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Original Article

Potential of Leaves of Eighteen Cultivars of *Ficus carica* as Antioxidants and Profiling of Phenolic Compounds as an Active Molecules

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Abstract

Ficus carica is a worldwide important crop. Its different parts such as leaves, stem barks, fruit and roots are used in traditional medicine from ancient age to treat the various diseases. In this research, total phenolic content, antioxidant activity, yield of extract and profiling of phenolic compounds of maturated leaves of different cultivars of *F. carica* were investigated by using two different extraction processes: ultrasound-assisted extraction (UAE) and maceration. The total phenolic content (TPC) was calculated by using Folin-Ciocalteu assay, while DPPH and ABTS assays were used for analysed the antioxidant capacity. Additionally, liquid chromatography (LC) coupled to mass spectrometry (MS) was used to study the individual phenolic compounds present in leaves. Using DPPH assay, the percentages of inhibition and Trolox equivalent antioxidant capacity were found to vary from 66.19 ± 1.01 to 87.06 ± 0.93 % and 53.67 ± 0.81 to 70.25 ± 0.74 mg TE/g DL, respectively, for extracts obtained by maceration. In the case of ultrasonication extraction, these values ranged from 72.84 ± 1.26 to 92.04 ± 1.24 % and 60.73 ± 0.92 to 74.19 ± 0.99 mg TE/g dry leaves (DL). While ABTS assay the latter parameters were found to vary from 96.61 ± 0.22 to 99.33 ± 0.23 % and 56.81 ± 0.12 to 58.32 ± 0.13 mg TE/g DL for maceration, as well as from 97.82 ± 0.23 to 99.79 ± 0.13 % and 57.48 ± 0.13 to 58.57 ± 0.07 mg TE/g DL for ultrasonication extraction. The total phenolic contents varied between 10.70 ± 0.20 to 21.18 ± 0.68 mg GAE/g DL, and 12.94 ± 0.23 to 23.04 ± 0.30 mg GAE/g DL, respectively for the latter extraction processes. The percentages of yields varied from 7.20 ± 0.20% to 13.05 ± 0.23% and 8.25 ± 0.25% to 15.20 ± 0.20 %, respectively, for maceration and ultrasonication extraction. In conclusion, extracts from leaves of *F. carica* obtained by UAE process showed the highest antioxidant activity, phenolic contents and extraction yields as compared to maceration process. In comparison amongst the leaves, the 'Violette solise' cultivar showed the highest antioxidant activity and contain highest phenolic contents. Finally, the extract of this cultivar contained phenolic acids (dihydroxybenzoic acid di-pentoside, caffeic acid, caffeoylmalic acid, coumaroylmalic acid, ferulic acid malate, and psoralic acid glucoside), *C*-glycosides flavones, rutin and prenylgenistein.

Keywords: *Ficus carica,* Moraceae, antioxidant activity, leaves, phenolic compounds and mass spectrometry.

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1. Introduction

Oxidation reactions naturally occur in the human body due to continuous respiration and oxidative metabolism in human cells. Although oxygen is necessary for aerobic cells to generate energy, it can produce various ageing and diseases causing substances by incorporating reactive oxygen species (ROS). These species mainly occurs in free radical forms (*OH, $^1O_2$, O_2*^-, $ONOO^-$) and non-free radical forms (R–OOH, NO and H_2O_2). These radicals can damage the macromolecules and imbalance the body that causes chronic degenerative diseases such as cancer, rheumatoid arthritis, atherosclerosis, emphysema, cirrhosis, diabetes and others [1, 2]. The human body is vulnerable to these reactive species; natural antioxidants are an important compound for reducing the concentration of these species and prevention of the above-mentioned chronic diseases. Antioxidants in the body are primarily derived from diet and can promote good health. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ) 2-tert-butyl-4-methylphenol (TBMP) and gallic acid esters, e.g. propyl gallate (PG), are the most widely used antioxidants in food products but these synthetic products are restricted due to its carcinogenic and other toxic properties [3]. Therefore, the attention of natural antioxidants has been raised considerably in the study of certain fruits, vegetables and leaves with high antioxidant contents to boost their consumption. Among natural antioxidants, phenolic compounds are one of the abundant and extensively distributed in the plants and over 8,000 phenolic compounds presently occur.

Ficus carica is one of the important crop for its dry and fresh consumption. Its different parts such as leaves, stem barks, fruit and roots are used in traditional medicine to treat several diseases such as gastrointestinal, respiratory, cardiovascular disorders [4], antidiabetic [5], antispasmodic remedy [6], and anti-inflammatory [7]. Fruits of *F. carica* are not only valuable from a nutritional point of view, but also they are source of phenolic compounds, which have a potential positive effect on human health and regulate its product quality. Phenolic compounds have positive correlation with antioxidant activity. The reported common polyphenolic compounds are gallic acid and syringic acid [8, 9], cyanidin-3-rhamnoglucoside [10, 11], chlorogenic acid, rutin, and psoralen may contribute to the antioxidant activity [12] of fig leaves and fruits. Most of the researchers investigated the antioxidant activity (DPPH, ABTH, FRAP, etc.), phenolic content, and others bioactivities of the fruits of different cultivars [10, 13-19]. On the contrary, few studies have focused on leaves [12, 20, 21] and others parts [22] of *F. carica*, studying also a low number of cultivars or from concrete regions. However, some studies suggest that leaves exhibited higher phenolic content and antioxidant capacity as against to others parts due to phenolic compounds [21-23]. Concomitantly, leaves of *F. carica* displayed stronger antioxidant activity by both the mechanisms of single electron transfer and hydrogen atom transfer [23]. Moreover, there are also a few reports dealing with the extraction processes to maximize antioxidant activity. However, this preliminary screening could help to develop antioxidant formulations for food and health applications,

Therefore, the aim of this work was to determine the antioxidant activity, total phenolic content and yield of extract of eighteen cultivars of *F. carica* and to compare these activities by using two extraction processes: ultrasonication and maceration, which are widely used. This is the first report for these cultivars and their activity. The cluster and correlation analysis were also performed for these cultivars. Finally, their phenolic profiles were comprehensively studied by LC-MS/MS spectrometer.

2. Material and Methods

2.1. Chemicals and Reagents

1,1-Diphenyl-2-picrylhydrazyl(DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich, USA. Folin-Ciocalteu reagent was purchased from Merck, Germany. Potassium persulfate, 99.9 % pure ethanol, monohydrate gallic acid and anhydrous sodium carbonate were purchased from Friendemann Schmidt (FS) Chemicals, Australia. All the chemicals used were analytical grade. 18 mΩ deionised water was used to prepare the standard materials and extraction.

2.2. Sample Preparations

The fresh leaves were collected from 18 different cultivars of *F. carica* from Fig garden, Living lab energy and Future crops laboratories at Kuala Pilah under the FKAB, UKM in February 2016 and are shown in Figure 1. Each cultivars leaves were washed with deionized water, given an airing at room temperature, and then dried at 35-40 $^{\circ}$C with the help of Septree Food Dehydrator, China. Finally, all the leaves were powdered using a special grinder (XY-2200B, Shenzhen Yason General Machinery Co., Ltd, Guangdong, China) and stored in an airtight container.

2.3. Extraction Procedures of Antioxidants

2.3.1. Ultrasound-Assisted Extraction (UAE)

Ultrasonication extraction was fulfilled in a Thermoline ultrasonic bath (220 V and 40 kHz) at 35 $^{\circ}$C. Two hundred fifty mg of dried and ground powdered sample was transferred in a capped long test-tube (50 mL) and 15 mL of 70% of the ethanolic solution was poured in the sample. Then the mixture was immersed in the ultrasonic bath and fixed well in the same position during sonication (60 minutes). After the extraction, the suspension sample was centrifuged at 4000 rpm for 15 min. Then the supernatant liquid was filtered and the extract thus obtained used directly for the determining of the required properties

Figure 1. Leaves of different cultivars of *Ficus carica*.

2.3.2. Maceration Extraction

The same amount of dried and powdered sample (250 mg) was kept in a capped long test-tube and extracted by the same volume and percentages of aqueous ethanol. The samples were extracted at room temperature for one hour with an orbital shaker at 200 rpm. After shaking, all the suspension samples were placed in a centrifuge machine at 4000 rpm for 15 min, and subsequently, the liquid was filtered and the aforementioned extracts were stored at 4 ^{0}C for the analysis of biological activity within 2 days.

2.4. Extract Yield Percentage

The yield of extract is a measure of the solvent's efficiency to extract specific components from the original material and it was defined as the amount of extract recovered in mass compared with the initial amount of whole plant. To measure the yields, the solvent was removed from the sample by a rotary evaporator (Buchi, Switzerland). The results were expressed in percentage (%) and were determined for each extraction techniques.

2.5. Total Antioxidant Capacity

2.5.1. DPPH Free Radical Scavenging Assay

The DPPH activity of leaves of different cultivars of *F. carica* were measured by using pre-reported methods with some modifications [24]. In brief, 0.1 mM of fresh DPPH was prepared with the 70% of aqueous ethanol. 100

μL of the standard Trolox solution (positive control) or appropriate dilutions of different extract were mixed with 2.9 mL of 0.1 mM DPPH solution. Then the control, standard and sample absorbance were measured at 520 nm after 30 minutes incubation at room temperature. Trolox equivalent antioxidant capacity (TEAC) was calculated by prepare a trolox curve (the standard curve equation: y = -0.0007x + 0.9396, R^2 = 0.9998) from 31.25 μg/mL to 1.5 mg/mL of standard trolox solution and the results were expressed as mg trolox equivalent (TE)/g dry leaves (DL). The DPPH scavenging activity was expressed as a percentage of inhibition. The scavenging capacity or inhibition of DPPH (%) was calculated by using the equation below:

$$\text{Antioxidant capacity (\% inhibition)} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (1)$$

Where $A_{control}$ is the absorbance of radical solution with 70% of aqueous ethanol; A_{sample} is the absorbance of radical solution mixed with sample extract or standard. Each sample and standard was measured in three replicates. The absorbance was measured with 756 PC UV–Visible spectrophotometer (Shanghai Yuefeng Instruments & Meters Co., Ltd.).

2.5.2. ABTS$^+$ Free Radical Scavenging Assay

The ABTS *radical scavenging* assay was calculated based on [25] with some modifications. First the radical solution was prepared by mixing both the stock solutions, 7 mM aqueous solution of ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) solution at a ratio of 1:1 [12]. The mixture was kept for 12-16 hours in the dark at room temperatures. Then the fresh working solution was prepared for each bioassay by diluting 1 ml ABTS radical solution with required amount of ethanol to obtain the absorbance of 0.700 ± 0.02 units at 745 nm. Afterwards, 100 μL of different extracts or different standard Trolox solution was added to 3.9 mL of an ABTS$^+$ solution. The absorbance was measured immediately at 745 nm after 6 minutes incubation at room temperature. 70% of aqueous ethanol and Trolox were used as blank and positive control respectively, and Trolox was taken as a positive control. TEAC was calculated by preparing a trolox curve for ABTS assay (the standard curve equation: y = -0.0006x + 0.5869, R^2 = 0.9998) from 31.25 μg/mL to 1.0 mg/mL of standard trolox solution and the results were designated as mg TE/g DL. The percentages of inhibition of ABTS was calculated by equation 1. The equipment used was described before.

2.6. Determination of Total Phenolic Content

The phenolic content of leaves of *F. carica* was analysed by using Folin-Ciocalteu (FC) reagent with some modifications [26]. FC reagent was used as an oxidising agent. Firstly, 100 μL of the standard gallic acid or leaves extract were mixed with 3.25 mL of 12 times pre-diluted of Folin-Ciocalteu reagent. The samples and standards were properly mixed and allowed to stand for 7 min; then 750 μL of 20% Na_2CO_3 was added to the test tube containing main solution and incubated for 2 hours for incubation at room temperature under dark conditions. Finally, the absorbance was recorded at 760 nm based on a colorimetric redox reaction from a standard curve ($y = 0.0027x + 0.0235$, $R^2 = 0.9998$), and using standard gallic acid solution of 31.25 µg/mL to 1.0 mg/mL. The results were presented as mg gallic acid equivalent (GAE)/g DL. Each standard and extract was measured in three times ($n = 3$). The equipment used was as described for the previous assays.

2.7. Determination of Phenolic Compounds Using LC-ESI-MS Analysis

The polyphenolic compounds were profiled using liquid chromatography (LC) coupled to mass spectrometry (MS) (Bruker micrOTOF-Q). A reverse phase C18 column (Phenomenex 250 mm, 5 µm particle size) was used. The eluting system consisted of H_2O acidified with 0.1% formic acid as solvent A and (1:1, v/v) CH_3CN/MeOH acidified with 0.1% formic acid as solvent B. The mobile phases were filtered through a 0.45 µm membrane disk filter and degassed by sonication before injection. Elution was performed using the following gradient: 5% B, 0–5 min; 5%–10% B, 5–10 min; 10%–50% B, 10–55 min; 50%–95% B, 55–65 min; 5% B, 65–70 min. The injection volume was 20 µL and the flow rate set to 0.4 mL/min. The parameters for analysis were carried out using negative ion mode as follows: source temperature 150 °C, cone voltage 50 eV, capillary voltage 3 kV, desolvation temperature 350 °C, cone gas flow 50 L/h, desolvation gas flow 600 L/h. Mass spectra were acquired between *m/z* 50–1000. The peaks and spectra were processed using the Bruker Daltonics Data Analysis 3.4 software. The tentative phenolic compounds were identified by comparing its retention time and reported mass spectrum data with literature on *F. carica*.

2.8. Statistical Analysis

To study the variance of antioxidant activity and phenolic content of various cultivar of the *F. carica,* data was processed by one-way analysis of variance (ANOVA) using STATGRAPHICS Centurion XVII (Version 17.2.00, StatPoints Technologies Inc. 1982 - 2016). Correlation, regression and cluster analysis were also carried out in STATGRAPHICS Centurion XVII. Statistically significant differences were determined by the Tukey honest significant difference (HSD) *post hoc* test. For the F values obtained, $p < 0.05$ were considered statistically significant. Pearson Product-Moment correlation matrix and regression analysis were used in order to evaluate the connection between DPPH, ABTS and total phenolic content in the extraction processes. The data of TEAC and GAE curve was done in Microsoft Excel 10 (Microsoft Inc., Redmond, WA, USA). Three replicates of each sample were used for statistical analysis. All data presented are expressed as means ± SD.

3. Results and Discussion

3.1. Antioxidant Activity

Antioxidant activity of plant materials can be analysed, and can be attributed to various reported mechanisms. Generally, two modes of action, such as single electron transfer and hydrogen atom transfer have been described [27, 28]. Therefore, in this research, the antioxidant activities of leaves of different cultivar were analysed by two most common *in vitro* assays namely DPPH and ABTS. Their results were expressed by different methods, such as percentage of inhibition (%) and trolox equivalent antioxidant capacity (mg TE /g DL), shown in Table 1.

The DPPH assay is a broadly used and reliable antioxidant determination method as against to other assays [29]. In this process, DPPH solution reduced to non-radical DPPH-H in presence of hydrogen-donating antioxidant. The antioxidant compound containing crude extract reduced the stable purple color to yellow-coloured diphenylpicrylhydrazine. The DPPH antioxidant activities of leaves from the studied cultivars of *F. carica* are presented in Figure 2.

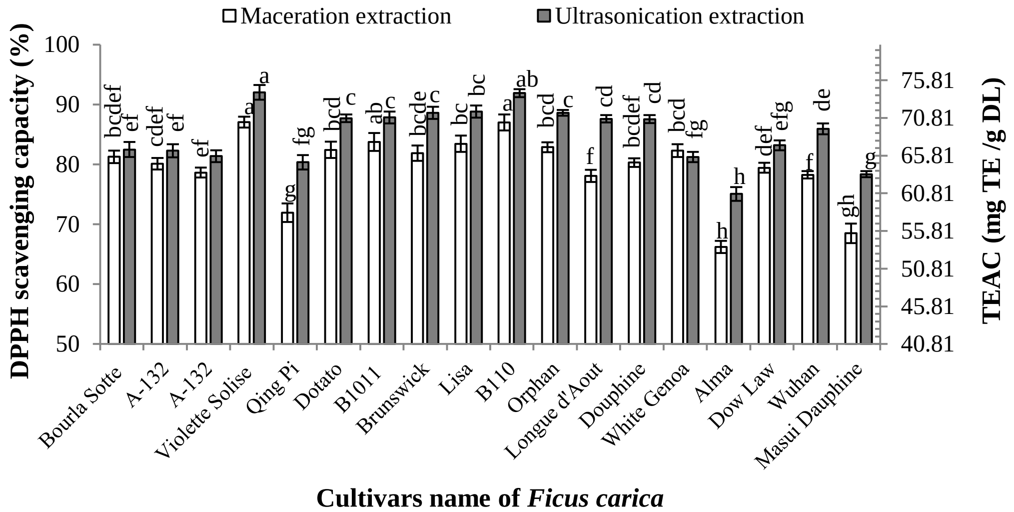

Figure 2. The percentages of inhibitions and TEAC of Fig cultivars in DPPH method.

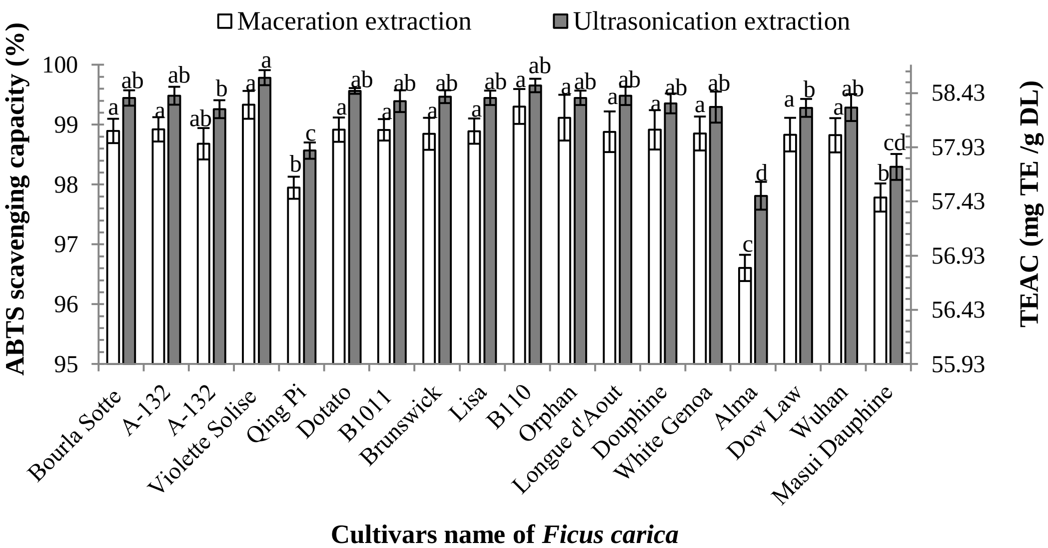

Figure 3. The percentages of inhibitions and TEAC of Fig cultivars in ABTS method.

The percentages of inhibition and trolox equivalent antioxidant capacity of leaves of eighteen (18) cultivars of *F. carica* were analysed in DPPH assay and ranged from 66.19 ± 1.01 to 87.06 ± 0.93 % and 53.67 ± 0.81 to 70.25 ± 0.74 mg TE/g DL respectively for maceration. On the other hand, in the extraction through ultrasound, these values range from 72.84 ± 1.26 to 92.04 ± 1.24 % and 60.73 ± 0.92 to 74.19 ± 0.99 mg TE/g DL respectively (Figure 2). While ultrasound-assisted extraction (UAE), all the cultivars of *F. carica* showed higher percentages of inhibition and trolox equivalent antioxidant capacity than the maceration extraction and showed in Table 1. These results agreed with studies elsewhere, as in fact solvent system, extraction process and drying temperature could effect on the recovery of phenolic compounds and the antioxidant activity [30]. The highest antioxidant activity was detected in ‘Violette solise’ and ‘B110’ as well as the lowest in ‘Alma’ and ‘Masui Dauphin’. From the ANOVA, there was no significant statistical difference between ‘Violette solise’ and ‘B110’ (Table 1). However, further studies should be performed to study the effect of growth location, cultivation practices, harvesting conditions, and seasonality, since these could be factors that potentially affect antioxidant activity [31]. Moreover, it was been reported that non-antioxidant compounds may also interfere the antioxidant activity [32], which also needs to be taken into consideration.

Our research showed the favourable results with previous studies on the leaves of fig cultivars [12, 20, 21, 33-35] and showed equivalent or higher antioxidant activity. As commented before, not only the cultivar may affect but also the extraction protocols used. Alternatively, antioxidant activities of our studied cultivars were lower than the Algerian cultivars reported by of Mahmoudi *et al* (2016) [34]. Moreover, our studied leaves extracts were higher than those of other fig plant parts, such as fruits, pulps or peels, including of commercial cultivars such as ‘Brown Turkey’, ‘Brunswick’, ‘Bursa’, Chechick’, ‘Kadota’, and ‘Mission’ [10, 33] and autochthonous Albanian fig ones [36].

The ABTS free radical scavenging method is another important assay for analysing the antioxidant activity. In ABTS assay, the percentages of inhibition of the leaves of eighteen (18) cultivars of *F. carica* were analysed, and the values ranged from 96.61 ± 0.22 to 99.33 ± 0.23 % and 97.82 ± 0.23 to 99.79 ± 0.13 % respectively, for maceration and ultrasonication extraction (Figure 3). The Trolox equivalent antioxidant capacity (TEAC) varied from 57.48 ± 0.13 to 58.57 ± 0.07 mg TE/g DL. The highest trolox equivalent antioxidant activity was detected in ‘Violette Solise’ and the lowest in ‘Alma’ (Table 1). This study showed favourable results with previous studies and presented equivalent or higher. Trolox equivalent antioxidant capacity of our studied cultivars were higher than those of extracts of fig plant from Tunisian fig cultivars (‘Temri’ and ‘Tounsi’) cultivars [37], some Albanian fig cultivars (‘Roshnik’, ‘Perdhikuli’, ‘Melacak’, ‘Bishtagjati’ and ‘Rotllar’ [36], and the aforementioned commercial fig cultivars (‘Brown Turkey’, ‘Brunswick’, Bursa’, ‘Chechick’, ‘Kadota’ and ‘Mission’) [10].

Overall, our experimental results showed significant differences in the same trends using ABTS and DPPH assay, but extracts showed higher inhibitory percentages in ABTS radical assays. This may be as a result of more susceptibility to the antioxidant compounds present in the extract. There major differences between the two methods in terms of the mechanism of action – in the ABTS assay, only single electron transfer reaction occurs, whereas both the single electron transfer and hydrogen atom transfer occurs in DPPH assay [38].

3.2. Total Phenol Content

The polyphenolic content of the 18 cultivars of *F. carica* obtained by ultrasound and maceration extraction process is shown in Table 2 and Figure 4. In the extraction of phenolic compounds of cultivars, using the UAE method obtained higher phenolic content, which ranged from 12.94 ± 0.23 to 23.04 ± 0.30 mg GAE/g DL). On the other hand, the TPC content extracted by maceration varied from 10.70 ± 0.20 to 21.18 ± 0.68 mg GAE/g DL. Therefore, at the studied environments, higher phenolic content observed for all the cultivars in case of ultrasound extraction. The highest TPC values were observed in ‘Violette solise’ and ‘B110’ as well as the lowest in ‘Alma’ and ‘Masui Dauphine’. One way ANOVA presented that there was no significant variance between ‘Violette solise’ and ‘B110’ (Table 2) in both the total phenolic content and antioxidant activity as commented before. Actually, ultrasound provokes a formation of tiny bubbles exposed to fast adiabatic expansions and compressions, which rises the temperatures and pressures within the system [39].

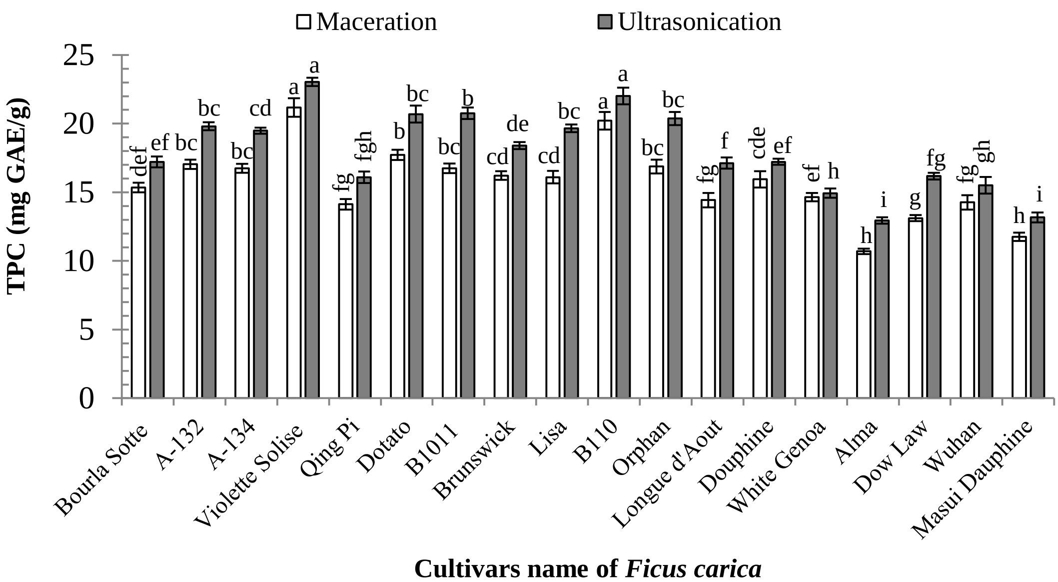

Figure 4. Total phenolic contents of fig cultivars in maceration and UAE extraction.

Thus, ultrasound irradiation process could contribute to the higher yields of phenolic content. However, the sonication process causes dried plants to swell by adsorbing higher amount extraction solvent [40] which enlarges the pores of cell walls , leading to greater diffusion. Finally, the process causes the breakdown the cell walls, which enables efficient washing of the cell wall content, allowing for more efficiency in phenolic release[41, 42].

Few researchers reported the total phenolic content for the cultivar of *Ficus carica* fruits [15, 16, 19] and a few researcher reported the TPC contents for leaves [12, 21, 34, 37] and others parts [22, 33] of *F. carica* cultivar. However the leaves exhibited higher phenolic content and antioxidant capacity as against others parts [21, 22, 33, 37]. This study showed favourable results with previous studies and presented equivalent or higher. It was observed that total phenolic content of our studied cultivars were lower than those of Belguith-Hadriche and co-workers reported Tunisian fig cultivars named 'Temri' and 'Tounsi' [37], Mahmoudi *et al.* (2016) for several Algerian cultivars of *Ficus carica* [34] and Harzallah and coauthors (2016) reported in another Tunisian cultivars [43]. However, our studied TPC content also higher than those of other fig plant parts Solomon *et al.* (2006) reported six commercial fig cultivars with 'Brown Turkey, Brunswick, Bursa, Chechick, Kadota and Mission' [10]; Jokić *et al.* (2014) repoerted five Croatian fig cultivars Bjelica, Termenjača, Crnica, Bružetka bijela and Šaraguja [44] and Hoxha *et al.* (2015) reported autochthonous Albanian fig cultivars and named Roshnik, Perdhikuli , Melacak, Bishtagjati and Rotllar [36].

Our studied total phenolic content of fig cultivars were also higher than those obtained by some researchers from others fruits such as apricots (3.05 mg GAE/g) and dates (2.42 mg GAE/g) [24], Turkish apple (0.76 mg GAE/g) and raisin (0.85 mg GAE/g) [45]. Algerian date varieties (0.42-0.85 mg GAE/g) [46], Georgia-Grown rabbiteye blueberries (5.56 mg GAE/g), southern highbush blueberries (3.99 mg GAE/ g FW), blackberries (4.87 mg GAE/ g) [47] and muscadines grapes (2.48 mg GAE/ g) [48]. From the TPC value listed previously, we can easily consider that the *F. carica* is a good source of natural antioxidants, phenolic compounds. In this sense, 'Violette Solise' and 'B110' cultivars of *F. carica* were the most interesting cultivars.

3.3 Yields of the Extract

The yield of extract obtained for ultrasound assisted extraction and maceration extraction of the cultivars of *F. carica* are shown in Table 2 and Figure 5. In ultrasound assisted extraction, the percentages of yields were calculated and ranged from 8.25 ± 0.25 to 15.20 ± 0.20 %. Whereas maceration extraction, these ranges from 7.20 ± 0.20 % to 13.05 ± 0.23 %. The leaves extract of cv. violette Solise had the highest yield (13.05 ± 0.23 %) and (15.20 ± 0.20 %), followed by B110 (12.78 ± 0.20 %) and (14.98 ± 0.23 %) respectively, for maceration and ultrasonication extraction. The extracts of Alma and Masui Dauphine cv. had the lowest yields. Most of the yield extracts of our studied cultivars were lower than those of Mahmoudi et al. (2016)

reported ten cultivars of *F. carica* (Bidha, Hamra, Onk Elhamam, Zarrouk, Chatwi, Boughandjo, Safra, Bakkor, Bither and Dhokkar) [34].

The extraction yield using UAE yielded a higher percentage compared to the maceration extraction for all the cultivars. The higher yield of extract in ultrasound assisted extraction is due to the ultrasound disrupting the cell walls and rapidly releasing cell content. Cavitation induced by sonication is the process in which bubbles with a negative pressure are formed near cell walls, grow, oscillate, and may split and implode. The collapse of cavitation bubbles near cell walls is expected to produce cell disruption, allowing penetration of the solvent into the cell through an ultrasonic jet [40]. However, the particle size of the plant materials are important features that have a significant effect on the yield of extract [49]. The lower the particle size increases the number of cells in plant materials, which directly exposes to the solvent and allows higher efficiency for penetration [50].

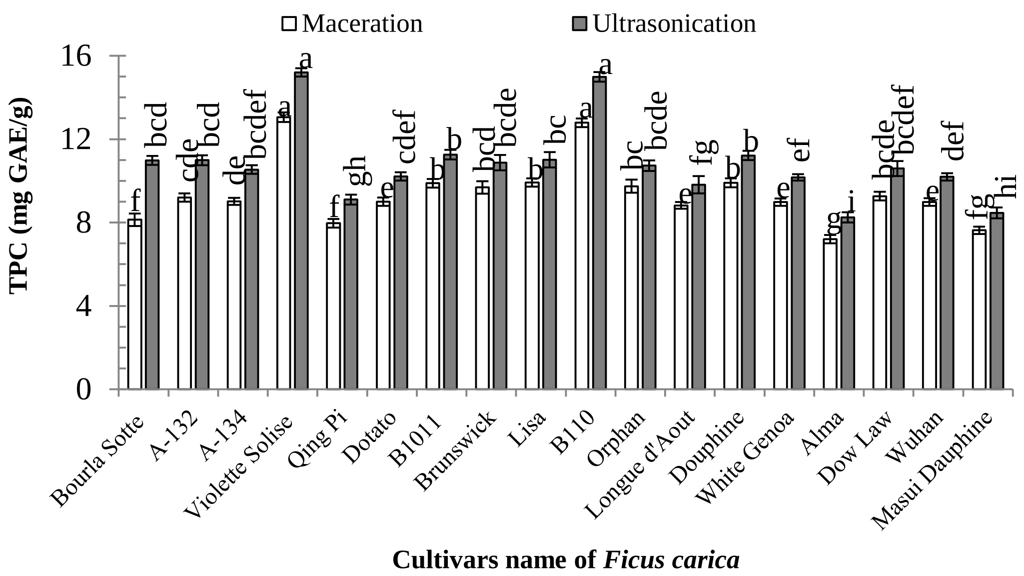

Figure 5. The percentages of yield of fig cultivars in maceration and UAE extraction.

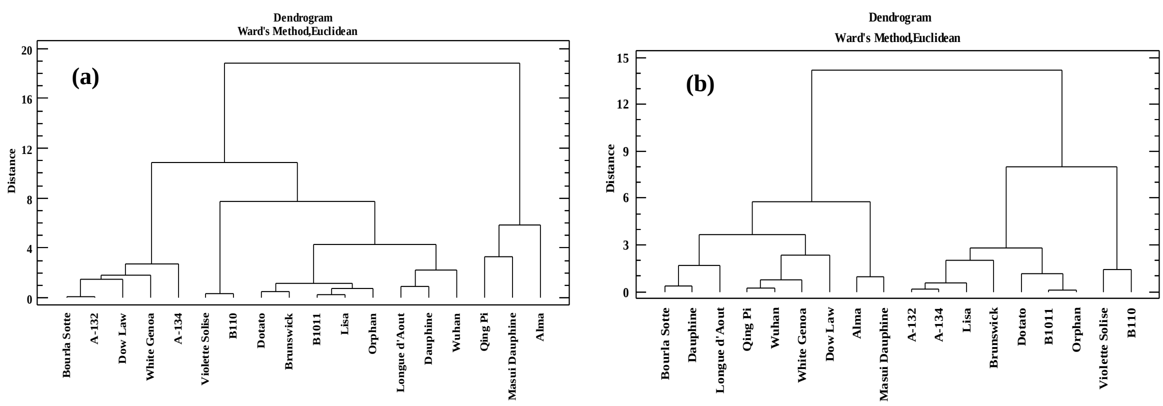

Figure 6. Dendrogram of *Ficus carica* cultivars on the basis of (a) antioxidant activity by and (b) total phenolic content.

Nonetheless, previous researchers reported that, high yield of extract does not necessarily prove high antioxidant activity and total phenolic content for all the sample [24, 45].

3.4. Cluster Analysis

The hierarchical cluster analysis is useful in solving classification problems. In our study, the objective of using cluster analysis was to reveal clusters based on the antioxidant activity and total phenolic content. It was observed that higher TPC content and antioxidant capacity (DPPH and ABTS) of fig cultivars are placed in same cluster, their linkage distance is higher than the lower antioxidant activity and TPC contents cultivars, which are placed in different cluster. In hierarchical cluster analysis, the dendrogram was created using Ward's method and Euclidean distances was measured the similarity between samples, considering all the cultivars experimental properties and shown in Figures 6 (a-b). In case of antioxidant activity, two main clusters can be distinguished at euclidean distances of about 11.0 (*Figure 6a*). The first cluster includes 15 cv. of *F. carica*, while second cluster contains residual 3 cv. of *F. carica*, which indicated the least amount of antioxidant activity. 'Violette Solise' and 'B110' cultivars showed the highest antioxidant activity at distances of about 8.0. The second cluster contained cultivars 'Alma', 'Masui Dauphine', and 'Qing Pi', which showed the lowest antioxidant activity. *From Figure 6b*, two main clusters also can be determined. The left cluster of the dendrogram includes 9 cultivars, whereas right cluster contains the remaining 9 cultivars. The highest total phenolic content were detected in 'Violette Solise' and 'B110', which were placed in same cluster and placed in the right side of the cluster. While 9 cultivars were placed in the left side of TPC dendrogram, this cluster showed lowest phenolic content and include 'Alma' and 'Masui Dauphine' cultivars (*Figure 6b*), as observed in antioxidant activity measured by both the scavenging method.

3.5. Correlation Analysis

The correlation analysis between ABTS, DPPH, and total phenolic contents of fig cultivars were displayed in Figure 7.

Pearson Product-Moment Correlations

-1.0 — 1.0

	DPPH-MAC	DPPH-ULT	ABTS-MAC	ABTS-ULT	TPC-MAC	TPC-ULT
DPPH-MAC		0.83	0.84	0.88	0.84	0.81
DPPH-ULT	0.83		0.74	0.78	0.78	0.78
ABTS-MAC	0.84	0.74		0.95	0.72	0.69
ABTS-ULT	0.88	0.78	0.95		0.77	0.74
TPC-MAC	0.84	0.78	0.72	0.77		0.93
TPC-ULT	0.81	0.78	0.69	0.74	0.93	

Figure 7. Pearson Product- Moment correlation matrix of the studied parameters (P <0.05).

A positive relationship ($p < 0.05$) was found between maceration DPPH assay and ultrasonic DPPH (r = 0.83), maceration ABTS (r = 0.84), and the ultrasonic ABTS (r = 0.88). Another significant correlation was observed between the maceration DPPH and maceration total phenolic content (TPC), and the ultrasonic TPC, but with poor r-values (r = 0.84 and 0.81 respectively). These significant correlations suggests that the outcomes of 84% and 81 % of the DPPH scavenging capacity of fig cultivars may be due to the involvement of phenolic compounds. The remaining percentage may also come from others antioxidant compounds such as volatile oils, amino acids, vitamins and others, which are not limited to phenolics [51]. Moreover, a significant correlation between ABTS and TPC contents, indicated that phenolic compounds may also contribute to the ABTS radical scavenging assay in the cultivars of fig. Several studies have previously reported the significant positive relationship between polyphenols and antioxidant activity [52-55]. Therefore, the present study showed that *F. carica* leaves are byproducts with strong radical scavengers and can be considered as good sources of natural antioxidants for food applications, among others.

3.6 Qualitative Profiling of Polyphenolic Compounds

The profiling of phenolic compounds was performed by using LC-MS spectrometer in the negative ionization mode. For that, the most active extract was studied in depth (Figure 8): leaves of ‘Violette solise’ cultivar extracted through the ultrasonication extraction. The retention time (RT), experimental *m/z* of negative molecular ions ($[M\text{-}H]^-$), molecular formula and the proposed compounds are shown in Table 3. The tentative compounds were compared with the reported literature and databases. A total of 10 phenolic compounds were characterized from this cultivar for the first time but reported from others cultivars.

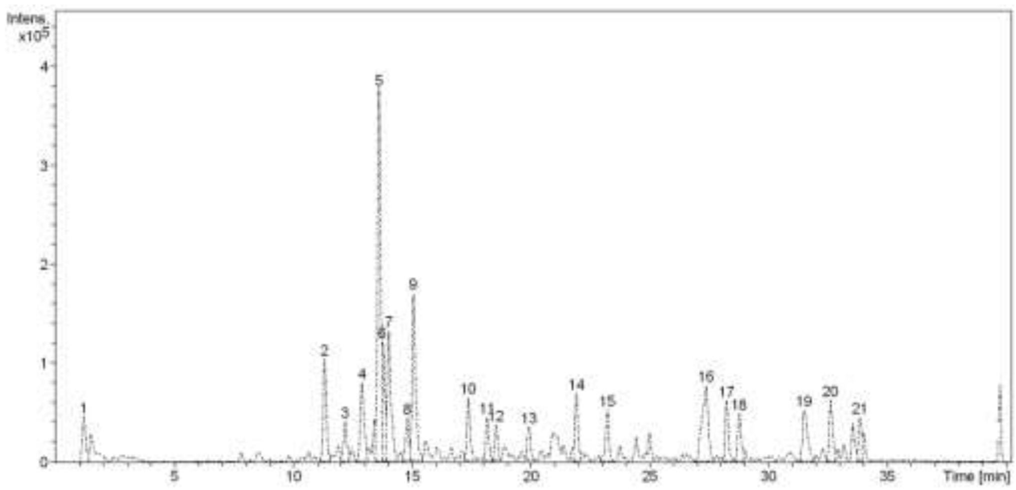

Figure 8. LC-MS fingerprinting analysis of leaves of ‘Violette solise’ cultivar analysed in the negative ionization mode.

The tentative identified compounds belonged to hydroxybenzoic acid, hydroxycinnamic acid, and flavonoids. The compound dihydroxybenzoic acid di-pentoside was reported by Ammar and co-workers for the first time in Tunisian fig cultivars of 'Temri', 'Tounsi' and 'Soltani' in 2015 [23, 30]. Five hydroxycinnamic acid derivatives named as caffeic acid, caffeoylmalic acid, coumaroylmalic acid and ferulic acid malate were also reported in the leaves of the latter cultivars of fig [23, 30]. Psoralic acid glucoside was also described in fig leaves recently [56]. A few previous studies have already been reported these hydroxycinnamic acid derivatives from others fig cultivars: caffeoylmalic acid from cv. Masui dauphine [56]; caffeic acid from the leaves of cv. Brown Turke [57], Concerning flavonoids, we characterized one flavonol compound named quercetin-3-*O*-rutinoside (rutin) [23, 30, 56], two *C*-glycosides of flavones, luteolin *C*-hexoside *C*-pentoside and apigenin *C*-hexoside *C*-pentoside [23, 30] and them isoflavone prenylgenistein, which was reported from *F. carica* [23, 30].

We also characterized other compounds such as oxylipins derivatives from this cultivar, which are first time report from this genus but few researcher reported from others genus. These oxylipins derivatives were hydroxy-octadecatrienoic acid and hydroxy-octadecadienoic acid/ 13-hydroxy-octadecadienoic[58]. Recently Jiménez-Sánchez *et al.* (2016) reported these compounds in green asparagus (*Asparagus officinalis*) [59].

4. Conclusion

Ultrasound-assisted extraction (UAE) is a new, simple, and economical extraction process. This was used to extract antioxidants from the leaves of different cultivars of *F. carica* and compared with the maceration extraction method. Based on the antioxidant activity, phenolic content and yield of extract, it can be summarised that the extracts obtained by UAE process showed potent antioxidant activity, may be related to their phenolic content. 'Violette solise' cultivar showed the highest antioxidant activity in both extractions methods and contain higher phenolic contents. Phenolic compounds characterized in this extract included phenolic acids, *C*-glycosides flavones, rutin (flavonol) and prenylgenistein (isoflavone). Another interesting cultivar was 'B110'. Therefore, the leaves of both cultivars of *F. carica* could be potential sources of antioxidants from a natural origin that could be significant contribution as a therapeutic agent (in preventing or slowing oxidative stress and chronic related disorders), as well as for food applications (as antioxidant additives). Therefore, the highest activity presented cultivars could be desirable to be used in *in vitro* and *in vivo* studies to elucidate their mode of action as antioxidant. Furthermore, these cultivars can be potential candidates for further phytochemical and pharmacological studies to isolate and identify secondary metabolites correlated to antiradical activity or other bioactivities.

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Table 1. Antioxidant activity of eighteen cultivars of *Ficus carica* in different extraction process

Name of *Ficus* species	DPPH radical scavenging capacity				ABTS radical scavenging capacity			
	Maceration		Ultrasonication		Maceration		Ultrasonication	
	Inhibition (%)	TEAC (mg TE/g DL)	Inhibition (%)	TEAC (mg TE/g DL)	Inhibition (%)	TEAC (mg TE/g DL)	Inhibition (%)	TEAC (mg TE/g DL)
Bourla Sotte	81.26 ± 1.04bcdef	65.64 ± 0.83bcdef	82.53 ± 1.24 de	66.61 ± 0.99de	98.89 ± 0.20a	58.08 ± 0.11a	99.45 ± 0.13ab	58.38 ± 0.07ab
A-132	80.10 ± 0.97cdef	64.72 ± 0.77cdef	82.33 ± 1.07de	66.46 ± 0.85de	98.92 ± 0.20a	58.09 ± 0.11a	99.48 ± 0.15ab	58.40 ± 0.08ab
A-134	78.63 ± 0.85ef	64.11 ± 0.65ef	78.90 ± 1.11fg	65.74 ± 0.78fg	98.68 ± 0.26ab	57.96 ± 0.14ab	99.26 ± 0.15b	58.28 ± 0.08b
Violette Solise	87.06 ± 0.93a	70.25 ± 0.74a	92.04 ± 1.24a	74.19 ± 0.99a	99.33 ± 0.23a	58.32 ± 0.13a	99.79 ± 0.13a	58.57 ± 0.07a
Qing Pi	71.92 ± 1.54g	58.23 ± 1.22g	80.43 ± 1.20ef	64.94 ± 0.96ef	97.95 ± 0.19bc	57.55 ± 0.10bc	98.57 ± 0.14c	57.90 ± 0.08c
Dotato	82.43 ± 1.39bcd	66.58 ± 1.1bcd	87.74 ± 0.65b	70.77 ± 0.51b	98.91 ± 0.20a	58.09 ± 0.11a	99.56 ± 0.05ab	58.45 ± 0.03ab
B1011	83.73 ± 1.49ab	67.61 ± 1.18ab	87.87 ± 0.99b	70.87 ± 0.79b	98.91 ± 0.18a	58.09 ± 0.10a	99.39 ± 0.18ab	58.36 ± 0.10ab
Brunswick	81.88 ± 1.29bcde	66.14 ± 1.03bcde	88.65 ± 1.00b	71.49 ± 0.80b	98.85 ± 0.27a	58.05 ± 0.15a	99.47 ± 0.10ab	58.40 ± 0.06ab
Lisa	83.43 ± 1.36bc	67.37 ± 1.08bc	88.87 ± 1.02b	71.67 ± 0.81b	98.89 ± 0.21a	58.08 ± 0.12a	99.45 ± 0.12ab	58.39 ± 0.07ab
B110	87.01 ± 1.34a	70.22 ± 1.06a	91.92 ± 0.66a	74.10 ± 0.53a	99.30 ± 0.29a	58.31 ± 0.16a	99.66 ± 0.11ab	58.50 ± 0.06ab
Orphan	82.87 ± 0.84bcd	66.93 ± 0.66bcd	88.65 ± 0.48b	71.49 ± 0.39b	99.12 ± 0.38a	58.20 ± 0.21a	99.45 ± 0.12ab	58.39 ± 0.07ab
Longue d'Aout	78.06 ± 1.02f	63.10 ± 0.81f	87.65 ± 0.63bc	70.69 ± 0.50bc	98.88 ± 0.34a	58.07 ± 0.19a	99.48 ± 0.15ab	58.40 ± 0.08ab
Dauphine	80.29 ± 0.72bcdef	64.88 ± 0.57bcdef	87.59 ± 0.68bc	70.65 ± 0.54bc	98.91 ± 0.33a	58.09 ± 0.18a	99.36 ± 0.17ab	58.34 ± 0.09ab
White Genoa	82.30 ± 1.09bcd	66.47 ± 0.87bcd	80.13 ± 1.37ef	65.62 ± 0.69ef	98.85 ± 0.28a	58.06 ± 0.16a	99.30 ± 0.26ab	58.30 ± 0.14ab
Alma	66.19 ± 1.01h	53.67 ± 0.81h	72.84 ± 1.26h	60.73 ± 0.92h	96.61 ± 0.22d	56.81 ± 0.12d	97.82 ± 0.23d	57.48 ± 0.13d
Dow Law	79.43 ± 0.83def	64.19 ± 0.66def	81.70 ± 0.91def	67.19 ± 0.66def	98.83 ± 0.28a	58.04 ± 0.15a	99.28 ± 0.15b	58.29 ± 0.08b
Wuhan	78.25 ± 0.60f	63.26 ± 0.48f	84.67 ± 1.00cd	69.35 ± 0.73cd	98.82 ± 0.28a	58.04 ± 0.16a	99.29 ± 0.23ab	58.30 ± 0.12ab
Masui Dauphine	68.48 ± 1.63gh	55.50 ± 1.30gh	76.41 ± 0.55g	63.33 ± 0.40g	97.78 ± 0.23c	57.46 ± 0.13c	98.30 ± 0.22cd	57.75 ± 0.12cd

Data were represented as mean ± SD of three measurements. Different letters symbolized significant differences [$P < 0.05$] by mean of the nonparametric Tukey HSD test.

Table 2. Total phenolic content and yields of extract of eighteen cultivars of *Ficus carica* in different extraction process.

Name of *Ficus carica* cultivars	Total phenolic content (mg GAE/gm DL)		Yields of extract (%)	
	Maceration	**Ultrasonication**	**Maceration**	**Ultrasonication**
Bourla Sotte	15.36 ± 0.35def	17.21 ± 0.41ef	8.13 ± 0.31f	10.98 ± 0.22bcd
A-132	17.04 ± 0.34bc	19.81 ± 0.29bc	9.20 ± 0.20cde	10.99 ± 0.23bcd
A-134	16.75 ± 0.33bc	19.49 ± 0.22cd	9.02 ± 0.17de	10.54 ± 0.21bcdef
Violette Solise	21.18 ± 0.68a	23.04 ± 0.30a	13.05 ± 0.23a	15.20 ± 0.20a
Qing Pi	14.13 ± 0.38fg	16.09 ± 0.43fgh	7.97 ± 0.21f	9.10 ± 0.24gh
Dotato	17.72 ± 0.38b	20.69 ± 0.62bc	9.00 ± 0.20e	10.22 ± 0.20cdef
B1011	16.74 ± 0.34bc	20.76 ± 0.41b	9.88 ± 0.20b	11.26 ± 0.23b
Brunswick	16.22 ± 0.32cd	18.40 ± 0.27de	9.68 ± 0.29bcd	10.88 ± 0.37bcde
Lisa	16.10 ± 0.46cd	19.66 ± 0.28bc	9.92 ± 0.20b	11.01 ± 0.37bc
B110	20.22 ± 0.64a	22.01 ± 0.61a	12.78 ± 0.20a	14.98 ± 0.23a
Orphan	16.88 ± 0.49bc	20.38 ± 0.47bc	9.74 ± 0.32bc	10.73 ± 0.25bcde
Longue d'Aout	14.44 ± 0.52fg	17.12 ± 0.41f	8.82 ± 0.16e	9.81 ± 0.42fg
Dauphine	15.95 ± 0.59cde	17.22 ± 0.22ef	9.90 ± 0.22b	11.21 ± 0.22b
White Genoa	14.64 ± 0.30ef	14.94 ± 0.34h	8.98 ± 0.18e	10.17 ± 0.15ef
Alma	10.70 ± 0.20h	12.94 ± 0.23i	7.20 ± 0.20g	8.25 ± 0.25i
Dow Law	13.12 ± 0.22g	16.18 ± 0.24fg	9.27 ± 0.21bcde	10.59 ± 0.36bcdef
Wuhan	14.27 ± 0.52fg	15.51 ± 0.61gh	8.99 ± 0.19e	10.20 ± 0.18def
Masui Dauphine	11.76 ± 0.31h	13.18 ± 0.36i	7.63 ± 0.17fg	8.47 ± 0.26hi

Data were represented as mean ± SD of three measurements. Different letters symbolized significant differences [P < 0.05] by mean of the nonparametric Tukey HSD test.

Table 3. Phenolic compounds characterized using LC-MS/MS in the extract of fig leaves of 'Violette solise' cultivar.

Peak N°	RT [min]	Experimental *m/z*	Ion type	Molecular formula	Theoretical *m/z*	Error [ppm]	MS/MS fragments [*m/z*]	Proposed compounds	Reference
1	1.2	272.9607	$[M-H]^-$	-	-	-	NF	Unknown	
2	11.3	417.1059	$[M-H]^-$	$C_{17}H_{22}O_{12}$	417.1039	-4.9	241.0727, 152.0101	Dihydroxybenzoic acid di-pentoside	[23, 30]
3a	12.2	459.1530	$[M-H]^-$	$C_{20}H_{28}O_{12}$	459.1508	-4.8	167.0357	Derivative of vanillic acid	[23]
3b		338.2450	$[M-H]^-$	-	-	-	320.2320, 224.1741	Unknown	
4a	12.9	731.1855	$[M-H]^-$	$C_{34}H_{36}O_{18}$	731.1829	-3.6	345.0767, 319.0970	Unknown	
4b		579.1376	$[M-H]^-$	$C_{26}H_{28}O_{15}$	579.1355	-3.6	399.0705, 369.0637	Luteolin *C*-hexoside *C*-pentoside	[16, 23, 30]
5	13.6	563.1423	$[M-H]^-$	$C_{26}H_{28}O_{14}$	563.1406	-3.0	383.0768, 353.0670	Apigenin *C*-hexoside *C*-pentoside isomer 1	[23, 30, 56]
6	13.7	591.1004	$[2M-H]^-$	$C_{26}H_{24}O_{16}$	591.0992	-2.1	179.0349	Caffeoylmalic acid	[23, 30, 56]
		295.0464	$[M-H]^-$	$C_{13}H_{12}O_8$	295.0459	-1.6			
7	14.0	563.1415	$[M-H]^-$	$C_{26}H_{28}O_{14}$	563.1406	-1.5	353.064	Apigenin *C*-hexoside *C*-pentoside isomer 2	
8a	14.8	577.1572	$[M-H]^-$	$C_{27}H_{30}O_{14}$	577.1563	-1.6	293.0447	Apigenin *C*-hexoside *C*-deoxyhexoside	[23, 30]
8b		451.3296	$[M-H]^-$	-	-	-	337.2578, 225.1601	Unknown	
9	15.1	609.1469	$[M-H]^-$	$C_{27}H_{30}O_{16}$	609.1461	-1.3	300.0276	Quercetin-3-*O*-rutinoside [rutin]	[16, 23, 30]
10	17.4	731.1850	$[2M-H]^-$	$C_{34}H_{36}O_{18}$	731.1829	-2.9	551.1154, 389.0665, 203.0303	Psoralic acid glucoside	[16, 23, 30]
11	18.2	949.6728	$[M-H]^-$	-	-	-	NF	Unknown	
12	18.5	551.1205	$[M-H]^-$	-	-	-	507.1309, 345.0768	Unknown	
13	19.9	375.2752	$[M-H]^-$	-	-	-	NF	Unknown	
14	21.9	345.0770	$[M-H]^-$	-	-	-	NF	Unknown	
15	23.2	309.2071	$[M-H]^-$	$C_{18}H_{30}O_4$	309.2071	0.1	NF	Dihydroxy-octadecatrienoic acid	[58]
16	27.4	293.2116	$[M-H]^-$	$C_{18}H_{30}O_3$	293.2122	2.1	NF	Hydroxy-octadecatrienoic acid isomer 1	[58,59]
17	28.2	295.2271	$[M-H]^-$	$C_{18}H_{32}O_3$	295.2279	2.6	NF	Hydroxy-octadecadienoic acid /13-hydroxy-octadecadienoic acid	[58,59]
18a	28.7	471.3479	$[M-H]^-$	-	-	-	NF	Unknown	
18b		293.2118	$[M-H]^-$	$C_{18}H_{30}O_3$	293.2122	1.4	NF	Hydroxy-octadecatrienoic acid isomer 2	[58,59]
19	31.5	277.2164	$[M-H]^-$	$C_{18}H_{30}O_2$	277.2173	3.3	NF	9,12,15-Octadecatrienoic acid [linolenic acid]	[59]
20	32.6	279.2321	$[M-H]^-$	$C_{18}H_{32}O_2$	279.2330	3.1	NF	9,12-Octadecadienoic acid [linoleic acid]	[59]
21	33.8	281.2479	$[M-H]^-$	$C_{18}H_{34}O_2$	281.2486	2.5	NF	Octadecenoic acid	[59]

NF, not fragmented under our experimental MS/MS conditions; RT, retention time