification  
497 of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of *Pistacia lentiscus* L.  
498 *Phytochemical analysis: PCA*, *13*, 79–86.

499 Slimestad, R., Fossen, T., & Vågen, I. M. (2007). Onions: a source of unique dietary flavonoids. *Journal*  
500 *of agricultural and food chemistry*, *55*, 10067–10080.

501 Tokuşoglu, O., Unal, M. K., & Yemiş, F. (2005). Determination of the phytoalexin resveratrol (3,5,4'-  
502 trihydroxystilbene) in peanuts and pistachios by high-performance liquid chromatographic diode  
503 array (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS). *Journal of agricultural*  
504 *and food chemistry*, *53*, 5003–5009.

505 Trabelsi, H., Cherif, O. A., Sakouhi, F., Villeneuve, P., Renaud, J., Barouh, N., Boukhchina, S., & Mayer,  
506 P. (2012). Total lipid content, fatty acids and 4-desmethylsterols accumulation in developing fruit  
507 of *Pistacia lentiscus* L. growing wild in Tunisia. *Food Chemistry*, *131*, 434–440.

508 Ullah, R., Khan, M., Shah, S. A., Saeed, K., & Kim, M. O. (2019). Natural Antioxidant Anthocyanins—A  
509 Hidden Therapeutic Candidate in Metabolic Disorders with Major Focus in Neurodegeneration.  
510 *Nutrients*, *11*, 1195.

511 Vaya, J., & Mahmood, S. (2006). Flavonoid content in leaf extracts of the fig (*Ficus carica* L.), carob  
512 (*Ceratonia siliqua* L.) and pistachio (*Pistacia lentiscus* L.). *BioFactors*, *28*, 169–175.

513 Wojdyło, A., & Oszmiański, J. (2020). Antioxidant Activity Modulated by Polyphenol Contents in Apple  
514 and Leaves during Fruit Development and Ripening. *Antioxidants*, *9*, 567.

515 Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2006).  
516 Concentrations of anthocyanins in common foods in the United States and estimation of normal  
517 consumption. *Journal of agricultural and food chemistry*, *54*, 4069–4075.

518 Yemmen, M., Landolsi, A., Hamida, J. B., Mégraud, F., & Ayadi, M. T. (2017). Antioxidant activities,  
519 anticancer activity and polyphenolics profile, of leaf, fruit and stem extracts of *Pistacia lentiscus*  
520 from Tunisia. *Cellular and Molecular Biology*, *63*, 87–95.

521 Yosr, Z., Imen, B. H. Y., Rym, J., Chokri, M., & Mohamed, B. (2018). Sex-related differences in essential  
522 oil composition, phenol contents and antioxidant activity of aerial parts in *Pistacia lentiscus* L.  
523 during seasons. *Industrial Crops and Products*, *121*, 151–159.

524 Zhang, Y., Yin, L., Huang, L., Tekliye, M., Xia, X., Li, J., & Dong, M. (2021). Composition, antioxidant  
525 activity, and neuroprotective effects of anthocyanin-rich extract from purple highland barley bran  
526 and its promotion on autophagy. *Food Chemistry*, *339*, 127849.

527

528

529 **Figure captions**

530 **Figure 1.** Images of *Pistacia lentiscus* L. fruits collected at different dates corresponding to  
531 different stages of maturity

532

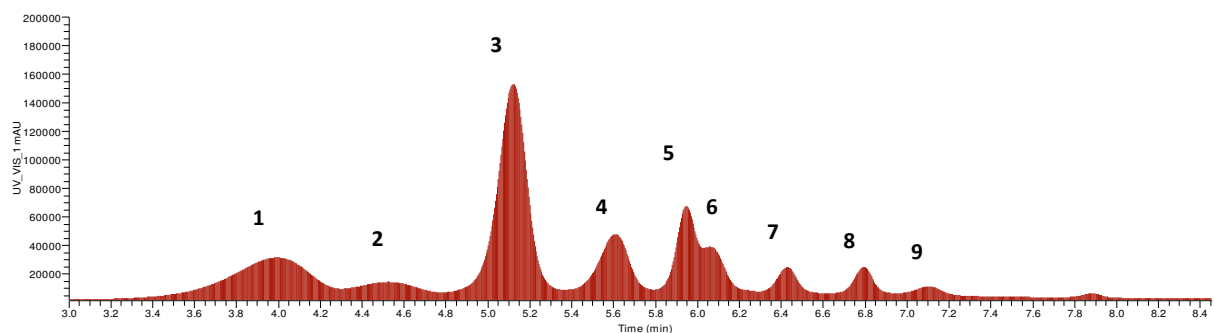


533

534

535

536 **Figure 2.** UHPLC-DAD chromatogram of *Pistacia lentiscus* anthocyanins. **1:** Dp dihex (1); **2:**  
537 Dp dihex (2); **3:** Dp 3-O-gal; **4:** Dp 3-O-glu; **5:** Cy 3-O-gal; **6:** Dp pent; **7:** Cy 3-O-glu; **8:** Cy  
538 pent; **9:** Dp pent. *Dp*=dephinidin; *Cy*=Cyanidin; *glu*=glucoside; *gal*=galactoside;  
539 *hex*=hexoside; *pent*=pentoside; *dihex*=dihexoside



540


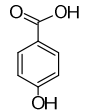
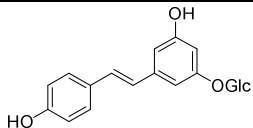
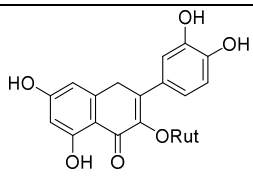
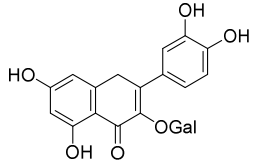
1 **Table 1.** UHPLC-QqQ-MS data (peak number, retention time,  $m/z$  values in positive mode) and LC-NMR data of *P. lentiscus* fruit anthocyanins.

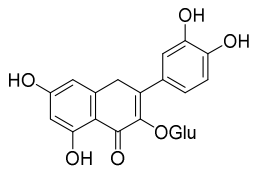
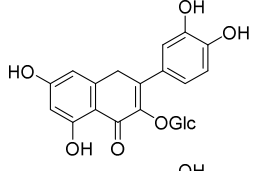
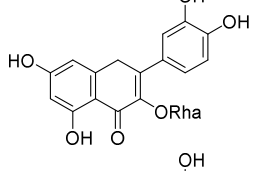
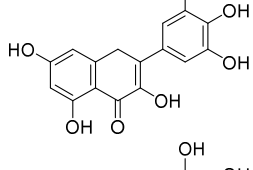
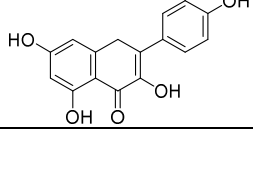
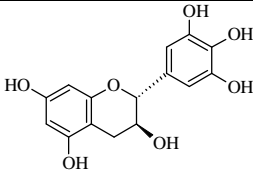
Peak	Compound	$t_R$ (min)	$MH^+$	$MS^2$	$^1H$ -NMR
1	delphinidin dihexoside 1	3.89	627	303	-
2	delphinidin dihexoside 2	4.48	627	303	-
3	delphinidin 3- <i>O</i> -galactoside	5.01	465	303	9.05 (1H, s, H-4), 7.87 (2H, s, H-2'/6'), 6.95 (1H, brs, H-8), 6.73 (1H, d, $J = 2$ Hz, H-6), 5.34 (1H, d, $J = 8$ Hz, H-1''), 4.10 (1H, dd, $J = 8$ and 10 Hz, H-2''), 4.05 (1H, brd, $J = 3$ Hz, H-4''), 3.90-3.84 (3H, H-5'', H-6a'', H-6b''), 3.78 (1H, dd, $J = 3$ and 10 Hz, H-3'')
4	delphinidin 3- <i>O</i> -glucoside	5.49	465	303	9.04 (1H, s, H-4), 7.85 (2H, s, H-2'/6'), 6.97 (1H, brs, H-8), 6.73 (1H, d, $J = 2$ Hz, H-6), 5.40 (1H, d, $J = 8$ Hz, H-1''), 3.98 (1H, dd, $J = 2$ and 12 Hz, H-6b''), 3.75 (1H, dd, $J = 6$ and 12 Hz, H-6a''), 3.70-3.50 (4H, H-2'', H-3'', H-4'', H-5'')
5	cyanidin 3- <i>O</i> -galactoside	5.84	449	287	9.03 (1H, s, H-4), 8.27 (1H, dd, $J = 2$ and 8 Hz, H-6'), 8.08 (1H, d, $J = 2$ Hz, H-2'), 7.02 (1H, d, $J = 8$ Hz, H-5'), 6.65 (1H, d, $J = 2$ Hz, H-6), 5.26 (1H, d, $J = 8$ Hz, H-1''), 3.98 (1H, dd, $J = 8$ and 10 Hz, H-2''), 3.94 (1H, brd, $J = 3$ Hz, H-4''), 3.85-3.75 (3H, H-5'', H-6a'', H-6b''), 3.77 (1H, dd, $J = 3$ and 10 Hz, H-3'')
6	delphinidin pentoside 1	6.00	435	303	-
7	cyanidin 3- <i>O</i> -glucoside	6.33	449	287	9.03 (1H, s, H-4), 8.27 (1H, dd, $J = 2$ and 8 Hz, H-6'), 8.08 (1H, d, $J = 2$ Hz, H-2'), 7.02 (1H, d, $J = 8$ Hz, H-5'), 6.65 (1H, d, $J = 2$ Hz, H-6), 5.40 (1H, d, $J = 8$ Hz, H-1''), 3.98 (1H, dd, $J = 2$ and 12 Hz, H-6b''), 3.75 (1H, dd, $J = 6$ and 12 Hz, H-6a''), 3.70-3.50 (4H, H-2'', H-3'', H-4'', H-5'')
8	cyanidin pentoside	6.69	419	287	-
9	delphinidin pentoside 2	7.00	435	303	-

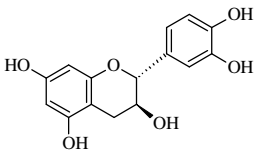
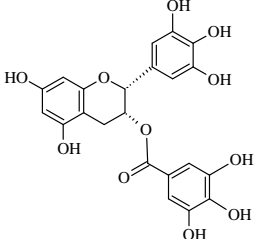
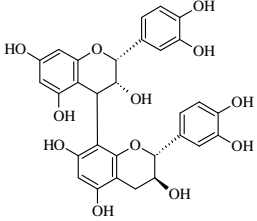
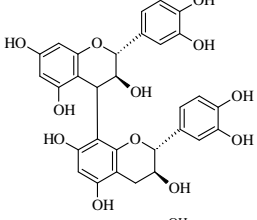
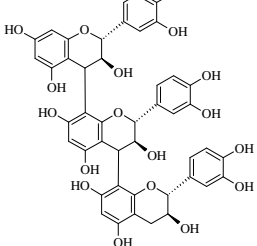
2

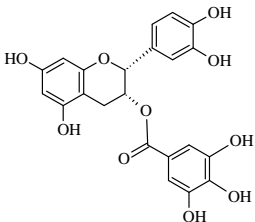
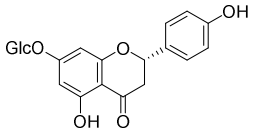
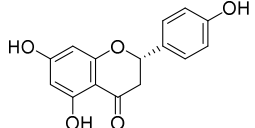
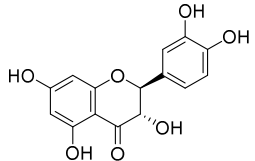
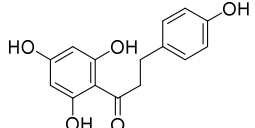
3

- 4 **Table 2.** Compound, structure, retention time, MRM-MS condition for the phenolic compounds identified in *P. lentiscus* fruits (Rut = rutinose;  
 5 Gal = galactoside; Glucur = glucuronide; Glu = glucoside; Rha = rhamnoside).

Compounds	Structure	$t_R$ (min)	Ion mode (+/-)	Precursor ion	Product ions <sup>a</sup>	Collision energies (V)	Fragmentor (V)
<b>Phenolic acids</b>							
protocatechuic acid		5.32	-	153	<b>109</b> 93 65	12 12 24	84
4-hydroxybenzoic acid		13.98	-	137	<b>93</b>	12	84
<b>Stilbenes</b>							
<i>trans</i> -piceid		13.01	-	389	<b>227</b> 143	4 44	150
<b>Flavonols</b>							
quercetin 3- <i>O</i> -rutinoside		13.62	-	609	<b>301</b> 271 255	28 60 60	150
quercetin 3- <i>O</i> -galactoside		13.69	+	463	<b>301</b> 271 255	16 44 40	150

quercetin 3- <i>O</i> -glucuronide		13.87	-	477	<b>301</b> 151	16 40	150
quercetin 3- <i>O</i> -glucoside		14.01	-	463	<b>301</b> 271 255	16 44 40	150
quercetin 3- <i>O</i> -rhamnoside		15.63	-	447	<b>301</b> 271 255	16 40 40	150
myricetin		16.01	-	317	<b>151</b> 137	16 20	125
quercetin		17.25	-	301	<b>151</b> 121	12 20	175
<b>Flavanols</b>							
gallocatechin		5.91	-	305	<b>125</b> 137	12 12	150

catechin		8.30	–	289	<b>109</b> 123	29 25	100
epigallocatechin gallate		10.32	–	457	<b>305</b> 169 125	12 8 40	118
procyanidin B1		7.90	–	577	<b>289</b> 425 407	8 15 15	160
procyanidin B3		8.17	–	577	<b>289</b> 425 407	8 15 15	160
Unknown trimer (quantified as procyanidin C1)		8.9 (11.07)	–	865	<b>125</b> 407 289	40 40 56	186

Epicatechin gallate		13.36	-	441	<b>289</b> 169 125			
<b>Flavanones</b>								
naringenin 7-O-glucoside		15.59	+	435	<b>273</b> 135	10 20	118	
naringenin		18.40	-	271	<b>151</b> 119	8 20	118	
<b>Flavanonols</b>								
taxifolin		13.13	-	303	<b>125</b> 285 57	4 12 40	118	
<b>Dihydrochalcone</b>								
phloretin		16.12	+	275	<b>107</b>	25	118	

6 <sup>a</sup>Value in bold denote quantification ion.

7

8 **Table 3:** Quantitative analysis of phenolic compounds in *Pistacia lentiscus* fruits (in mg/100g DW).

Compounds	Stage 1 <sup>a</sup>	Stage 2	Stage 3	Stage 4	Stage 5
<b>Anthocyanins</b>					
delphinidin dihexoside 1	<LOD	<LOQ	<LOQ	122 ± 3	248 ± 6
delphinidin dihexoside 2	<LOD	<LOD	<LOD	30 ± 4	10 ± 1
delphinidin galactoside	0.8±0.1	1.0±0.2	3 ± 1	513 ± 16	646 ± 8
delphinidin glucoside	0.7±0.1	0.7±0.1	0.8±0.1	319 ± 4	71 ± 1
cyanidin galactoside	0.7±0.2	0.8±0.3	5 ± 1	201 ± 9	103 ± 3
delphinidin pentoside 1	<LOD	<LOD	<LOD	37 ± 2	123 ± 3
cyanidin glucoside	0.8±0.1	0.8±0.1	1.1±0.2	93 ± 5	20 ± 1
cyanidin pentoside	0.7±0.1	0.8±0.1	3 ± 1	42 ± 2	35 ± 5
delphinidin pentoside 2	0.7±0.1	0.7±0.1	0.7±0.1	17 ± 1	19 ± 1
<b>Total anthocyanins</b>	<b>4.4±0.7</b>	<b>4.8±0.9</b>	<b>13.6 ± 3.4</b>	<b>1373 ± 45</b>	<b>1273 ± 27</b>
<b>Phenolic acids</b>					
protocatechuic acid	49 ± 4	39 ± 2	22 ± 2	7 ± 1	2 ± 1
4-hydroxybenzoic acid	<LOD	0.20 ± 0.05	<LOD	<LOD	<LOD
<b>Total phenolic acids</b>	<b>49 ± 4</b>	<b>39 ± 2</b>	<b>22 ± 2</b>	<b>7 ± 1</b>	<b>2 ± 1</b>
<b>Stilbenes</b>					
<i>trans</i> -piceid	0.21 ± 0.01	0.10 ± 0.05	0.22 ± 0.04	0.11 ± 0.04	0.10 ± 0.04
<b>Flavonols</b>					
quercetin rutinoside	23 ± 3	5.1 ± 0.2	20 ± 1	4.0 ± 0.4	5.0 ± 0.3
quercetin galactoside	93 ± 5	29 ± 3	36 ± 2	24 ± 2	10 ± 1
quercetin glucuronide	34 ± 7	11 ± 1	21 ± 1	13 ± 1	7 ± 1
quercetin glucoside	64 ± 8	20 ± 1	29 ± 1	23 ± 2	11 ± 1
quercetin rhamnoside	1.1± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.3 ± 0.1
myricetin	0.5 ± 0.1	0.2 ± 0.1	1 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
quercetin	0.4 ± 0.1	0.2 ± 0.1	1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
<b>Total flavonols</b>	<b>216 ± 23</b>	<b>66 ± 4</b>	<b>108 ± 5</b>	<b>65 ± 6</b>	<b>34 ± 2</b>
<b>Flavanols</b>					
catechin	37 ± 1	16 ± 3	17 ± 0.1	21 ± 1	18 ± 1
gallocatechin	104 ± 9	19 ± 0.7	34 ± 2	48 ± 2	104 ± 8
(epi)gallocatechin gallate	1.5 ± 0.1	0.4 ± 0.1	3.0 ± 0.1	2.0 ± 0.2	3.0 ± 0.2
procyanidin B1	0.6 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	<LOQ	<LOQ
procyanidin B3	2.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
procyanidin C1	2.0 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.2 ± 0.1
(epi)catechin gallate	1 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	1 ± 0.1	1 ± 0.1
<b>Total flavanols</b>	<b>148 ± 10</b>	<b>38 ± 4</b>	<b>56 ± 2</b>	<b>72 ± 3</b>	<b>127 ± 9</b>
<b>Flavanones</b>					
naringenin glucoside	15.1 ± 0.1	5.2 ± 0.1	3.3 ± 0.1	5.0 ± 0.2	7.0 ± 0.2
naringenin	0.13±0.02	0.11±0.02	<LOQ	0.14±0.02	<LOD
<b>Flavanonols</b>					
taxifolin	0.7 ± 0.1	1.0 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
<b>Dihydrochalcone</b>					
Phloretin	47 ± 5	8 ± 1	20 ± 1	18 ± 1	22 ± 1

9

10