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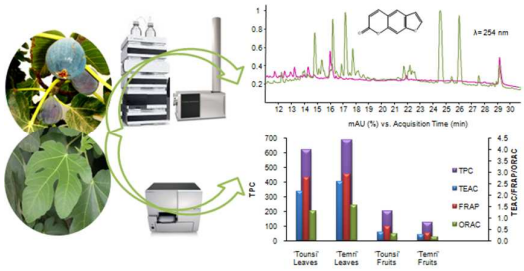

RP–UHPLC–DAD-QTOF–MS gives new insights into the fig phenolic constituents that correlate with their antioxidant potency.

New insights into the qualitative phenolic profile of *Ficus carica* L. fruits and leaves from Tunisia using ultra-high-performance liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry and their antioxidant activity

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Abstract

Ficus carica L. fruits have been consumed from the earliest times, and other parts of the tree have been used for traditional medicinal purposes. Nowadays, the beneficial properties of this and other *Ficus* species are attributed to the presence of key phytochemicals. To increase our knowledge about this topic, the present study has conducted the phenolic profiling of leaves and whole fruits from two Tunisian cultivars, 'Temri' and 'Tounsi', using reversed-phase ultra-high-performance liquid chromatography (RP-UHPLC) coupled to two detection systems: diode-array detection (DAD) and quadrupole time-of-flight (QTOF) mass spectrometry (MS). The UV/Vis absorption was a valuable tool for classifying phenolic compounds into families, while MS using electrospray ionization (ESI) and MS/MS allowed the molecular formula to be established and structural information to be obtained. The total phenol content and the antioxidant activity were also assessed. As result, in the negative ionization mode 91 phenolic compounds were characterized including hydroxybenzoic acids, hydroxycinnamic acids, hydroxycoumarins and flavanoids (flavonols, flavones, flavanones, flavanonols, flavanols and isoflavones). This work was complemented by the detection of other 18 phenolic compounds in the positive ionization mode, including anthocyanins and furanocoumarins. To the best of our knowledge, this is the first time most of these compounds have been tentatively reported in *F. carica*. These results indicate the complexity of this family of secondary metabolites in *F. carica*, as well as the potential of this analytical method for characterization purposes. In conclusion, the qualitative phenolic profile, total phenolic content and antioxidant activity differed especially between leaves and fruits.

Keywords

Antioxidant activity; *Ficus carica*; furanocoumarins; mass spectrometry; Moraceae; phenolic compounds

1. Introduction

Moraceae is an angiosperm plant family, very rich in edible species and characterized by milky latex in all parenchymatous tissue, unisexual flowers, anatropous ovules, and aggregate drupes or achenes.[1] *Ficus* is one of the thirty-seven genera of this family, which comprises about 800 species.[2] Among them, the fig tree or common fig (*Ficus carica* L.) is the most well known species. This plant is a native of the Middle East and one of the first plants cultivated by humans. Fig fruits are consumed either fresh or dried,[3,4] and today *F. carica* continues to be an important crop worldwide, especially in the Mediterranean basin,[5] which includes Tunisia.

In general, figs have the best nutrient score among dried fruit, being an important source of minerals and vitamins,[4] as well as containing relatively higher amounts of crude fibre than all other common fruits.[6,7] Among its phytochemicals, some phenolic classes have been reported in Spanish, Italian and Turkish commercial figs such as the furanocoumarins psoralen and bergapten (5-methoxypsoralen),[8] the flavonoid rutin,[8–10] hydroxycinnamic acids like ferulic and chlorogenic acids[8,9,11] and anthocyanins.[4] The analytical techniques to perform these studies include gas chromatography (GC) coupled to mass spectrometry (MS) and a flame ionization detector (FID)[12], as well as high-performance liquid chromatography (HPLC) coupled to UV, diode array detection (DAD) and mass spectrometry (MS) in a negative or positive ionization mode depending on the target phenolic class.[4,7,8,10–14]

Regarding the potential health benefits, *F. carica* exhibits antioxidant,[2,6,7] and remarkably hypolipidemic and hypoglycemic properties[15] that could be of interest for managing metabolic syndrome. In fact, the antidiabetic effects of *F. carica* leaves extracts have evoked great interest as a natural therapy[15] since diabetes is one of the most common diseases in nearly all countries. It also continues to increase in number and significance as changing lifestyles lead to reduced physical activity and increased obesity.[16] Pèrez and co-workers confirmed that the

71 water extract of fig leaves and its chloroform fraction tend to normalize the antioxidant status
72 of diabetic rats.[17] Although several studies have related the bioactivity of this and other *Ficus*
73 species to the phenolic constituents,[15] more studies are needed to clarify this issue. Thus,
74 novel analytical methodologies may help in the elucidation of the bioactive molecules.

75 In the case of Tunisia, more than 70 different fig ecotypes were recently reported with a
76 wide phenotypic diversity and distinguished by taste, colour and flavour of fruits. However,
77 little is known about their bioactivity and minor phytochemical composition. Two examples
78 of cultivars, known as the 'Temri' and 'Tounsi' cultivars, are commonly cultivated in the
79 centre and south of Tunisia,[18] respectively. Therefore, as potential bioactive markers, the total
80 phenolic content (TPC) and antioxidant capacity of leaves and dried whole fruits from these
81 two Tunisian cultivars of *F. carica* were firstly evaluated. Secondly, their phenolic profiles
82 were extensively studied by ultra-high-performance liquid chromatography (UHPLC) coupled
83 with two detection systems, DAD and quadrupole time-of-flight (QTOF)-MS using
84 electrospray ionization in complementary negative and positive ionization modes.

85 **2. Results and discussion**

86 **2.1 Total phenolic content and antioxidant activity of the 'Tounsi' and 'Temri' fig**
87 **cultivars**

88 **Total phenolic content**

89 In general, the leaves were richer in phenolic compounds than fruits, the TPC value being
90 the highest in the **'**Temri**'** cultivar (686.88 mg of gallic acid/100 g of leaves; Fig. 1).
91 However, the dried whole fruits from the **'**Tounsi**'** cultivar presented a higher TPC value
92 (200.18 mg of gallic acid/100 g of dried fruits) than **'**Temri**'** (124.48 mg of gallic acid/100 g
93 of dried fruits) (Fig. 1). Concerning the fig fruits, Solomon *et al.*[7] evaluated the TPC of six
94 common commercial figs, which had values ranging from 48.6 to 281.1 mg of gallic acid/100

g of fresh fruits. These authors showed that cultivars with skins with dark purple colours, such as Mission and Chechick, were richer in phenolic compounds than those with clearer skins, which explain our results since the skin from the **'**Tounsi**'** fruits presents a darker purple colour than **'**Temri**'** fruits.

In vitro antioxidant activity

Three different methods were used to evaluate the antioxidant capacity: trolox equivalent antioxidant capacity (TEAC), which is also known as the ABTS method; ferric ion reducing antioxidant power (FRAP); and oxygen radical absorbance capacity (ORAC). The TEAC and FRAP methods are based on single electron transfer (SET) mechanisms, whereas the ORAC method is based on a hydrogen atom transfer (HAT) reaction. In this regard, it is now recommended that *in vitro* antioxidants should be determined by at least two methods, preferably with different mechanisms.[19,20] The results are depicted in Fig. 1. Caffeic acid was used as control due to the lack of standardization of these protocols in the literature, with the TEAC, FRAP and ORAC values in agreement with those in studies by Rice-Evans *et al.*[21], Ozgen *et al.*[22] and Ou *et al.*[23], respectively.

According to aforementioned results for TPC, the leaves showed higher antioxidant activity values than fruits by the three methods assayed. In the same manner, the highest TEAC, FRAP and ORAC values were measured in the 'Temri' cultivar, being 2.58 mmol equivalent of Trolox/100 g of sample, 2.93 mmol equivalents of $FeSO_4$/100 g of sample and 1.56 mmol equivalents of Trolox/100 g of sample, respectively. In general, the antioxidant potential of leaves from the *Ficus* genus is higher than that of the fruits.[24]

Previous studies on the antioxidant activity were only conducted on fresh fruits, with results ranging from 0.025 to 0.716 mmol equivalent of trolox/100 g for TEAC, and 0.36 to 1.61 mmol equivalent of $FeSO_4$/100 g for FRAP[7,25], so it is not appropriate to compare these

with our values. Furthermore, the drying process may partially alter the total fruits phenolic content[10], anthocyanins[26], as well as antioxidant activity.[26] In the case of the ORAC, this activity has not been studied before in this fruit. This method is interesting since it is based on the scavenging of peroxyl radicals that are physiologically relevant radicals.[19]

Correlation between TPC and antioxidant activity

Overall, the leaves of both cultivars possessed the strongest antioxidant activity and the fruits had the weakest activity. This may be explained by the occurrence of the highest amounts of phenolic compounds in leaves, since our results indicate an excellent correlation between TPC content and TEAC ($r = 0.994$), FRAP ($r = 0.997$) and ORAC ($r = 0.993$) at $p < 0.01$ (Table 1). On the other hand, the antioxidant activities determined by these three methods also correlated well between each other ($r > 0.98$; Table 1). These results are in accordance with previous studies that have also shown a strong correlation between the TPC, TEAC[7] and FRAP[25] of fig fruits. However, in other foods little or no relationship has been found and other antioxidant compounds may contribute greatly.[20] Thus, our results indicate that phenolic compounds are determinants of antioxidant agents in the *F. carica* samples.

2.2 Qualitative profiling of leaves and fruits

General identification process

In the present work, a qualitative analysis of the phenolic composition was performed using RP-UHPLC-DAD-QTOF-MS and MS/MS, using electrospray ionization in negative and positive ionization modes. Respectively, Tables 2 and 3 show the general results for the following: retention time (RT), molecular monoisotopic mass, experimental *m/z*, molecular formula, UV data (nm), MS score, error (ppm), main MS/MS fragments and the proposed assignment. Additionally, Tables S1 and S2 provide the species, plant family and previous studies that have reported on each compound.

143 On the one hand, the UV/Vis was a valuable tool for classifying phenolic compounds into
144 families and subfamilies according to the presence of one or two charecteristic absorption
145 bands in the UV: band I and band II that come from the B-ring cinnamoyl structure and the A-
146 ring benzoyl or benzene structure, respectively. In this regard, the wavelength of maximum
147 absorption for the characterized phenolic compounds is depicted in Tables 2 and 3, as
148 commented above. Besides, as an example, Fig. S1 shows the UV spectra of several phenolic
149 types from *F. carica*, where band I ranged from 325−371 nm, aproximately, and band II was
150 around 260−298 nm. In the case of flavonoids, at the same time that the heterocyclic C-ring
151 structure serves for their sub-classification, the most intensive band also depends on this ring.
152 For example quercetin (flavonol) showed a prominent band I with a maximum at 371 nm,
153 whereas naringenin (flavanone) presented a maximum at 289 nm that comes from the band II
154 (Fig. S1). Genistein (isoflavone) was characterized by a maximum around 260 nm with higher
155 intensity than the second maximum at 330 nm. This UV absorption behaviour enabled to
156 differentiate isoflavones from flavones, preliminarily. In addition, anthocyanins presents a
157 maximum absorption at visible wavelengths, around 520 nm, that is a characteristic feature of
158 this flavonoid subclass.

159 On the other hand, the QTOF mass analyzer delivers accurate mass mesurements and
160 isotopic fidelity (see experimental section) that allow the molecular formula of the target
161 compound to be obtained. Therefore, in order to procure confident formula assignments for
162 target molecular ions, the lower mass error value and the higher MS score the better (see
163 values in Tables 2 and 3). Afterwards, databases as well as literature were consulted for the
164 retrieval of chemical structure information taking the MS and UV data into account. Finally,
165 using MS/MS analyses, the structure of the parent compound may be tentatively confirmed
166 through studying the fragmentation pattern: fragment ions and neutral losses, which are also
167 accuratly measured. As an example, this general identification process is summarized in Fig.

168 S2. Moreover, the RT served as criterion of polarity and elution order. In this way, a total of
169 13 phenolic compounds were confirmed with standards by comparison of the RT, UV spectra
170 and MS/MS data in order to validate our characterization process (see Table 2).

171 Briefly, with a concise data mining, 91 phenolic compounds were characterized in the
172 negative ionization mode (method 1) including hydroxybenzoic acids, hydroxycinnamic
173 acids, flavonoids that were represented by flavonols, flavones, flavanones, flavanonols,
174 flavanols and isoflavones subclasses, and hydroxycoumarins (Table 2). Several of these
175 compounds were also detected using the analytical method 2 (data not shown). These data
176 were complemented with 18 phenolic compounds tentatively characterized using the positive
177 ionization mode, belonging to anthocyanins, furanocoumarins (Table 3) and a isoflavone,
178 which were either poorly ionized in the negative ionization mode or undetectable.
179 Furthermore, the major part of the characterized phenolic compounds were tentatively
180 reported in *F. carica* for the first time in this work (Fig. S3), and other unreported phenolic
181 structures were proposed as well according to their UV, MS and MS/MS information. Several
182 previous studies on *F. carica* applied GC and HPLC coupled to several detectors, including
183 mass analyzers such as quadrupole and ion trap.[4,7,8,10–14] However, there were few compounds
184 identified in these works, which are in the range from 4 to 15. One of the reason is that the
185 authors focused on a particular phenolic subclass, e.g. anthocyanins[4,7], or target phenolic
186 compounds.[8,11] Therefore, at this point, our findings remark the potencial of RP-HPLC-DAD-
187 QTOF-MS in order to perform successful and extensive characterization works of plant
188 extracts and as starting point for structure elucidation of new molecules. In this regard, the
189 MS analysis *via* electrospray ionization in the negative and positive ionization modes was
190 complementary and enabled the detection and characterization of a large number of
191 compounds. However, the analyst must be cautious in offering interpretations until all the
192 information is evaluated. It is probably the most critical and long time-consuming part since,

193 although there are efforts to generate spectral libraries using standards, unfortunately LC-ESI-
194 MS methods often lack the consistency, standardization or reproducibility that characterizes
195 GC-MS or nuclear magnetic resonance spectroscopy.[27]

196 The chromatographic profiles are depicted in Fig. 2 that show the base peak
197 chromatograms (BPC) of leaves and fruits of both cultivars that represent the ions detected in
198 negative ionization mode using method 1, and the UV chromatograms at 254 and 520 nm, at
199 which furanocoumarins and anthocyanins, respectively, show absorption,[4,8] using method 2
200 (see Experimental section). These chromatographic profiles, BPC and UV at 254 nm, show
201 that the leaves presented richer qualitative and quantitative profiles, explaining our previous
202 results for TPC and antioxidant activity. However, not surprinsingly, anthocyanins were only
203 detected in fruits.

204 Regarding non-phenolic compounds, several organic acids, amino acids and other
205 compounds were also characterized, and these are additionally described in the supplementary
206 information, see Table S1 and non phenolic compounds information. Furthermore, Table S3
207 also contains information about certain unknown compounds that their MS/MS spectra is
208 related to the MS/MS of hydroxybenzoic derivatives. Double bond equivalents (DBE) are
209 reported in this table since this value is related to the total number of combined rings and
210 double bonds in the molecule, and so it is useful as indicator of aromaticity or unsaturation.
211 For example, a benzene ring has 4 DBE, that is one ring and three double bonds.

212 **Phenolic acids: hydroxybenzoic, hydroxycinnamic acids and others**

213 Overall, 45 phenolic acids were found in *F. carica* (Tables 2 and S1), belonging to
214 hydroxybezoic and hydroxycinnamic acids. The main qualitative differences were found
215 between leaves and fruits. The first phenolic class with a more polar feature eluted over a
216 period of 10.61 to 15.90 min, whereas the second class compounds eluted between 11.19 and

19.62 min. In general, phenolic acids and their derivatives ionized better in the negative ionization mode and most of them presented a loss of 18.0106 u (H_2O) and 43.9898 u (CO_2) in MS/MS, which is consistent with previous findings in Gómez Romero *et al*.[28] and Abu-Reidah *et al*.[29] Interestingly, the leaves were richer in phenolic derivatives formed by conjugation with sugars and organic acids, including malic and quinic acid. However, the free forms of hydroxybenzoic, caffeic and ferulic acids, except vanillic acid, were only present in fruits.

It is worth mentioning that all hydroxybenzoic acids except dihydroxybenzoic acid were reported for the first time in *F. carica*. The new compounds were derivatives of hydroxybenzoic, dihydroxybenzoic and trihydroxybenzoic acid (e.g. gallic acid), being *O*-methylated (e.g. vanillic and syringic acids) or conjugated with hexose, pentose and malic acid. These moieties were assigned according to their respective neutral losses established on the basis of the fragmentation pattern in MS/MS, as previously reported.[28–31] As an example, Fig. 3a shows the MS/MS spectra of the isomer of syringic acid malate (isomer I) (*m/z* 313.0569): 197.0462 ($[C_9H_{10}O_5-H]^-$), 133.0145 ($[C_4H_6O_5-H]^-$) and 115.0039 ($[C_4H_4O_4-H]^-$), which correspond to free syringic acid ion after the loss of malic acid, malic acid ion and its fragment ion generated by the loss of H_2O, respectively. In addition, the presence of fragments at *m/z* 167.0354 and 153.0559 indicated the loss of CH_2O and CO_2 from the methoxy group and the carboxylic acid moiety of the phenolic acid, respectively. This compound was detected only in the leaves of both cultivars.

A total of 24 hydroxycinnamic acids were derivatives of coumaric, caffeic, ferulic and sinapic acids. Overall, hydroxycinnamics also presented a higher signal in leaves than in fruits (Fig. S4a and d). The presence of caffeic acid and *trans*-ferulic acid in fruits and chlorogenic acid in fruits and leaves was confirmed with standards and presented the same RT, molecular formula, UV maximum and fragmentation pattern. These compounds have been previously

242 reported in this species.[8,9,32–34] Moreover, other three isomers of chlorogenic acid were also
243 found. Recently, Olivera *et al.*[9] described the isomers 3-*O*- and 5-*O*-caffeoylquinic acids
244 (chlorogenic acid) in Portuguese white fig samples.

245 Interestingly, as for the aforementioned phenolic acid class, several conjugated forms
246 were reported for the first time in *F. carica* and as well as in the Moraceae family (Tables 2
247 and S1). For example, three isomers of caffeoylquinic acid hexoside were characterized based
248 on their molecular formula and UV and MS/MS spectra, which was in agreement with
249 previous studies on other plant families.[30,35,36] In a similar manner, the fragmentation pattern
250 of caffeic, coumaric and ferulic acids conjugated with hexose or organic acids were in
251 accordance with previous studies.[28–30,32,35] Overall, these conjugations could be established on
252 the basis of the MS/MS spectra, because the moieties of the latter and/or the free phenolic
253 acid were observed (Tables 2 and S1). For example, fragment ions with *m/z* values of
254 191.0561 ($[C_7H_{12}O_6-H]^-$) (quinic acid) and 179.0350 ($[C_9H_8O_4-H]^-$) (caffeic acid) were
255 released from caffeoylquinic acid isomers.

256 Finally, a phenylpropanoid acid related to furanocoumarin psoralen was assigned as
257 psoralic acid glucoside, according to the recent findings in *F. carica* leaves (Takahashi *et*
258 *al.*[32]), which also suggested that this compound could be a precursor of psoralen. Their
259 fragmentation pattern agreed with the Takahashi's study, as we also observed the loss of
260 glucose (*m/z* 203.0347) and the loss of CO_2 (*m/z* 159.0453) as the main fragments in MS/MS.
261 This compound was detected in leaves (Fig. S4b and e). Furthermore, a compound related to
262 this was detected in negative and positive ion modes (e.g. see compound with *m/z* value at
263 205.0502 and RT 17.21 min in Tables 3 and S2), which could be the aglycone or a dihydro
264 form of hydroxypsoralen. The MS data, the UV spectra and published literature were not
265 enough to elucidate the structure of this compound.

Flavonoids

As commented above, UV-Vis spectra can be used as an indicative tool for the primary characterization of flavonoids, whereas MS and MS/MS information can provide additional and significant information.[37] In this way, the flavonols, flavones, flavanones, flavanonols and isoflavones were characterized in the negative ionization mode (Tables 2 and S1) and two anthocyanins in positive ionization mode (Tables 3 and S2).

The flavonols quercetin, quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside were confirmed by standards and previously reported in fresh and dried figs.[8–10,32–34,38] A malonyl derivative of quercetin was found at RT 18.68 min, the fragmentation pattern (Fig. 3b) being in agreement with Takahashi *et al.*[32] and so characterized as quercetin 3-*O*-(6"-malonyl)glucoside. In the case of quercetin di-deoxyhexoside hexoside, it is tentatively described here in *F. carica* for the first time, and has been previously reported in other plant families (e.g. Table S1). In general, new quercetin derivatives in *F. carica* probably contain at least a sugar at the position 3 of the C-ring that produces a shift of λmax from band I, which comes from the B-ring cinnamoyl structure, of quercetin to a lower wavelength (< 20 nm).[36]

Flavones were among the most qualitatively abundant fig flavonoids and presented slight distribution differences between leaves and fruits. In the case of luteolin-7-*O*-glucoside, luteolin and apigenin, their identification was resolved by means of comparison of the RT, UV absorption and MS/MS spectra with commercial standards When standards were unavailable, MS/MS helps in the assignment, together with the previous literature. For example, consecutive neutral losses of 18.0106 u (H_2O), 60.0211 u ($C_2H_4O_2$), 90.0317 u ($C_3H_6O_3$), 120.0423 u ($C_4H_8O_4$), 180.0634 u ($C_6H_{12}O_6$) and/or 210.0740 u ($C_7H_{14}O_7$) are considered to be characteristic of the fragmentation pattern of *C*-glycosylated compounds. The MS/MS spectra for these compounds are in good agreement with previous studies.[39] In

290 contrast to *C*-glycosides, the MS/MS spectra of the *O*-glycosidic forms of apigenin and
291 luteolin showed more abundant fragment ions corresponding to the aglycones after the neutral
292 loss of 162.0526 u (hexose) and 308.1122 u (hexose-deoxyhexose), respectively (Tables 2 and
293 S1).

294 The third group of flavonoids identified was flavanones (Table 2 and S1). Among them,
295 eryodictiol and naringenin have been previously reported in other *Ficus* species.[40,41] It should
296 be mentioned that the glycosylated flavanones were reported here for the first time in the
297 Moraceae family. For example, eriodictyol di-hexoside (*m/z* 611.1624, RT 16.09 min) was
298 characterized according to its fragmentation pattern, which agrees with the findings of Iswaldi
299 *et al*.[42] for eriodictyol 5,3'-di-*O*-glucoside in *Aspalathus linearis* (Fabaceae). Moreover, the
300 UV-Vis spectra of these compounds showed a main maximum close to 280 nm related to a
301 strong UV band II absorption from the A ring benzoyl structure.[36,37] Two isomers of
302 eriodictyol hexoside, with *m/z* at 449.1099 and 449.1086 and RT 17.95 and 19.87,
303 respectively, were reported in the Cucurbitaceae family.[43] It was not possible to distinguish
304 between both isomers, since no commercial standards were available for these compounds.
305 Interestingly, the last three compounds were found only in fruits of cultivar 'Temri', being
306 putative characteristic biomarkers of its consumption.

307 Altough isoflavonoids are widely distributed in the Moraceae family,[44,45] there is no
308 mention in the literature about this class in *F. carica*. Our methodology allows ten isoflavones
309 to be tentatively characterized (Tables 2 and S1), including several prenylated forms.
310 Genistein and methylated derivatives of genistein and prenylgenistein have been previously
311 described in other *Ficus* species.[45–47] Only genistein (RT 24.46 min, *m/z* 269.0459) could be
312 confirmed with standards and was found in the leaves and fruits of both cultivars. The UV
313 data clearly show a main maximum close to 260 nm, which is in accordance with the findings
314 of Shen *et al.*[48] Overall, among other fragments found in the MS/MS spectra of the genistein

315 derivatives, aglycone at *m/z* value of 269.1190 (even electron) or 268.0374 (odd electron)
316 were detected, and also the characteristic fragment ions at *m/z* 151.0031 ($^{1,3}A^-$), 133.0658
317 ($^{0,3}B^-$) (even electron) or 132.0214 (odd electron) and 117.0346 ($^{1,3}B^-$) released from the
318 breakage of genistein backbone. In the case of the malonylhexoside derivative of
319 hidroxygenistein methyl ether, the loss of CO_2 from the malonyl group and the subsequent
320 loss of 204.0634 u ($C_8H_{12}O_6$, acetyl hexosyl rest) were also observed in MS/MS. A dimethyl
321 ether isoflavone was only detected in positive ionization mode (Tables 3 and S2), exhibiting
322 the UV maximums related to the isoflavone core, and the MS/MS spectrum was in agreement
323 with 7-hydroxy-6,4'-dimethoxyisoflavone (afromosin).[49] Since there was no information
324 about this compound in the Moraceae literature and the MS/MS data were not enough to
325 distinguish the exact position of the free hydroxyl or methoxy groups, the compound was
326 denominated as hydroxy-dimethoxyisoflavone.

327 Interestingly, the prenylated isoflavones presented a remarkably higher signal in leaves
328 than in fruits (Fig. S4c and f). Not surprisingly, they eluted at higher RT due to the presence
329 of this lipophilic prenyl side chain, with an RT from 26.49 to 29.10 min. The UV data and
330 main MS/MS fragments agreed with prenylated forms of genistein (6-, 3'-, and 8-
331 prenylgenistein) present in the *Lupinus* species.[48] In this regard, 6- and 8-prenylgenistein was
332 reported in stem barks and fruits of other *Ficus* species (e.g. *Ficus tikoua*).[47,50] Furthemore,
333 related prenylated forms linked to hydroxygenistein were also tentatively characterized, and
334 the UV data agreed with the findings of Shen *et al*.[48] In general, these prenylated compounds
335 were characterized by the neutral loss of C_4H_7 (55.0548 u) and C_5H_9 (69.0704 u) from the
336 prenyl moiety, amongst others. In this regard, prenylated flavonoids possess unique
337 bioactivities relative to their unmodified parent compounds, particularly potent antifungal
338 activity.[47,48]

Finally, using the analytical method 1, the flavanol (+)-catechin, which was confirmed with the standard, and the flavanonol dihydroquercetin were also detected, in accordance with previous studies on the *Ficus* species.[10,13,33,40,41]

Using analytical method 2, two anthocyanins could be detected in dried fig fruits at 520 nm (see Fig. 2e and f). According to the MS and MS/MS data obtained in the positive ionization mode and the published studies on fig fruits,[4] they were assigned to cyanidin 3,5-diglucoside (*m/z* 611.1613, $C_{27}H_{31}O_{16}$) and cyanidin 3-rutinoside (*m/z* 595.1667, $C_{27}H_{31}O_{15}$) (Tables 3 and S2).

Hydroxycoumarins

The presence of 7-hydroxycoumarin (umbelliferone) was confirmed with the standard. This compound was previously reported in *F. carica*.[45] and it is suggested that it is the precursor of furanocoumarins.[51] The rest of the hydroxycoumarins were putatively characterized on the basis of the MS/MS spectra, UV data and literature.[40,45] All of these compounds were found in the leaves and some of them in fruits, too. Their fragmentation pattern was characterized by the loss of CO (27.9949 u), CO_2 (43.9898 u) and, subsequently, C_2H_4 (28.0313 u) from the aglycone, in agreement with our previous findings for lettuce (*Lactuca sativa*) leaves.[29] A prenylated form of 7-hydroxycoumarin was also tentatively charaterized at RT 27.95 min and *m/z* 229.0872 ($C_{14}H_{14}O_3$), which showed the characteristic loss of C_4H_7 (55.0548 u) from the prenyl moiety in MS/MS at *m/z* 174.0319, as observed above. Several fragments were also in agreement with the findings of Yang *et al*.[52]

Furanocoumarins

There are two type of furanocoumarins in nature, linear and angular ones.[53] *F. carica* contains mainly the first class, with psoralen and 8-methoxypsoralen (bergapten) being the major representatives.[12,24,32] In this regard, a total of 14 furanocoumarins were tentatively

363 characterized in Tunisian figs in positive ionization mode, including the aforementioned
364 compounds (Tables 3 and S2). The major part of the characterized furanocoumarins are
365 described here for the first time in *F. carica* and also in the Moraceae family (e.g.
366 isopentenoxypsoralen, at RT 28.25 min and *m/z* 271.098). Alternatively, others have been
367 previously reported in other *Ficus* species, such as marmesin isomers, 4',5'-dihydropsoralen
368 and oxypeucedanin hydrate (Table S2). In agreement with Yang *et al.'s* study on *Radix*
369 *glehniae*,[52] we detected a characteristic series of fragment ions for furanocoumarins that were
370 mainly generated by consecutive losses of CO (e.g. Fig. 3c). As stated above, in a similar way
371 the loss of C_3H_6 (42.0470 u), C_4H_8 (56.0626 u) and C_5H_8 (68.0626 u) from the prenyl moiety
372 was also observed in the MS/MS spectra of prenylated furanocoumarins,
373 isopentenoxypsoralen and prenyl methoxypsoralen.[52,54] In addition, the UV data of
374 furanocoumarins also agreed with that of Frérot *et al*.[55]

375 Linear furocoumarins have received great attention since these compounds, used
376 medicinally and in a controlled way, may represent a novel class of natural drugs that are
377 potentially useful for the photodynamic treatment of several skin diseases.[12] In this regard, *F.*
378 *carica* leaves could be of interest, thanks to their qualitatively rich profiles (Tables 3 and S2;
379 Fig. 2).

380 **3. Conclusions**

381 Despite the popularity of the consumption of dried fig fruits, there is little information
382 about its antioxidant activity. Moreover, in the case of its qualitative phenolic composition,
383 previous studies have only focused on target phenolic compounds. In our study, a total of 109
384 phenolic compounds were characterized in *F. carica* samples. Most of them were reported for
385 the first time in *F. carica* species. In addition, fig leaves presented a richer phenolic
386 qualitative profile with also a higher total phenol content in comparison to fruits. In this

regard, phenolic acids conjugated with sugars and organic acids as well as furanocoumarins were mainly present in leaves, but not in fruits. Concurrently, *F. carica* leaves exhibited stronger antioxidant capacity by both electron or hydrogen transfer mechanisms. Therefore, our results are of interest to further studies on the phytochemical composition of *F. carica* and the Moraceae family; additionally, the antioxidant values may be used as references for future researches to make comparisons with other fig cultivars. Overall, these results contribute to explaining the past and current usage of *F. carica* in folk medicine, as leaves extracts can be regarded as a promising source of antioxidant phenolic compounds for further uses in pharmacology and cosmetology.

4. Experimental

Chemical and reagents

Ethanol, acetonitrile, formic acid and glacial acetic acid were purchased from Fisher Chemicals (ThermoFisher, Waltham, MA, USA). Solvents used for extraction and analysis were of analytical and HPLC-MS grades, respectively. Ultrapure water was obtained by a Milli-Q system (Millipore, Bedford, MA, USA).

The reagents used to measure the TPC and the antioxidant capacity were Folin & Ciocalteu's, sodium carbonate (Na_2CO_3), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), fluorescein, potassium persulphate ($K_2S_2O_8$) and ferric sulphate ($FeSO_4$). They were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dehydrated sodium phosphate, trihydrated sodium acetate, sodium acetate, ferric chloride ($FeCl_3 \cdot 6H_2O$) and hydrochloric acid (HCl) were obtained from Panreac (Barcelona, Spain). Phenolic standards available in our laboratory were bought from Sigma-Aldrich:

411 chlorogenic acid, caffeic acid, vanillic acid, *trans*-ferulic acid, rutin, quercetin-3-*O*-glycoside,
412 quercetin, luteolin-7-*O*-glucoside, apigenin, luteolin, (+)-catechin, genistein, 7-
413 hydroxycoumarin and gallic acid. The degree of purity of the standards was around 95%
414 (w/w).

415 **Fig samples**

416 Leaves and fruits from the *F. carica* cultivars 'Tounsi' and 'Temri' were collected in
417 Sfax region (southeast Tunisia) in August 2013. The samples (about 0.5 kg) was randomly
418 harvested and immediately transferred to the laboratory where they were dried at room
419 temperature in the dark, and then they were finely ground prior to extraction.

420 **Sample preparation**

421 Dried fig leaves and fruits (3 g) were put in amber glass bottle homogenized in 100 mL of
422 70:30 (v/v) ethanol/water placed on a stirring hot plate for 24 hours at 37 °C and 150 rpm.
423 Each mixture was centrifuged at 8000 rpm for 15 min and the supernatant was collected.
424 Afterwards, the solvent was evaporated to dryness using a rotary evaporator under vacuum at
425 40 °C, and the residue was redissolved in 3 mL of 70:30 (v/v) ethanol/water. Finally, the
426 supernatants were filtered with a syringe filter (regenerated cellulose, 0.45 μm pore size) and
427 stored at −20 °C until analysis. The extraction was repeated in duplicate.

428 **Total phenol content (TPC)**

429 The TPC of the extracts was determined in triplicate by the colorimetric assay using the
430 Folin–Ciocalteu reagent[57] modified according to Romero-de Soto *et al.*[58] with 96-well
431 polystyrene microplates (ThermoFisher) and a Synergy Mx Monochromator-Based Multi-
432 Mode Microplate reader (Bio-Tek Instruments Inc, Winooski, VT, USA). The absorbance of
433 the solution at a wavelength of 760 nm was measured after incubation for 2 hours in the dark

and compared with a calibration curve of serially diluted gallic acid, which was elaborated in the same manner. The results were expressed as the equivalents of gallic acid.

Antioxidant capacity assays

TEAC assay

This antioxidant method measures the reduction of the radical cation of ABTS by antioxidants, and is based on Miller *et al*.'s approach[59] The method was modified as described Laporta *et al*.[60] Briefly, the ABTS radical cation ($ABTS^{+\bullet}$) was produced by reacting the ABTS stock solution with 2.45 mM of potassium persulfate and keeping the mixture in darkness at room temperature for 12 to 24 h before use. For the antioxidant assay with vegetable extracts, the $ABTS^{+\bullet}$ solution was diluted with water until an absorbance value of 0.70 (± 0.02) at 734 nm was reached. Afterwards, 300 µL of the $ABTS^{+\bullet}$ solution and 30 µL of the extract were mixed for 45 s and measured immediately after 5 min (absorbance did not change significantly up to 10 min). The readings were performed at 734 nm and 25 °C. The result of each sample was then compared with a standard curve made from the corresponding readings of Trolox (0.625–30 µM in the microplate wells). Caffeic acid was used as a positive control. The results are expressed in mmol of trolox equivalents/100 g of sample.

FRAP assay

The FRAP assay was conducted following the method described by Benzie and Strain.[61] The stock solutions included 300 mM acetate buffer (1.23 g $C_2H_3NaO_2$ + 0.8 mL $C_2H_4O_2$ + 49.2 mL of water, pH = 3.6 adjusted with HCl), 10 mM of TPTZ solution in 40 mM HCl and 20 mM $FeCl_3$ in water. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL of TPTZ and 2.5 mL of $FeCl_3$ solutions. Briefly, 40 µL of the extracts was mixed with 250 µL of freshly prepared FRAP reagent on a 96-well plate. Samples were incubated for 10 min at 37 °C; then, absorbance was recorded at 593 nm for 4 min on the

458 microplate reader. The final absorbance of each sample was compared with those from the
459 standard curve made from $FeSO_4 \cdot 7H_2O$ (12.5–200 μM, final concentration in wells). Caffeic
460 acid was used as a positive control. The results are expressed in mmol of $FeSO_4$ equivalents
461 /100 g of sample.

462 *ORAC assay*

463 The method used was based on that of Ou *et al*.[23] modified by Laporta *et al*.[60] The
464 reaction was carried out in 75 mM phosphate buffer (pH = 7.4), and the final reaction mixture
465 was 200 μL fluorescein and AAPH, which was used at 40 nM and 19 mM, respectively. A
466 freshly prepared AAPH solution was used for each experiment. The temperature of the
467 incubator was set at 37 °C and the fluorescence was recorded 15 min after the addition of
468 AAPH. The microplate was immediately placed in the reader and the fluorescence recorded
469 every minute for 180 min. The microplate was automatically shaken prior to each reading. All
470 the fluorescent measurements are expressed relative to the initial reading (AUC for each
471 well). A blank (phosphate buffer instead of the antioxidant solution), several dilutions of
472 trolox (0.625-15 μM, final concentration in wells) and samples (at least four valid dilution
473 points) were measured. All the reaction mixtures were prepared in triplicate, and at least two
474 independent assays were performed for each sample. The net area under curve (AUC)
475 corresponding to the trolox or samples was calculated by subtracting the AUC corresponding
476 to the blank. Caffeic acid was used as a positive control. ORAC values were expressed as
477 trolox equivalents by using the standard curve calculated for each assay. The final results
478 were in mmol of trolox equivalents/100 g of samples.

479 **Characterization of phenolic compounds by UHPLC−DAD-QTOF-MS**

480 Analyses were made with an Agilent 1200 series rapid resolution (Palo Alto, CA, USA)
481 equipped with a binary pump, an autosampler and a DAD. The system was coupled to a 6540

Agilent Ultra-High-Definition (UHD) Accurate-Mass Q-TOF LC/MS, equipped with an Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface.

To characterize phenolic compounds, the mobile phases consisted of water plus 0.5% acetic acid (phase A) and acetonitrile (phase B), according to the approach of Abu-Reidah *et al*.[62] (method 1). The following multistep linear gradient was applied: 0 min, 0% B; 10 min, 20% B; 15 min, 30% B; 20 min, 50% B; 25 min, 75% B; 30 min, 100% B; 31 min, 100% B; 34 min, 0% B; 40 min, 0% B. The flow rate was set at 0.50 mL/min throughout the gradient. To characterize anthocyanins and furanocoumarins, the mobile phases were water plus 5% formic acid (phase A) and acetonitrile (phase B), according to the approach of Gómez-Caravaca *et al*.[63] (method 2). Separation was carried out with a Zorbax Eclipse Plus C18 column (150 × 4.6 mm, 1.8 μm of particle size) at room temperature. The UV spectra were recorded from 190 to 600 nm. The injection volume was 5 μL. Samples were diluted by 1/4 with an ethanol:water mix of 70:30 (v/v).

The operating conditions in negative ionization mode were as follows: gas temperature, 325 °C; drying gas, nitrogen at 10 L/min; nebulizer pressure, 20 psig; sheath gas temperature, 400 °C; sheath gas flow, nitrogen at 12 L/min; capillary voltage, 4000 V; skimmer, 45 V; octopole radiofrequency voltage, 750 V; focusing voltage, 500 V, with the corresponding polarity automatically set. Spectra were acquired over a mass range from *m/z* 100 to 1700 and for MS/MS experiments from *m/z* 70 to 1700. In the case of anthocyanins and furanocoumarins, MS analyses were performed in positive ionization mode based on several studies,[8,64] with the parameters set as commented above, but with the corresponding polarity. Reference mass correction of each sample was performed with a continuous infusion of Agilent TOF mixture containing trifluoroacetic acid (TFA) ammonium salt (*m/z* 112.9856 corresponding to TFA) and hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine (*m/z* 1033.9881 corresponding to the TFA adduct) for negative ionization mode, while using

purine (*m/z* 121.0508) and hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine (*m/z* 922.0098) for positive ionization mode. The detection window was set to 100 ppm.

Data analysis was performed on a Mass Hunter Qualitative Analysis B.06.00 (Agilent technologies). For characterization, the isotope model selected was common organic molecules with a peak spacing tolerance of *m/z* 0.0025 and 7 ppm. Then, the characterization of the compounds was done taking into account the generation of candidate molecular formula with a mass error limit of 5 ppm and also considering RT, experimental and theoretical masses, and MS/MS spectra. The MS score related to the contribution to mass accuracy, isotope abundance and isotope spacing for the generated molecular formula was set at ≥80. Confirmation was made through a comparison with standards, whenever these were available in-house. Consequently, the literature on Moraceae and the following chemical structure databases were consulted: PubChem (http://pubchem.ncbi.nlm.nih.gov), ChemSpider (http://www.chemspider.com), SciFinder Scholar (https://scifinder.cas.org), Reaxys (http://www.reaxys.com), Phenol-Explorer (www.phenol-explorer.eu) and KNApSAcK Core System (http://kanaya.naist.jp/knapsack jsp/top.html).

Statistical analysis

Microsoft Excel 2007 (Redmond, WA, USA) was employed for statistical analysis. The correlation between TPC and antioxidant activity was performed using SPSS Statistics 22 (Armonk, NY, USA).

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Conflicts of interest

The authors declare no competing financial interest.

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666

667 Table 1. Correlation between the total phenolic content (TPC) and antioxidant activity of
668 leaves and fruits of *F. carica* cultivars 'Temri' and 'Tounsi'.

Correlations					
		TPC	TEAC	FRAP	ORAC
TPC	Pearson Correlation	1	0.994**	0.997**	0.993**
	Sig. (2-tailed)		0.000	0.000	0.000
TEAC	Pearson Correlation	0.994**	1	0.991**	0.985**
	Sig. (2-tailed)	0.000		0.000	0.000
FRAP	Pearson Correlation	0.997**	0.991**	1	0.984**
	Sig. (2-tailed)	0.000	0.000		0.000
ORAC	Pearson Correlation	0.993**	0.985**	0.984**	1
	Sig. (2-tailed)	0.000	0.000	0.000	

669 Antioxidant activity: TEAC, trolox equivalent antioxidant capacity; FRAP, ferric ion reducing
670 antioxidant power; ORAC, oxygen radical absorbance capacity.
671 **Correlation is significant at the 0.01 level (2-tailed).
672
673

674 Table 2. Phenolic compounds characterized using the negative ionization mode in leaves and fruits of *F. carica* cultivars 'Tounsi' and 'Temri'.

RT (min)	[M-H]$^-$	Formula	Score	Error (ppm)	UV (nm)	Main fragments *via* MS/MS	Proposed compound	Presence			
								'Tounsi'		'Temri'	
Hydroxybenzoic acids and derivatives								L	F	L	F
10.61	359.0976	$C_{15}H_{20}O_{10}$	94.7	1.6	280	197.0455; 179.0346; 153.0549; 135.0452; 85.0292	Syringic acid hexoside I	+	-	+	-
10.76	315.0725	$C_{13}H_{16}O_9$	98.9	-1.2	-	153.0190; 152.0109; 108.0212; 109.0293	Dihydroxybenzoic acid hexoside I	+	+	+	+
10.76	313.0569	$C_{13}H_{14}O_9$	84.0	-1.2	280	197.0462; 167.0354; 153.0559; 135.0454; 133.0145; 123.0455; 115.0039	Syringic acid malate I	+	-	+	-
10.94	359.0993	$C_{15}H_{20}O_{10}$	89.3	-2.9	280	197.0458; 179.0352; 153.0352; 135.0454; 123.0453; 85.0297	Syringic acid hexoside II	+	-	+	-
11.07	329.0886	$C_{14}H_{18}O_9$	80.3	-2.4	255; 291	167.0345; 152.0111; 123.0450; 108.0213	Vanillic acid glucoside	+	+	-	+
11.07	313.0573	$C_{13}H_{14}O_9$	82.7	-2.7	280	179.0350; 135.0450; 133.0142; 115.0037	Syringic acid malate II	+	-	+	-
11.09	475.1473	$C_{20}H_{28}O_{13}$	86.7	-3.8	-	329.0880; 167.0347; 109.0293	Vanillic acid hexoside deoxyhexoside	+	-	+	-
11.19	433.1002	$C_{17}H_{22}O_{13}$	95.3	-3.2	280	301.0564; 169.0138; 168.0061; 151.0035; 125.0242	Gallic acid di-pentoside I	+	-	+	-
11.23	315.0726	$C_{13}H_{16}O_9$	98.9	-1.5	-	153.0188; 109.0294	Dihydroxybenzoic acid hexoside II	+	+	+	+
11.50	433.0996	$C_{17}H_{22}O_{13}$	98.2	-1.6	280	301.0568; 169.0130; 168.0064; 151.0041; 125.0245	Gallic acid di-pentoside II	+	+	+	+
11.57	447.1143	$C_{18}H_{24}O_{13}$	99.3	0.4	305	315.0714; 271.0816; 152.0112; 109.0291; 108.0217	Dihydroxybenzoic acid hexoside pentoside I	+	+	+	+
12.32	447.1143	$C_{18}H_{24}O_{13}$	97.4	0.2	260; 297	152.0114; 109.0291	Dihydroxybenzoic acid hexoside pentoside II	+	-	+	-
12.54	153.0198	$C_7H_6O_4$	92.5	-4.1	260; 290	109.0296; 108.0220	Dihydroxybenzoic acid	+	+	+	+
12.56	315.0723	$C_{13}H_{16}O_9$	99.7	-0.5	-	153.0194; 152.0194; 109.0291; 108.0219	Dihydroxybenzoic acid hexoside II	+	-	+	-
12.62	285.0613	$C_{12}H_{14}O_8$	97.3	1.2	260; 300	152.0115; 153.0191; 108.0217; 109.0295	Dihydroxybenzoic acid pentoside I	+	+	+	+
12.68	447.1151	$C_{18}H_{24}O_{13}$	94.3	-1.6	-	153.0192; 109.0295	Dihydroxybenzoic acid hexoside pentoside III	+	+	+	+
13.17	285.0621	$C_{12}H_{14}O_8$	85.2	-1.4	230; 300	153.0191; 152.0113; 109.0294; 108.0219	Dihydroxybenzoic acid pentoside II	+	+	+	+
13.25	417.1043	$C_{17}H_{22}O_{12}$	98.2	-1.0	310	285.0613; 241.0718; 153.0165; 152.0119; 108.0217; 109.0287	Dihydroxybenzoic acid di-pentoside	+	+	+	+
14.66	137.025	$C_7H_6O_3$	96.1	-5.0	-	109.0294; 108.0221; 93.0349; 92.0273	Hydroxybenzoic acid I	+	-	+	-

15.17	137.0245	$C_7H_6O_3$	95.3	-1.7	-	93.0344	Hydroxybenzoic acid II	+	+	+	+
15.90	167.0349	$C_8H_8O_4$	96.9	0.9	261; 292	152.0110; 123.0431; 124.0163; 108.0214	Vanillic acid*	+	+	+	+
Hydroxycinnamic acids and derivatives											
11.19	515.1408	$C_{22}H_{28}O_{14}$	88.4	-0.9	264; 327	353.0881; 191.0560; 179.0346	Caffeoylquinic acid hexoside I	+	-	+	-
11.75	515.1408	$C_{22}H_{28}O_{14}$	98.2	-0.7	262; 324	341.0872; 323.0771; 191.0559; 179.0348; 173.0451; 135.0451	Caffeoylquinic acid hexoside II	+	+	+	+
12.31	353.0884	$C_{16}H_{18}O_9$	98.3	-1.3	264; 328	191.0560; 179.0349; 135.0448	Caffeoylquinic acid I	+	+	+	+
12.37	343.1043	$C_{15}H_{20}O_9$	95.9	-2.7	282	181.0508; 163.0400; 137.0609; 135.0443	Dihydrocaffeic acid hexose	+	+	+	+
12.64	515.1408	$C_{22}H_{28}O_{14}$	98.5	-1.0	280; 320	341.0773; 323.0773; 191.0560; 179.0347; 135.0446	Caffeoylquinic acid hexoside III	+	+	+	+
12.89	355.1038	$C_{16}H_{20}O_9$	99.1	-1.0	322	193.0502; 178.0267; 149.0606; 134.0369	Ferulic acid hexoside I	-	+	-	+
13.79	337.0926	$C_{16}H_{18}O_8$	81.3	0.8	300; 320	191.0557; 173.0454; 163.0399	Coumaroylquinic acid I	+	-	+	-
13.90	353.0883	$C_{16}H_{18}O_9$	98.2	-1.3	298; 325	191.0566; 179.0347	Caffeoylquinic acid II* (chlorogenic acid)	+	+	+	+
14.11	325.0929	$C_{15}H_{18}O_8$	92.4	-0.5	326	163.0397; 119.0499	Comaroyl hexoside	-	+	-	+
14.21	353.0882	$C_{16}H_{18}O_9$	83.7	-1.1	325	-	Caffeoylquinic acid III	+	+	+	+
14.72	355.1036	$C_{16}H_{20}O_9$	99.3	-0.4	323	193.0502; 178.0267; 149.0602; 134.0370	Ferulic acid hexoside II	-	+	-	+
15.28	353.0885	$C_{16}H_{18}O_9$	83.2	-1.6	272; 328	191.0565	Caffeoylquinic acid IV	+	+	+	+
15.61	337.0929	$C_{16}H_{18}O_8$	98.4	0.5	272; 328	191.0568	Coumaroylquinic acid II	+	-	+	-
15.84	179.036	$C_9H_8O_4$	94.5	-5.5	295; 324	135.056; 134.0377; 89.0399	Caffeic acid*	-	+	-	+
16.03	295.0467	$C_{13}H_{12}O_8$	95.9	-2.5	298; 330	179.0350; 133.0143; 115.0038	Caffeoylmalic acid	+	-	+	-
16.83	337.0929	$C_{16}H_{18}O_8$	99.8	0.0	272; 326	191.0558	Coumaroylquinic acid III	+	-	+	-
17.30	385.1144	$C_{17}H_{22}O_{10}$	82.0	-0.9	268; 326	267.0724; 249.0617; 223.0458; 205.0353; 147.0294; 113.0241; 91.0551; 85.0294	Sinapic acid hexoside	+	+	+	+
18.01	279.0513	$C_{13}H_{12}O_7$	99.0	-1.0	291; 324	163.0398; 133.0139; 119.0499; 115.0033	Coumaroylmalic acid I	+	-	+	-
18.10	339.0729	$C_{15}H_{16}O_9$	98.1	-1.7	286; 330	309.0621; 223.0616; 208.0372; 193.0507; 164.0480; 149.02543; 133.0142; 115.0039	Sinapic acid malate	+	-	+	-
18.25	279.051	$C_{13}H_{12}O_7$	99.2	0.0	298; 320	163.0401; 133.0139; 119.0500; 115.0033	Coumaroylmalic acid II	+	-	+	-
18.40	309.0625	$C_{14}H_{14}O_8$	96.0	-2.8	286; 325	193.0510; 178.0267; 149.0607; 133.0146; 115.0039	Ferulic acid malate I	+	-	+	-

18.67	309.0623	$C_{14}H_{14}O_8$	98.0	-2.0	288; 320	193.0556; 134.0371	Ferulic acid malate II	+	-	+	-
19.09	193.0511	$C_{10}H_{10}O_4$	98.3	-2.0	293; 325	134.0373	*Trans*-ferulic acid*	-	+	-	+
19.62	193.0503	$C_{10}H_{10}O_4$	84.7	0.3	282; 325	134.0373	Ferulic acid isomer	-	+	-	+
Flavonoids-Flavonols											
13.09	771.2002	$C_{33}H_{40}O_{21}$	97.5	-1.7	356	609.1459; 462.0801; 463.0871; 301.0352; 300.0258	Quercetin *O*-deoxyhexoside di-hexoside	+	+	+	+
13.39	625.141	$C_{27}H_{30}O_{17}$	87.5	-1.0	346	463.0893; 462.0814; 301.0360	Quercetin *O*-di-hexoside	+	+	+	+
15.59	755.2052	$C_{33}H_{40}O_{20}$	94.1	-1.6	356	301.0359; 300.0279	Quercetin di-deoxyhexoside hexoside	+	+	+	+
17.18	609.1486	$C_{27}H_{30}O_{16}$	93.8	-1.9	354	463.0890; 300.0278; 273.0398; 257.0448; 229.0502; 178.9983; 121.0297; 151.0036; 107.0142	Quercetin-3-*O*-rutinoside* (rutin)	+	+	+	+
17.94	463.0888	$C_{21}H_{20}O_{12}$	99.8	-0.3	354	301.0349; 300.0278; 151.0037	Quercetin-3-*O*-glucoside* (isoquercetin)	+	+	+	+
18.68	549.0882	$C_{24}H_{22}O_{15}$	99.2	0.6	354	505.0986; 463.0874; 301.0351; 300.0276	Quercetin 3-*O*-(6"-malonyl)glucoside	+	+	+	+
23.05	301.0373	$C_{15}H_{10}O_7$	83.2	-0.8	371	273.0399; 178.9983; 151.0034; 121.0296; 107.0139	Quercetin*	-	+	-	+
Flavonoids-Flavones											
14.76	579.1367	$C_{26}H_{28}O_{15}$	87.3	-3.3	344	561.1251; 519.1156; 489.1044; 459.0938; 429.0834; 399.0727; 369.0623; 285.0499; 133.0289	Luteolin *C*-hexoside *C*-pentoside I	+	+	+	+
14.89	579.136	$C_{26}H_{28}O_{15}$	96.3	-0.7	354	561.1254; 519.1153; 489.1049; 459.0939; 429.0834; 399.0723; 369.0624; 285.0400; 133.0297	Luteolin *C*-hexoside *C*-pentoside II	+	+	+	+
15.10	563.1415	$C_{26}H_{28}O_{14}$	98.3	-1.5	336	545.1321; 503.1212; 473.1097; 443.0988; 383.0786; 353.0669; 325.0733; 297.0766; 117.0347	Apigenin *C*-hexoside *C*-pentoside I[b]	+	+	+	+
15.60	563.1435	$C_{26}H_{28}O_{14}$	88.3	-4.7	335	545.1312; 503.1203; 473.1104; 443.0999; 383.07858; 353.0680; 325.0726; 297.0778; 117.0343	Apigenin *C*-hexoside *C*-pentoside II[b]	+	+	+	+
16.00	447.0937	$C_{21}H_{20}O_{11}$	98.7	-1.0	350	429.0821; 387.2027; 357.0615; 327.0512; 285.0404; 133.0138	Luteolin 6-*C*-glucoside (isoorientin)[c]	+	+	+	+
16.21	563.142	$C_{26}H_{28}O_{14}$	84.5	-3.3	330	545.1302; 503.1195; 473.1092; 443.0989; 383.0777; 353.0670; 297.0766; 117.0357	Apigenin 6-*C*-hexose-8-*C*-pentose III[b]	+	+	+	+
16.58	447.0938	$C_{21}H_{20}O_{11}$	98.7	-1.3	350	357.0608; 327.0507; 285.0398; 133.0291	Luteolin 8-*C*-glucoside (orientin)[c]	+	+	+	+
16.80	577.1579	$C_{27}H_{30}O_{14}$	98.2	-2.0	330	457.1140; 413.0880; 293.0455	Apigenin *C*-hexoside *C*-deoxyhexoside	+	+	+	+
17.42	431.0989	$C_{21}H_{20}O_{10}$	99.4	-1.2	326	341.0663; 311.0553; 283.0603; 269.0444; 268.0372; 117.0342	Apigenin 8-*C*-glucoside (vitexin)	+	+	+	+
17.82	447.0932	$C_{21}H_{20}O_{11}$	89.9	-1.0	352	285.0406; 284.0327; 197.0806; 175.0282; 133.0294	Luteolin 7-*O*-glucoside* (cynaroside)	+	+	+	+

24.29	269.0459	$C_{15}H_{10}O_5$	98.8	0.0	336	241.0495; 227.0352; 225.0553; 201.0551; 183.0445; 181.650; 159.0457; 151.0033; 149.0240; 117.0344; 107.0137	Apigenin*	+	+	+	+
22.46	285.0407	$C_{15}H_{10}O_6$	95.7	-1.8	349	267.0298; 257.0453; 243.0297; 241.504; 217.0506; 213.0549; 199.0396; 197.0604; 175.0395; 151.0031; 133.0295	Luteolin *	+	+	+	+
Flavonoids-Flavanones												
16.09	611.1624	$C_{27}H_{32}O_{16}$	94.2	-1.5	280	449.1094; 287.0563; 151.0036; 135.0445	Eriodictyol di-hexoside	-	-	-	+
17.95	449.1099	$C_{21}H_{22}O_{11}$	96.2	-2.3	280	287.0565; 151.0039; 135.0451; 107.0142	Eriodictyol hexoside I	-	-	-	+
19.87	449.1086	$C_{21}H_{22}O_{11}$	96.0	0.9	286	287.058; 151.0033; 135.0450; 107.0138	Eriodictyol hexoside II	-	-	-	+
22.91	287.0569	$C_{15}H_{12}O_6$	97.7	-2.5	282	151.0039; 135.0449; 125.0241; 107.0139; 83.0137	Eriodictyol	-	+	-	+
24.46	271.0617	$C_{15}H_{12}O_5$	98.8	-2.0	289	177.0183; 151.0034; 119.0499; 107.0137	Naringenin	+	+	+	+
Flavonoids-Flavanols												
14.52	289.0717	$C_{15}H_{14}O_6$	81.5	0.6	278	245.0821; 205.0497; 203.0707; 161.0606; 125.0245	(+)-catechin*	+	+	+	+
Flavonoid-Flavanonols												
19.50	303.0510	$C_{15}H_{12}O_7$	98.7	-0.1	283	285.0399; 151.0034; 125.0240	Dihydroquercetin (taxifolin)	-	+	-	+
Flavonoid-Isoflavones												
22.68	547.1093	$C_{25}H_{24}O_{14}$	88.6	-0.7	-	503.1204; 299.0558; 284.0320; 165.0191; 149.9954; 133.0294; 121.0292	Hydroxygenistein methyl ether malonylhexoside	+	-	+	-
24.46	269.0459	$C_{15}H_{10}O_5$	85.5	-0.9	260; 330	241.0492; 225.0556; 201.0552; 151.0031;133.0292; 119.0504; 117.0349; 107.0139	Genistein*	-	-	+	-
25.82	299.0555	$C_{16}H_{12}O_6$	99.6	2.2	260; 335	298.0475; 285.0357; 284.0310; 256.0370; 240.0419; 239.0343; 165.0190; 149.9955; 133.0289; 121.0291	7-methoxy 2'-hydroxy genistein (cajanin)	+	+	+	+
26.49	353.1037	$C_{20}H_{18}O_6$	97.9	-1.9	266	325.1074; 298.0472; 283.0604; 219.0655; 175.0397; 133.0290; 133.0658	Prenylhydroxygenistein I	+	-	+	-
27.19	353.1034	$C_{20}H_{18}O_6$	84.2	-2.2	264	325.1074; 285.1127; 284.0322; 219.0657; 175.0398; 151.0761; 133.0657; 133.0295	Prenylhydroxygenistein II	+	+	+	+
27.62	353.1037	$C_{20}H_{18}O_6$	97.5	-2.0	264; 344	325.1078; 285.1127; 284.0320; 219.0660; 175.0762; 151.0761; 151.0032; 133.0657; 133.0293	Prenylhydroxygenistein III	+	+	+	+
27.69	337.1087	$C_{20}H_{18}O_5$	94.8	-2.7	-	293.0462; 282.0534; 269.1190; 254.0516; 133.0658; 117.0346	Prenylgenistein I	+	+	+	+
27.82	283.0614	$C_{16}H_{12}O_5$	99.7	-0.5	-	268.0374; 239.0348; 151.0040; 132.0214; 107.0133	Genistein 4'-methyl ether (biochanin A)	+	+	+	+

28.54	337.1082	$C_{20}H_{18}O_5$	98.2	0.2	265; 339	293.0449; 282.0526; 269.0436; 268.0368; 254.0564; 238.0622; 225.0469; 133.0287	Prenylgenistein II	+	+	+	+
29.10	337.1084	$C_{20}H_{18}O_5$	99.0	-0.3	266; 340	293.0452; 282.0528; 269.0446; 268.0370; 253.0500; 254.0574; 238.0624; 133.0923	Prenylgenistein III	+	+	+	+
Hydroxycoumarins											
13.09	339.0728	$C_{15}H_{16}O_9$	94.8	-2.4	279; 330	177.0191; 133.0293	Esculetin hexoside I	+	+	+	+
13.71	339.075	$C_{15}H_{16}O_9$	83.4	-0.1	279; 335	177.0197	Esculetin hexoside II	+	-	+	-
15.39	177.0187	$C_9H_6O_4$	97.0	-3.9	-	149.0241; 133.0293; 105.0346	Dihydroxycoumarin I	+	+	+	+
18.32	205.0146	$C_{10}H_6O_5$	98.6	-1.8	286	161.0243; 133.0295; 117.0348; 105.0347; 89.0396; 77.0398	6-carboxyl-umbelliferone	+	-	+	-
19.34	161.0244	$C_9H_6O_3$	97.4	-1.7	283; 324	133.0291; 117.0342; 105.034	7-Hydroxycoumarin* (umbelliferone)	+	+	+	+
20.86	177.0194	$C_9H_6O_4$	93.5	0.7	285	149.0247; 133.02937; 105.0346	Dihydroxycoumarin II	+	+	+	+
22.60	205.0517	$C_{11}H_{10}O_4$	92.2	-5.2	244; 252sh; 289; 338	187.0400; 161.0607; 146.0372; 133.0657; 118.0419; 105.0709	Phellodenol A/hydrated form of 4',5'-dihydropsoralen	+	-	+	-
22.94	235.0616	$C_{12}H_{12}O_5$	97.8	-1.7	255; 282	217.0499; 201.0189; 191.0712; 176.0477; 161.0241; 148.0523; 133.0293; 117.0345	Murrayacarpin B/di-hydrated form of bergapten	+	-	+	-
27.95	229.0872	$C_{14}H_{14}O_3$	99.5	-0.4	-	213.0553; 185.0603; 146.0368; 130.0420; 118.0426	Prenyl-7-hydroxycoumarin	+	+	+	-
Others											
17.88	365.0964	$C_{17}H_{18}O_9$	97.8	-1.7	244; 288; 334	203.0347; 159.0453; 131.0497; 130.0421; 103.0552	(2Z)-3-[6-(β-D-glucopyranosyloxy)-1-benzofuranyl]-2-propenoic acid (psoralic acid glucoside)	+	-	+	-

[a]Compounds described here for first time in family Moraceae. Several saccharide combinations and conjugation posititons are reported in different plant families (see KNApSAck, Reaxys or SciFinder databases).

[b]Apigenin *C*-hexoside pentoside could be schaftoside (apigenin 6-*C*-glucoside 8-*C*-arabinoside) or isochaftoside (apigenin 6-*C*-arabinoside 8-*C*-glucoside). The latter were previously described in *F. carica* leaves (Takahashi *et al.*[32]).

[c]The identification was based on the elution pattern under similar analytical conditions (Tahir *et al.*[39]).

[d]Compounds described here for first time in family Moraceae and common in the family Fabaceae (see KNApSAck, Reaxys or SciFinder databases)

[e]6-, 8- and 3'-prenylgenistein were previously reported in other *Ficus* species.

*Identification confirmed by comparison with standards. RT, retention time; Exp., experimental. L, leaves; F, fruits. The UV data agreed with Gómez-Romero *et al.*[28]; Lin *et al.*[36]; Tsimogiannis *et al.*[37]

684 Table 3. Other phenolic compounds characterized using the positive ionization mode in leaves and fruits of *F. carica* cultivars 'Tounsi' and

685 'Temri'.

RT (min)	$[M+H]^+$	Formula	Score	Error (ppm)	UV (nm)	Main fragments *via* MS/MS	Proposed compound	Presence			
								'Tounsi'		'Temri'	
Anthocyanins								L	F	L	F
11.51	611.1603	$C_{27}H_{31}O_{16}$	93.9	-0.3	520	449.1078; 287.0565	Cyanidin 3,5-diglucoside	-	+	-	+
13.13	595.1667	$C_{27}H_{31}O_{15}$	98.5	0.5	282; 520	449.1073; 287.0547	Cyanidin 3-rutinoside	-	+	-	+
Furanocoumarins											
15.97	365.0872	$C_{17}H_{16}O_9$	98.6	-0.9	250; 264; 308	203.0336; 175.0438; 147.0438; 131.0387; 119.0487; 101.0387; 91.0540	Hydroxypsoralen hexoside I [a]	+	-	+	-
16.65	365.0871	$C_{17}H_{16}O_9$	96.9	-1.0	252; 264; 310	203.0336; 175.0389; 147.0440; 131.0395; 119.0485; 91.0539	Hydroxypsoralen hexoside II[a]	+	-	+	-
17.58	247.0969	$C_{14}H_{14}O_4$	94.3	-2.4	-	229.0845; 213.0548; 189.0574; 175.0393; 147.0438; 119.0489; 103.0545	Marmesin isomer I[b]	+	+	+	+
17.64	409.1496	$C_{20}H_{24}O_9$	96.2	-1.0	-	247.0962; 229.0862; 213.0545; 185.0602; 175.0389; 147.0348; 119.0487; 91.0543	Marmesinin	+	-	+	-
17.77	235.0606	$C_{12}H_{10}O_5$	93.2	-3.3	256; 303	217.0505; 202.0259; 174.0547; 131.0489; 115.0537	Methoxypsoralen derivative (hydrate)	+	-	+	-
21.8	189.0549	$C_{11}H_8O_3$	86.8	-1.5	250; 290	161.0605; 147.0441; 133.0644; 119.0489; 105.0700	4',5'-Dihydropsoralen	+	-	+	-
22.05	247.0971	$C_{14}H_{14}O_4$	95.0	-2.6	255	229.0858; 213.0545; 189.0537; 175.0392; 147.0442; 119.0492; 103.0544	Marmesin isomer II[b]	+	+	+	+
22.30	305.1030	$C_{16}H_{16}O_6$	95. 7	-3.0	257; 266; 310	203.0344; 175.0391; 159.0441; 147.0438; 131.0489; 119.0490	Oxypeucedanin hydrate	+	-	+	-
22.48	203.0343	$C_{11}H_6O_4$	85.8	-2.0	254; 269; 306	147.0442; 131.0494; 129.0332; 119.0496; 101,0376; 91.0541	Hydroxypsoralen[d]	+	-	+	-
24.46	187.0317	$C_{11}H_6O_3$	80.0	-1.7	254; 296; 328	159.0440; 131.0492; 115.0542; 103.0543	Psoralen	+	+	+	+
26.01	217.0502	$C_{12}H_8O_4$	97.6	-2.4	258; 266; 310	202.0259; 174.0311; 159.0447; 146.0359; 131.0490; 118.0410; 115.0486	Methoxypsoralen	+	+	+	+
26.26	287.0918	$C_{16}H_{14}O_5$	99.4	-1.2	-	203.0338; 175.1124; 159.0429; 147.0430; 131.0477; 119.0487; 103.0550	Oxypeucedanin	+	-	+	-
28.25	271.0980	$C_{16}H_{14}O_4$	91.8	-5.3	-	229.0503; 215.0349; 203.0349; 201.0554, 187.0397;	Isopentenoxypsoralen	+	-	+	-

						173.0603; 159.0448; 131.0495; 117.0702					
31.16	285.1131	$C_{17}H_{16}O_4$	95.5	-3.4	268; 309	243.0638; 229.0478; 217.0473; 201.0530; 186.0293; 115.0521	Prenyl methoxypsoralen	+	+	+	+
Isoflavones[e]											
30.85	299.0906	$C_{17}H_{14}O_5$	96.5	2.9	262; 329	284.0660; 267.0633; 256.0711; 243.0998; 166.0242; 137.0576	Hydroxy-dimethoxyisoflavone	+	+	+	+
Others											
17.21	205.0502	$C_{11}H_8O_4$	96.1	-3.5	255; 290; 335	187.0401; 133.0648; 131.0491; 115.0537; 107.0491; 105.0700; 103.0541	Psoralic acid/dihydro-hydroxypsoralen	+	-	+	-

686 RT, retention time; Exp., experimental. L, leaves; F, fruits. The UV data agreed with Dueñas *et al.*[4]; Teixeira *et al.*[8]; Frerot *et al.*[55]; Tang *et al.*[65]

687 [a]Hydroxypsoralen hexoside could be 5-hydroxypsoralen hexoside (bergaptol hexoside) or 8-hydroxypsoralen hexoside (xanthoxol hexoside).

688 [b]Marmesin was previously described in *F. carica* and its enantiomeric form nodakenetin in *Ficus tsiangii*.

689 [c]Hydroxypsoralen could be 5-hydroxypsoralen (bergaptol) or 8-hydroxypsoralen (xanthoxol) according to Yang *et al.*[52]

690 [d]Compounds described here for first time in *F. carica* but described in the family Moraceae and other families (see KNApSAck, Reaxys or SciFinder databases).

691 [e]Non detected in the negative ionization mode.

692 [f]Compounds described here for first time in the family Moraceae and common in the family Fabaceae (see KNApSAck, Reaxys or SciFinder databases).

693

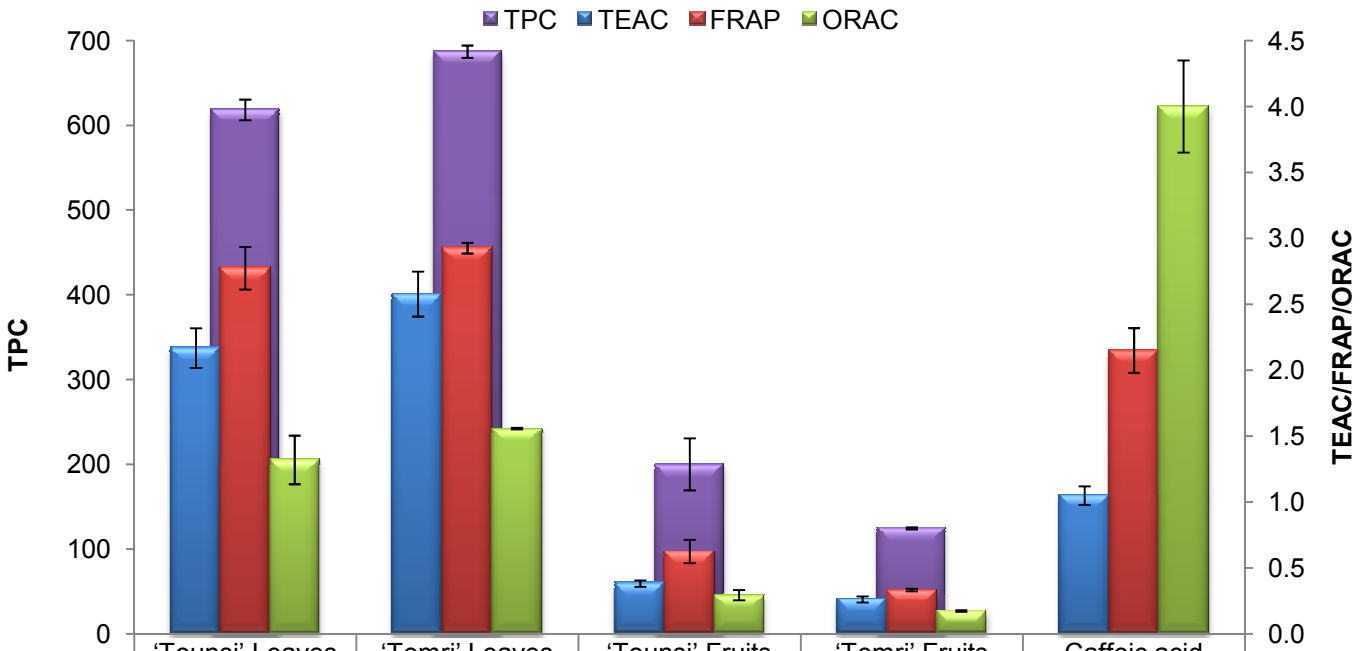
694

695 **Figure captions**

696 Fig. 1. Bar graph of total phenol content (TPC) (mg of gallic acid/100 g sample) of leaves and
697 fruits from *F. carica* cultivars 'Tounsi' and 'Temri' and antioxidant activity evaluated by:
698 trolox equivalent antioxidant capacity (TEAC) (mmol eq. Trolox/100 g of sample), ferric ion
699 reducing antioxidant power (FRAP) (mmol eq. $FeSO_4$/100 g sample) and oxygen radical
700 absorbance capacity (ORAC) (mmol eq. Trolox/100 g sample) assays. The primary *Y* axis
701 corresponds to TPC and the secondary *Y* axis corresponds to antioxidant activity. Data are
702 given as mean ± standard deviation. Caffeic acid was used as the control and expressed as
703 mmol eq. Trolox or $FeSO_4$/mmol of compound.

704 Fig. 2. Chromatographic profiles of the leaves and fruits from *F. carica* cultivars 'Tounsi' and
705 'Temri' obtained by RP-UHPLC-DAD-QTOF-MS: base peak chromatogram (BPC) in
706 negative ionization mode using analytical method 1 and UV chromatograms at 254 and 520
707 nm using analytical method 2. In each figure, the intensity was scaled to the largest area.

708 Fig. 3. Examples of MS/MS spectra of phenolic compounds highlighting the main fragments
709 from *F. carica*: (a) syringic acid malate (isomer I), (b) quercetin 3-*O*-(6"-malonyl) glucoside,
710 and (c) methoxypsoralen.



	'Tounsi' Leaves	'Temri' Leaves	'Tounsi' Fruits	'Temri' Fruits	Caffeic acid
TPC	618.29	686.88	200.18	124.48	
TEAC	2.17	2.58	0.38	0.26	1.05
FRAP	2.77	2.93	0.63	0.33	2.15
ORAC	1.32	1.56	0.29	0.17	4.00

Fig. 2