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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, flavanons, 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
water extract of fig leaves and its chloroform fraction tend to normalize the antioxidant status of diabetic rats.\textsuperscript{17} Although several studies have related the bioactivity of this and other \textit{Ficus} species to the phenolic constituents,\textsuperscript{15} more studies are needed to clarify this issue. Thus, novel analytical methodologies may help in the elucidation of the bioactive molecules.

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

2. Results and discussion

2.1 Total phenolic content and antioxidant activity of the ‘Tounsi’ and ‘Temri’ fig cultivars

Total phenolic content

In general, the leaves were richer in phenolic compounds than fruits, the TPC value being the highest in the ‘Temri’ cultivar (686.88 mg of gallic acid/100 g of leaves; Fig. 1). However, the dried whole fruits from the ‘Tounsi’ cultivar presented a higher TPC value (200.18 mg of gallic acid/100 g of dried fruits) than ‘Temri’ (124.48 mg of gallic acid/100 g of dried fruits) (Fig. 1). Concerning the fig fruits, Solomon \textit{et al.}\textsuperscript{7} evaluated the TPC of six common commercial figs, which had values ranging from 48.6 to 281.1 mg of gallic acid/100 g.
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. 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\textsuperscript{10}, anthocyanins\textsuperscript{26}, as well as antioxidant activity.\textsuperscript{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.\textsuperscript{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\textsuperscript{7} and FRAP\textsuperscript{25} of fig fruits. However, in other foods little or no relationship has been found and other antioxidant compounds may contribute greatly.\textsuperscript{20} Thus, our results indicate that phenolic compounds are determinants of antioxidant agents in the \textit{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.
On the one hand, the UV/Vis was a valuable tool for classifying phenolic compounds into families and subfamilies according to the presence of one or two characteristic absorption bands in the UV: band I and band II that come from the B-ring cinnamoyl structure and the A-ring benzoyl or benzene structure, respectively. In this regard, the wavelength of maximum absorption for the characterized phenolic compounds is depicted in Tables 2 and 3, as commented above. Besides, as an example, Fig. S1 shows the UV spectra of several phenolic types from *F. carica*, where band I ranged from 325−371 nm, approximately, and band II was around 260−298 nm. In the case of flavonoids, at the same time that the heterocyclic C-ring structure serves for their sub-classification, the most intensive band also depends on this ring. For example quercetin (flavonol) showed a prominent band I with a maximum at 371 nm, whereas naringenin (flavanone) presented a maximum at 289 nm that comes from the band II (Fig. S1). Genistein (isoflavone) was characterized by a maximum around 260 nm with higher intensity than the second maximum at 330 nm. This UV absorption behaviour enabled to differentiate isoflavones from flavones, preliminarily. In addition, anthocyanins presents a maximum absorption at visible wavelengths, around 520 nm, that is a characteristic feature of this flavonoid subclass.

On the other hand, the QTOF mass analyzer delivers accurate mass measurements and isotopic fidelity (see experimental section) that allow the molecular formula of the target compound to be obtained. Therefore, in order to procure confident formula assignments for target molecular ions, the lower mass error value and the higher MS score the better (see values in Tables 2 and 3). Afterwards, databases as well as literature were consulted for the retrieval of chemical structure information taking the MS and UV data into account. Finally, using MS/MS analyses, the structure of the parent compound may be tentatively confirmed through studying the fragmentation pattern: fragment ions and neutral losses, which are also accurately measured. As an example, this general identification process is summarized in Fig.
S2. Moreover, the RT served as criterion of polarity and elution order. In this way, a total of
13 phenolic compounds were confirmed with standards by comparison of the RT, UV spectra
and MS/MS data in order to validate our characterization process (see Table 2).

Briefly, with a concise data mining, 91 phenolic compounds were characterized in the
negative ionization mode (method 1) including hydroxybenzoic acids, hydroxycinnamic
acids, flavonoids that were represented by flavonols, flavones, flavanones, flavanonols,
flavanols and isoflavones subclasses, and hydroxycoumarins (Table 2). Several of these
compounds were also detected using the analytical method 2 (data not shown). These data
were complemented with 18 phenolic compounds tentatively characterized using the positive
ionization mode, belonging to anthocyanins, furanocoumarins (Table 3) and a isoflavone,
which were either poorly ionized in the negative ionization mode or undetectable.
Furthermore, the major part of the characterized phenolic compounds were tentatively
reported in F. carica for the first time in this work (Fig. S3), and other unreported phenolic
structures were proposed as well according to their UV, MS and MS/MS information. Several
previous studies on F. carica applied GC and HPLC coupled to several detectors, including
mass analyzers such as quadrupole and ion trap. However, there were few compounds
identified in these works, which are in the range from 4 to 15. One of the reason is that the
authors focused on a particular phenolic subclass, e.g. anthocyanins, or target phenolic
compounds. Therefore, at this point, our findings remark the potencial of RP-HPLC-DAD-
QTOF-MS in order to perform successful and extensive characterization works of plant
extracts and as starting point for structure elucidation of new molecules. In this regard, the
MS analysis via electrospray ionization in the negative and positive ionization modes was
complementary and enabled the detection and characterization of a large number of
compounds. However, the analyst must be cautious in offering interpretations until all the
information is evaluated. It is probably the most critical and long time-consuming part since,
although there are efforts to generate spectral libraries using standards, unfortunately LC-ESI-MS methods often lack the consistency, standardization or reproducibility that characterizes GC-MS or nuclear magnetic resonance spectroscopy.\textsuperscript{27}

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

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

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

Overall, 45 phenolic acids were found in \textit{F. carica} (Tables 2 and S1), belonging to hydroxybenzoic and hydroxycinnamic acids. The main qualitative differences were found between leaves and fruits. The first phenolic class with a more polar feature eluted over a 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\(_2\)O) 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 \(F. \text{carica}\). 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 ([\(C_9H_{10}O_5-H\)])\(\) and 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\(_2\)O, respectively. In addition, the presence of fragments at \(m/z\) 167.0354 and 153.0559 indicated the loss of CH\(_2\)O 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, hydroxycinnamnics also presented a higher signal in leaves than in fruits (Fig. S4a and d). The presence of caffeic acid and \textit{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
reported in this species.\textsuperscript{8,9,32–34} Moreover, other three isomers of chlorogenic acid were also found. Recently, Olivera \textit{et al.}\textsuperscript{9} described the isomers 3-\textit{O}- and 5-\textit{O}-caffeoylquinic acids (chlorogenic acid) in Portuguese white fig samples.

Interestingly, as for the aforementioned phenolic acid class, several conjugated forms were reported for the first time in \textit{F. carica} and as well as in the Moraceae family (Tables 2 and S1). For example, three isomers of caffeoylquinic acid hexoside were characterized based on their molecular formula and UV and MS/MS spectra, which was in agreement with previous studies on other plant families.\textsuperscript{30,35,36} In a similar manner, the fragmentation pattern of caffeic, coumaric and ferulic acids conjugated with hexose or organic acids were in accordance with previous studies.\textsuperscript{28–30,32,35} Overall, these conjugations could be established on the basis of the MS/MS spectra, because the moieties of the latter and/or the free phenolic acid were observed (Tables 2 and S1). For example, fragment ions with \textit{m/z} values of 191.0561 ([C\textsubscript{7}H\textsubscript{12}O\textsubscript{6}−H]) (quinic acid) and 179.0350 ([C\textsubscript{9}H\textsubscript{8}O\textsubscript{4}−H]) (caffeic acid) were released from caffeoylquinic acid isomers.

Finally, a phenylpropanoid acid related to furanocoumarin psoralen was assigned as psoralic acid glucoside, according to the recent findings in \textit{F. carica} leaves (Takahashi \textit{et al.}\textsuperscript{32}), which also suggested that this compound could be a precursor of psoralen. Their fragmentation pattern agreed with the Takahashi’s study, as we also observed the loss of glucose (\textit{m/z} 203.0347) and the loss of CO\textsubscript{2} (\textit{m/z} 159.0453) as the main fragments in MS/MS. This compound was detected in leaves (Fig. S4b and c). Furthermore, a compound related to this was detected in negative and positive ion modes (e.g. see compound with \textit{m/z} value at 205.0502 and RT 17.21 min in Tables 3 and S2), which could be the aglycone or a dihydro form of hydroxypsoralen. The MS data, the UV spectra and published literature were not 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 $\lambda_{\text{max}}$ 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\(_2\)O), 60.0211 u (C\(_2\)H\(_4\)O\(_2\)), 90.0317 u (C\(_3\)H\(_6\)O\(_3\)), 120.0423 u (C\(_4\)H\(_8\)O\(_4\)), 180.0634 u (C\(_6\)H\(_{12}\)O\(_6\)) and/or 210.0740 u (C\(_7\)H\(_{14}\)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.\(^{39}\)
contrast to C-glycosides, the MS/MS spectra of the O-glycosidic forms of apigenin and luteolin showed more abundant fragment ions corresponding to the aglycones after the neutral loss of 162.0526 u (hexose) and 308.1122 u (hexose-deoxyhexose), respectively (Tables 2 and S1).

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

Although isoflavonoids are widely distributed in the Moraceae family,\textsuperscript{44,45} there is no mention in the literature about this class in \textit{F. carica}. Our methodology allows ten isoflavones to be tentatively characterized (Tables 2 and S1), including several prenylated forms. Genistein and methylated derivatives of genistein and prenylgenistein have been previously described in other \textit{Ficus} species.\textsuperscript{45-47} Only genistein (RT 24.46 min, m/z 269.0459) could be confirmed with standards and was found in the leaves and fruits of both cultivars. The UV data clearly show a main maximum close to 260 nm, which is in accordance with the findings of Shen \textit{et al.}\textsuperscript{48} Overall, among other fragments found in the MS/MS spectra of the genistein
derivatives, aglycone at $m/z$ value of 269.1190 (even electron) or 268.0374 (odd electron) were detected, and also the characteristic fragment ions at $m/z$ 151.0031 ($^{1,3}\text{A}^-$), 133.0658 ($^{0,3}\text{B}^-$) (even electron) or 132.0214 (odd electron) and 117.0346 ($^{1,3}\text{B}^-$) released from the breakage of genistein backbone. In the case of the malonylhexoside derivative of hidroxygenistein methyl ether, the loss of CO$_2$ from the malonyl group and the subsequent loss of 204.0634 u ($\text{C}_8\text{H}_{12}\text{O}_6$, acetyl hexosyl rest) were also observed in MS/MS. A dimethyl ether isoflavone was only detected in positive ionization mode (Tables 3 and S2), exhibiting the UV maximums related to the isoflavone core, and the MS/MS spectrum was in agreement with 7-hydroxy-6,4'-dimethoxyisoflavone (afmosin). Since there was no information about this compound in the Moraceae literature and the MS/MS data were not enough to distinguish the exact position of the free hydroxyl or methoxy groups, the compound was denominated as hydroxy-dimethoxyisoflavone.

Interestingly, the prenylated isoflavones presented a remarkably higher signal in leaves than in fruits (Fig. S4c and f). Not surprisingly, they eluted at higher RT due to the presence of this lipophilic prenyl side chain, with an RT from 26.49 to 29.10 min. The UV data and main MS/MS fragments agreed with prenylated forms of genistein (6-, 3',- and 8-prenylgenistein) present in the Lupinus species. In this regard, 6- and 8-prenylgenistein was reported in stem barks and fruits of other Ficus species (e.g. Ficus tikoua). Furthermore, related prenylated forms linked to hydroxygenistein were also tentatively characterized, and the UV data agreed with the findings of Shen et al. In general, these prenylated compounds were characterized by the neutral loss of C$_4$H$_7$ (55.0548 u) and C$_5$H$_9$ (69.0704 u) from the prenyl moiety, amongst others. In this regard, prenylated flavonoids possess unique bioactivities relative to their unmodified parent compounds, particularly potent antifungal activity.
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.\textsuperscript{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,\textsuperscript{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*.\textsuperscript{45} and it is suggested that it is the precursor of furanocoumarins.\textsuperscript{51} The rest of the hydroxycoumarins were putatively characterized on the basis of the MS/MS spectra, UV data and literature.\textsuperscript{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.\textsuperscript{29} A prenylated form of 7-hydroxycoumarin was also tentatively characterized 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.*\textsuperscript{52}

**Furanocoumarins**

There are two type of furanocoumarins in nature, linear and angular ones.\textsuperscript{53} *F. carica* contains mainly the first class, with psoralen and 8-methoxypsoralen (bergapten) being the major representatives.\textsuperscript{12,24,32} In this regard, a total of 14 furanocoumarins were tentatively
characterized in Tunisian figs in positive ionization mode, including the aforementioned compounds (Tables 3 and S2). The major part of the characterized furanocoumarins are described here for the first time in *F. carica* and also in the Moraceae family (e.g. isopentenoxypsoralen, at RT 28.25 min and *m/z* 271.098). Alternatively, others have been previously reported in other *Ficus* species, such as marmesin isomers, 4',5'-dihydropsoralen and oxypeucedanin hydrate (Table S2). In agreement with Yang *et al.*'s study on *Radix glehniae*, we detected a characteristic series of fragment ions for furanocoumarins that were mainly generated by consecutive losses of CO (e.g. Fig. 3c). As stated above, in a similar way the loss of C$_3$H$_6$ (42.0470 u), C$_4$H$_8$ (56.0626 u) and C$_5$H$_8$ (68.0626 u) from the prenyl moiety was also observed in the MS/MS spectra of prenylated furanocoumarins, isopentenoxypsoralen and prenyl methoxypsoralen. In addition, the UV data of furanocoumarins also agreed with that of Frérot *et al.*

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

3. Conclusions

Despite the popularity of the consumption of dried fig fruits, there is little information about its antioxidant activity. Moreover, in the case of its qualitative phenolic composition, previous studies have only focused on target phenolic compounds. In our study, a total of 109 phenolic compounds were characterized in *F. carica* samples. Most of them were reported for the first time in *F. carica* species. In addition, fig leaves presented a richer phenolic 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 (Na₂CO₃), 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 (K₂S₂O₈) and ferric sulphate (FeSO₄). They were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dehydrated sodium phosphate, trihydrated sodium acetate, sodium acetate, ferric chloride (FeCl₃ · 6H₂O) and hydrochloric acid (HCl) were obtained from Panreac (Barcelona, Spain). Phenolic standards available in our laboratory were bought from Sigma-Aldrich:
chlorogenic acid, caffeic acid, vanillic acid, *trans*-ferulic acid, rutin, quercetin-3-*O*-glycoside, quercetin, luteolin-7-*O*-glucoside, apigenin, luteolin, (+)-catechin, genistein, 7-hydroxycoumarin and gallic acid. The degree of purity of the standards was around 95% (w/w).

**Fig samples**

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

**Sample preparation**

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

**Total phenol content (TPC)**

The TPC of the extracts was determined in triplicate by the colorimetric assay using the Folin–Ciocalteu reagent modified according to Romero-de Soto *et al.* with 96-well polystyrene microplates (ThermoFisher) and a Synergy Mx Monochromator-Based Multi-Mode Microplate reader (Bio-Tek Instruments Inc, Winooski, VT, USA). The absorbance of 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. The method was modified as described by Laporta et al. Briefly, the ABTS radical cation (ABTS⁺) 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⁺ 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⁺ 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. The stock solutions included 300 mM acetate buffer (1.23 g C₂H₃NaO₂ + 0.8 mL C₂H₄O₂ + 49.2 mL of water, pH = 3.6 adjusted with HCl), 10 mM of TPTZ solution in 40 mM HCl and 20 mM FeCl₃ in water. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL of TPTZ and 2.5 mL of FeCl₃ 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
The final absorbance of each sample was compared with those from the standard curve made from FeSO$_4$ · 7H$_2$O (12.5–200 µM, final concentration in wells). Caffeic acid was used as a positive control. The results are expressed in mmol of FeSO$_4$ equivalents /100 g of sample.

**ORAC assay**

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

**Characterization of phenolic compounds by UHPLC–DAD-QTOF-MS**

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

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.\textsuperscript{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.\textsuperscript{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,\textsuperscript{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) phosphazene (\( m/z \) 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).

Acknowledgements

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Research and Information and Communication Technologies, Tunisia, for its support of this research work.

Conflicts of interest

The authors declare no competing financial interest.

References


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

<table>
<thead>
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<th>TPC</th>
<th>TEAC</th>
<th>FRAP</th>
<th>ORAC</th>
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<td>0.994**</td>
<td>0.997**</td>
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<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
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<tr>
<td>TEAC</td>
<td>Pearson Correlation</td>
<td>0.994**</td>
<td>1</td>
<td>0.991**</td>
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<td>Sig. (2-tailed)</td>
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<tr>
<td>FRAP</td>
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<td>0.991**</td>
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<td>Sig. (2-tailed)</td>
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<tr>
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<td>0.985**</td>
<td>0.984**</td>
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</table>

Antioxidant activity: TEAC, trolox equivalent antioxidant capacity; FRAP, ferric ion reducing antioxidant power; ORAC, oxygen radical absorbance capacity.

**Correlation is significant at the 0.01 level (2-tailed).**
Table 2. Phenolic compounds characterized using the negative ionization mode in leaves and fruits of *F. carica* cultivars ‘Tounsi’ and ‘Temri’.

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<th>[M-H]</th>
<th>Formula</th>
<th>Score</th>
<th>Error (ppm)</th>
<th>UV (nm)</th>
<th>Main fragments via MS/MS</th>
<th>Proposed compound</th>
<th>Presence ‘Tounsi’</th>
<th>Presence ‘Temri’</th>
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<td>C_{13}H_{16}O_{9}</td>
<td>98.9</td>
<td>-1.2</td>
<td>-</td>
<td>153.0190; 152.0109; 108.0212; 109.0293</td>
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<td>+</td>
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Hydroxybenzoic acid II

Vanillic acid*

Hydroxycinnamic acids and derivatives

Caffeoylquinic acid hexoside I

Caffeoylquinic acid hexoside II

Caffeoylquinic acid III

Caffeoylquinic acid IV

Caffeoylquinic acid II* (chlorogenic acid)

Caffeoylquinic acid V

Dihydrocaffeic acid hexose

Ferulic acid hexoside I

Ferulic acid hexoside II

Ferulic acid hexoside III

Ferulic acid hexoside IV

Sinapic acid hexoside

Sinapic acid malate

Coumaroylquinic acid I

Coumaroylquinic acid II

Coumaroylquinic acid III

Coumaroylquinic acid IV

Coumaroylquinic acid V

Coumaroylmalic acid I

Coumaroylmalic acid II

Coumaroylmalic acid III

Caffeic acid*
| 18.67 | 309.0623 | C\textsubscript{14}H\textsubscript{12}O\textsubscript{8} | 98.0 | -2.0 | 288; 320 | 193.0556; 134.0371 | Ferulic acid malate II | + | - | + | - |
| 19.09 | 193.0511 | C\textsubscript{10}H\textsubscript{14}O\textsubscript{4} | 98.3 | -2.0 | 293; 325 | 134.0373 | Trans-ferulic acid* | - | + | - | - |
| 19.62 | 193.0530 | C\textsubscript{10}H\textsubscript{14}O\textsubscript{4} | 84.7 | 0.3 | 282; 325 | 134.0373 | Ferulic acid isomer | - | + | - | + |

**Flavonoids-Flavonols**

| 13.09 | 771.2002 | C\textsubscript{33}H\textsubscript{48}O\textsubscript{21} | 97.5 | -1.7 | 356 | 609.1459; 462.0801; 463.0871; 301.0352; 300.0258 | Quercetin O-deoxyhexoside di-hexoside | + | + | + | + |
| 13.39 | 625.1411 | C\textsubscript{22}H\textsubscript{39}O\textsubscript{17} | 87.5 | -1.0 | 346 | 463.0893; 462.0814; 301.0360 | Quercetin O-di-hexoside | + | + | + | + |
| 15.59 | 755.2052 | C\textsubscript{33}H\textsubscript{48}O\textsubscript{20} | 94.1 | -1.6 | 356 | 301.0359; 300.0279 | Quercetin di-hexoside hexoside | + | + | + | + |
| 17.18 | 609.1486 | C\textsubscript{22}H\textsubscript{39}O\textsubscript{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\textsubscript{21}H\textsubscript{30}O\textsubscript{12} | 99.8 | -0.3 | 354 | 301.0349; 300.0278; 151.0037 | Quercetin-3-O-glucoside* (isoquercetin) | + | + | + | + |
| 18.68 | 549.0882 | C\textsubscript{24}H\textsubscript{32}O\textsubscript{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\textsubscript{17}H\textsubscript{10}O\textsubscript{7} | 83.2 | -0.8 | 371 | 273.0399; 178.9983; 151.0034; 121.0296; 107.0139 | Quercetin* | - | + | - | + |

**Flavonoids-Flavones**

| 14.76 | 579.1367 | C\textsubscript{26}H\textsubscript{30}O\textsubscript{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 | 579.1366 | C\textsubscript{26}H\textsubscript{30}O\textsubscript{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\textsubscript{26}H\textsubscript{30}O\textsubscript{14} | 98.3 | -1.5 | 336 | 545.1321; 503.1212; 473.1097; 443.0998; 383.0786; 353.0669; 325.0733; 297.0766; 117.0347 | Apigenin C-hexoside C-pentoside I\textsuperscript{a} | + | + | + | + |
| 15.60 | 563.1435 | C\textsubscript{26}H\textsubscript{30}O\textsubscript{14} | 88.3 | -4.7 | 335 | 545.1312; 503.1203; 473.1104; 443.0999; 383.0785; 353.0680; 325.0726; 297.0778; 117.0343 | Apigenin C-hexoside C-pentoside II\textsuperscript{a} | + | + | + | + |
| 16.00 | 447.0937 | C\textsubscript{21}H\textsubscript{20}O\textsubscript{11} | 98.7 | -1.0 | 350 | 429.0821; 387.0207; 357.0615; 327.0512; 285.0404; 133.0138 | Luteolin 6-C-glucoside (isorientin)\textsuperscript{f} | + | + | + | + |
| 16.21 | 563.1422 | C\textsubscript{26}H\textsubscript{30}O\textsubscript{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\textsuperscript{b} | + | + | + | + |
| 16.58 | 447.0938 | C\textsubscript{21}H\textsubscript{20}O\textsubscript{11} | 98.7 | -1.3 | 350 | 357.0608; 327.0507; 285.0398; 133.0291 | Luteolin 8-C-glucoside (orientin)\textsuperscript{f} | + | + | + | + |
| 16.80 | 577.1579 | C\textsubscript{25}H\textsubscript{30}O\textsubscript{14} | 98.2 | -2.0 | 330 | 457.1140; 413.0880; 293.0455 | Apigenin C-hexose C-deoxyhexoside | + | + | + | + |
| 17.42 | 431.0989 | C\textsubscript{21}H\textsubscript{20}O\textsubscript{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\textsubscript{21}H\textsubscript{20}O\textsubscript{11} | 89.9 | -1.0 | 352 | 285.0406; 284.0327; 197.0806; 175.0282; 133.0294 | Luteolin 7-O-glucoside* (cyanarin) | + | + | + | + |

31
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<th>Mass</th>
<th>Formula</th>
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<th>M</th>
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<td>Genistein 4’-methyl ether (biochanin A)</td>
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**Flavonoids-Flavanones**

- **Apigenin***
- **Luteolin***
- **Eriodictyol***
- **Naringenin***

**Flavonoids-Flavanols**

- **Dihydroquercetin (taxifolin)**

**Flavonoid-Flavanonols**

- **(+)-catechin***

**Flavonoid-Isoflavones**

- **Genistein***
- **Eriodictyol hexoside I**
- **Eriodictyol hexoside II**
- **Eriodictyol**
- **Dihydroquercetin (taxifolin)**
- **Hydroxygenistein methyl ether malonylhexoside**
- **Genistein***
- **7-methoxy 2’-hydroxy genistein (cajanin)**
- **Prenylhydroxygenistein I**
- **Prenylhydroxygenistein II**
- **Prenylhydroxygenistein III**
- **Prenylenstein I**
- **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 + + + +
13.71 339.0754 C_{15}H_{16}O_{9} 83.4 -0.1 279; 335 177.0197 Esculetin hexoside II + - + -
15.39 177.0187 C_{6}H_{4}O_{4} 97.0 -3.9 - 149.0241; 133.0293; 105.0346 Dihydroxycoumarin I + + + +
18.32 205.0146 C_{10}H_{6}O_{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_{9}H_{6}O_{3} 97.4 -1.7 283; 324 133.0291; 117.0342; 105.034 7-Hydroxycoumarin* (umbelliferone) + + + +
20.86 177.0194 C_{9}H_{6}O_{3} 93.5 0.7 285 149.0247; 133.0293; 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_{10}O_{4} 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-benzo[d]furan-5-yl]-2-propenoic acid (psoralic acid glucoside) + - + -

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

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).

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

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

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

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<th>RT (min)</th>
<th>[M+H]+</th>
<th>Formula</th>
<th>Score</th>
<th>Error (ppm)</th>
<th>UV (nm)</th>
<th>Main fragments via MS/MS</th>
<th>Proposed compound</th>
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<td>Hydroxypsoralen hexoside Ia</td>
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<td>252; 264; 310</td>
<td>203.0336; 175.0389; 147.0440; 131.0395; 119.0485; 91.0539</td>
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</table>

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 aHydroxypsoralen hexoside could be 5-hydroxypsoralen hexoside (bergaptol hexoside) or 8-hydroxypsoralen hexoside (xanthoxol hexoside).

688 bMarmesin was previously described in *F. carica* and its enantiomeric form nodakenetin in *Ficus tsiangii*.

689 cHydroxypsoralen could be 5-hydroxypsoralen (bergaptol) or 8-hydroxypsoralen (xanthoxol) according to Yang et al.⁵²

690 dCompounds described here for first time in *F. carica* but described in the family Moraceae and other families (see KNApSACK, Reaxys or SciFinder databases).

691 eNon detected in the negative ionization mode.

692 fCompounds described here for first time in the family Moraceae and common in the family Fabaceae (see KNApSACK, Reaxys or SciFinder databases).
Figure captions

Fig. 1. Bar graph of total phenol content (TPC) (mg of gallic acid/100 g sample) of leaves and fruits from *F. carica* cultivars ‘Tounsi’ and ‘Temri’ and antioxidant activity evaluated by: trolox equivalent antioxidant capacity (TEAC) (mmol eq. Trolox/100 g of sample), ferric ion reducing antioxidant power (FRAP) (mmol eq. FeSO$_4$/100 g sample) and oxygen radical absorbance capacity (ORAC) (mmol eq. Trolox/100 g sample) assays. The primary Y axis corresponds to TPC and the secondary Y axis corresponds to antioxidant activity. Data are given as mean ± standard deviation. Caffeic acid was used as the control and expressed as mmol eq. Trolox or FeSO$_4$/mmol of compound.

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

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

<table>
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<tr>
<th></th>
<th>'Tounsi' Leaves</th>
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<th>'Temri' Fruits</th>
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